LIMNOLOGICA
Ecology and Management of Inland Waters

In cooperation with the German Limnological Society

Influence of stock origin and environmental conditions on the survival and growth of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in a cross-exposure experiment[☆]

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ARTICLE INFO

ABSTRACT

The freshwater pearl mussel (*Margaritifera margaritifera*) is a highly specialized and sensitive freshwater bivalve, whose survival in the juvenile phase is indicative of high quality habitats. This contribution investigates the use of juvenile freshwater pearl mussels as bioindicators, considering the influence of mussel stock and study stream conditions on juvenile performance, as described by survival and growth rates. A standardized cross experiment was carried out investigating juvenile performance in four different pearl mussel stocks originating from the Rhine, Danube and Elbe drainages, representing distinct genetic conservation units. The juveniles were exposed in five study streams which were selected to integrate pearl mussel streams with different water qualities and recruitment status of the mussel population. Per study stream, five standard mesh cages containing an equal number of 20 (10 × 2) juvenile pearl mussels per stock in separate chambers were installed. Survival and growth rates of juveniles were checked after three months (*i.e.* before their first winter) and after nine months (*i.e.* after their first winter). Mussel stock and study stream conditions significantly influenced juvenile performance. Growth rates were determined by study stream conditions and increased with stream water temperature, organic carbon and C/N ratios. Survival rates varied stock-specifically, indicating different levels of local adaptation to their native streams. Due to the detection of stream-specific differences in juvenile performance, freshwater pearl mussels appear suitable as bioindicators. However, a careful consideration of stock-specificity is necessary to avoid false interpretation of bioindication results. The comparison of stock-specific survival in native versus non-native streams implicates that exposure of juveniles outside their native habitats is able to increase breeding success or else serve for risk spreading in breeding programs.

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Introduction

The freshwater pearl mussel (*Margaritifera margaritifera*) is a highly specialized and sensitive freshwater bivalve, inhabiting oligotrophic, high quality stream habitats (Geist and Auerswald, 2007; Hastie et al., 2000; Oesterling et al., 2010). The species has a complex life cycle including an obligate parasitic stage on suitable host fish and a juvenile phase in the interstitial zone (Taeubert et al., 2010; Young and Williams, 1984). The most sensitive life stage appears to be the early post-parasitic phase, during which the juveniles need a stable, but well oxygenized interstitial for up to 5 years (Buddensiek et al., 1993). At present, land-use changes have resulted in severe siltation of pearl mussel rivers degrading juvenile habitats by clogging of macropores with subsequent reduction of oxygen supply to the interstitial zone (Denic and Geist, 2014; Leitner et al., 2014; Oesterling et al., 2008; Scheder et al., 2015). As a consequence, many populations are on the brink of extinction, as they lack juveniles and have not recruited for decades (Geist, 2010).

As catchment restoration is time consuming, (semi-)artificial propagation and captive breeding are currently implemented as

short term conservation action to preserve the overaged populations (Gum et al., 2011). In semi-artificial breeding, juveniles are kept in mesh cages and exposed in the free-flowing water of rivers, serving as bioindicators for ambient water quality at the same time. The captive breeding effort in endangered mussel species such as the freshwater pearl mussel has led to an increasing availability of artificially bred juvenile mussels for reintroduction and bioindication studies. However, the variable survival rates of these mussels and the unknown reasons for this observation have led to a controversy about the suitability of juvenile pearl mussels as bioindicators (Gum et al., 2011; Schmidt and Vandre, 2010).

It is known that different stocks of the freshwater pearl mussel can show high rates of genetic differentiation even at small spatial scales (Geist and Kuehn, 2005; Geist et al., 2010; Karlsson et al., 2014), suggesting that specialization and local adaptation may occur. However, the phenomenon of local adaptation is controversially discussed in the literature with an approximately equivalent amount of studies demonstrating or disconfirming local adaptation (Jones, 2013; Lajeunesse and Forbes, 2002). Kaltz and Shykoff (1998) proposed that local adaptation and its detection depend on the spatial scale on which experiments are conducted. Jones (2013) argued that local populations may not always be optimally adapted to their native habitats, especially in degraded environments. Experiments investigating the correlation between genetic differentiation, local adaptation and ecological performance are mainly restricted to terrestrial plants or parasites and are often based on theoretical models exclusively (*e.g.* Bennington et al., 2012; Gandon and Michalakis, 2002; Jones, 2013; Leimu and Fischer, 2008; Malagnini et al., 2013). Knowledge on the interaction between genetics and performance may help to increase conservation success for endangered species such as the freshwater pearl mussel. In this contribution, we therefore tested the influence of freshwater pearl mussel stock and study stream conditions on juvenile mussel performance. A standardized cross experiment was carried out investigating growth and survival of four different pearl mussel stocks from three large Central European drainage systems, the Rhine, Elbe and Danube. Specifically the following hypotheses were tested: (i) environmental factors determine growth and survival rates independent of mussel stock, (ii) the probability of winter survival increases with mussel shell length, (iii) stocks are locally adapted and juvenile mussels from native stocks exposed to the streams of parental origin exhibit higher growth and survival rates than non-native stocks and (iv) freshwater pearl mussel juveniles are suitable bioindicators separating high and low quality habitats.

Material and methods

Study area

Mussel stocks and study streams were selected to cover three large Central European drainage systems of the Rivers Rhine, Elbe and Danube. Their location, names and codes, which are referred to throughout the text, are visualized and explained in Fig. 1. Mussel stocks were selected to represent distinct genetic conservation units (Geist and Kuehn, 2005). The study streams were chosen according to the following criteria: The streams had to be pearl mussel streams of different status with respect to water chemistry and the mussel population. Basic parameters of the study streams are summarized in Table 1. Three of the study streams (DG, ER, RO) were native streams with non-recruiting mussel stocks used in the experiment. In addition, one stream with a recruiting pearl mussel population (DW) and one stream where the freshwater pearl mussel is considered extinct (EH) were included.

Study design

Infestation of host fish was carried out by on site collection of glochidia (Gum et al., 2011) and subsequent preparation of an infestation bath to which host fish were exposed. As host fish, local brown trout (*Salmo trutta*) strains were used to ensure high infestation rates (Taeubert et al., 2010). Infestation measures were performed in areas of mussel stock origin. Before the start of the experiment, infested host fish were transferred to the Aquatic Systems Biology Laboratories at Technische Universitaet Muenchen, where collection of juvenile mussels was carried out. Juvenile excystment occured from 04 June 2012 to 13 July 2012, with
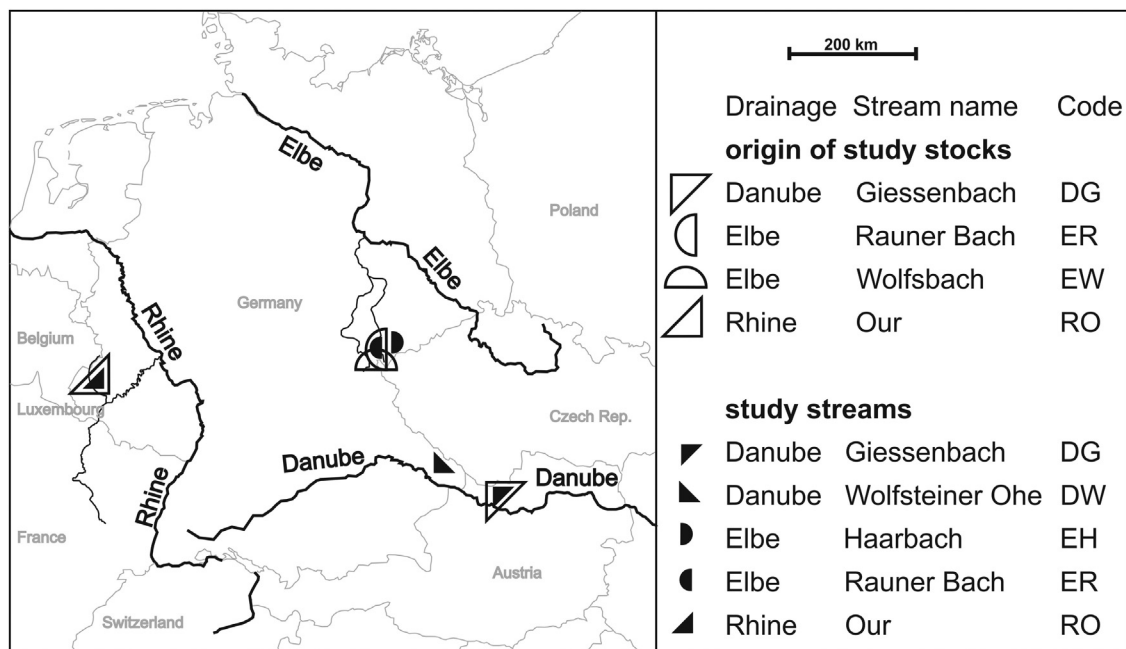

Fig. 1. Location of mussel stock origin and study streams in relation to major Central European drainage areas. Codes are composed of initial letters of major drainage (first code letter) and mussel stock origin or study stream, respectively (second letter).

peak collection between 26 June 2012 and 06 July 2012 following the procedure described by Taeubert et al. (2013). Only juveniles collected during the peak collection period were used in the experiment to avoid the introduction of a bias by using incompletely developed juveniles. Since not all mussels excysted at the same day, freshly dropped off juveniles were maintained for a maximum of 14 days and supplied with food following Eybe et al. (2013) until they were randomly transferred to the mesh cages. Per study stream, five standard mesh cages, so-called *Buddensiek cages* (Buddensiek, 1995), were installed. Each cage contained an equal number of $20 (10 \times 2)$ juvenile pearl mussels per stock in separate chambers. Due to the restricted number of individuals from the Elbe populations, the mesh cages exposed in the ER did not contain mussels of stock EW and cages exposed in RO did contain juveniles from RO and DG only. Performance of juvenile mussels (described by growth and survival rates) was checked after three (before the first winter) and nine months (after the first winter) of exposure under a binocular microscope. Growth was defined as the ratios of the pooled maximal shell lengths of all living individuals of specific stocks and at specific sites at different time points. To minimize the potential effects of the measurements on the mussels, they were always kept covered by original stream water and handling times between retrieval and replacement in the streams were kept minimal. Juvenile mussel size was determined by measuring the maximum total shell length ($\pm 2\,\mu m$) using a binocular microscope connected to the cell D software program.

Water temperature at the study sites was measured continuously once per hour with temperature loggers (EL-USB-1, Lascar Electronics, Salisbury, UK). Detritus samples were collected from the streams every three months and stored at $-20\,°C$ until analysis. In the laboratory, organic carbon was determined by burning the samples for 5 h at 550 °C in a muffle oven. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was carried out for determination of detritus origin and quality as a food source for juvenile mussels. Samples were dried for 48 h at 40 °C, ground to fine powder and packed into tin capsules. $\delta^{13}C$ and $\delta^{15}N$ were measured with an isotope ratio mass spectrometer (IRMS, Delta plus, Finnigan MAT, MasCom GmbH, Bremen, Germany). The IRMS was connected to (via ConFlo II, Finnigan MAT, MasCom GmbH, Bremen, Germany) an elemental analyser (EA 1108, Carlo Erba, Thermo Fisher SCIENTIFIC, Milan, Italy). Stable isotope ratios are expressed in delta (d) notation, as parts per thousand (‰) relative to a Vienna-PeeDee Belemnite (V-PDB) standard for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Analyses of a solid internal laboratory standard (bovine horn, run after each 10 samples) were used to calibrate C and N isotope determination revealing maximum standard deviations of 0.19‰ for $\delta^{13}C$ and 0.17‰ for $\delta^{15}N$. Deltas were calculated as follows: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where δX is $\delta^{13}C$ or $\delta^{15}N$, and R is the respective $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

Statistical analysis

Relative survival and growth rates were calculated per stock and study stream. Univariate general linear models (GLM) were

calculated to quantify the contribution of study stream (*i.e.* environmental factors) and mussel stock to the variation in survival and growth rates, with separate models for the total, before winter and over winter periods: $Y = \mu + A + B + C + \varepsilon$ and $Z = \mu + A + B + C + \varepsilon$ where Y is the survival rate and Z the growth rate. μ represents the intercept and ε the random error term. Study stream (A) and mussel stock (B) are fixed factors and C is their interaction. Differences of survival and growth rates between streams and mussel stocks were analyzed with one-way ANOVA and Tukey's post-hoc test in case of normal distribution and homogeneity of data. Kruskal–Wallis test and Tamhane-T2 test were used in case of non-homogenous data. Stream specific differences in RO were tested by Student's *t*-test, as only two groups were compared. Linear regression analyses were carried out to assess the dependence of survival on size parameters (growth rates, shell length). Relative comparisons of stock specific survival and growth rates were expressed as differences between survival and growth of stocks in native versus non-native study streams. Analogously, stream-specific survival and growth rates (differences in survival and growth rates of the non-native versus native mussel stocks in a study stream) were computed, with positive values indicating higher growth or survival of non-native versus native stocks, or of stocks in non-native versus native streams (*i.e.* where mussel stocks originated from). All statistical analyses were performed with IBM SPSS 20.

Results

After nine months of exposure, mean survival of juveniles was 16.1%, mean growth rate was 79.4% and mean shell length was 0.77 mm. Mean values of stock and stream specific total survival and growth rates ranged from 1 (stock RO in ER) to 33% (stocks DG and EW in DG) and from 60 (stock ER in EH) to 102% (stock ER in DW), respectively.

Analysis of univariate general linear models revealed that study stream and mussel stock contributed significantly to the variation in juvenile performance, though model contributions varied by date and variable. Significant contributions of models to the variation in total, before winter and over winter survival were detected with $p < 0.001$ and $r^2 = 0.536$, 0.802 and 0.665, respectively. In contrast, the model parameters only contributed significantly to total and before winter growth rates ($p < 0.001$, $r^2 = 0.588$ and $p = 0.01$, $r^2 = 0.412$) but not to over winter growth ($p = 0.799$). In all models, variation in growth rates was explained mainly by study stream, whereas mussel stock had a stronger influence on variation in survival rates. For instance, mussel stock explained 40.9% of the variation in total survival ($p < 0.001$) whereas study stream accounted only for 15.0% ($p = 0.034$).

Multiple comparisons of stock and stream-specific performance supported the results of GLM analyses. Overall, the highest growth rates were found in warmer study streams with mean water temperatures of at least 13.5 °C during summer (DW, DG, RO), whereas cooler water temperatures (12.8 °C) as in the EH resulted in reduced growth (Table 1). The highest growth rate and shell length before winter were detected in the DW, where high water temperatures

Table 1
Basic characteristics of the study streams used in the cross exposure experiments: exposed study stocks, recruitment status of the native mussel populations and arithmetic mean values of NO_3-N, NH_4-N, pH, specific conductance, water temperature, organic carbon, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ are provided.

Study stream	Exposed study stocks	Native mussel population	NO_3-N [mg/l]	NH_4-N [mg/l]	pH	Sp. conductance [µS/cm]	Water temperature [°C]	Organic carbon [%]	C/N ratio	$\delta^{13}C$ [%]	$\delta^{15}N$ [%]
DG	DG, ER, EW, RO	Non-recruiting	2.4	0.02	7.2	165	13.5	12.1	13.9	−28.7	2.9
DW	DG, ER, EW, RO	Recruiting	1.5	0.15	7.2	103	13.5	11.8	14.3	−28.6	3.1
EH	DG, ER, EW, RO	Extinct	3.6	0.05	7.2	117	12.8	9.2	13.8	−29.1	3.5
ER	DG, ER, RO	Non-recruiting	1.8	0.05	7.4	210	n.d.	8.1	11.3	−28.5	4.3
RO	DG, RO	Non-recruiting	4.4	0.05	7.3	166	14.3	8.1	11.9	−28.5	3.9

and the highest C/N ratios in detritus samples were found. However, differences for C/N ratios were not significant between streams (Kruskal–Wallis test, $p = 0.284$). $\delta^{13}C$ values of detritus samples ranged closely around -28 ‰ and $\delta^{15}N$ values varied around 3 ‰ in all streams.

A comparison of stream-specific total growth rates of single study stocks revealed significant differences for all stocks except RO (one-way ANOVA, $p < 0.040$ and $p = 0.612$). Total growth of the stocks DG, ER and EW was highest in the study stream DW. However, in case of stock DG, total growth was similarly high in the native stream DG and RO (Tukey-HSD, $p = 0.119$ and 0.931, respectively). The highest total survival rates were detected in the streams DW and the DG (Fig. 2; one-way ANOVA $p = 0.003$). Differences were

significant between DW and ER (Tukey-HSD, $p = 0.022$) as well as between DG and ER and RO, respectively (Tukey-HSD, $p = 0.005$ and 0.028). Over-winter survival rates in the DW (94.1%) were significantly higher than in the other study streams (Tukey-HSD, $p < 0.001$) suggesting a correlation between winter survival and shell length before winter. This finding is supported by regression analysis revealing highly significant, positive relationships of winter and total survival rates to shell lengths and growth rates before winter. In contrast, survival before winter did not depend on these factors (Fig. 3). Mussels of the RO stock had the lowest growth rates and shell lengths before winter. Consequently, over-winter and total survival rates were significantly lower in the Rhine stock compared to the Danube and Elbe stocks (Tukey-HSD, $p = 0.02$
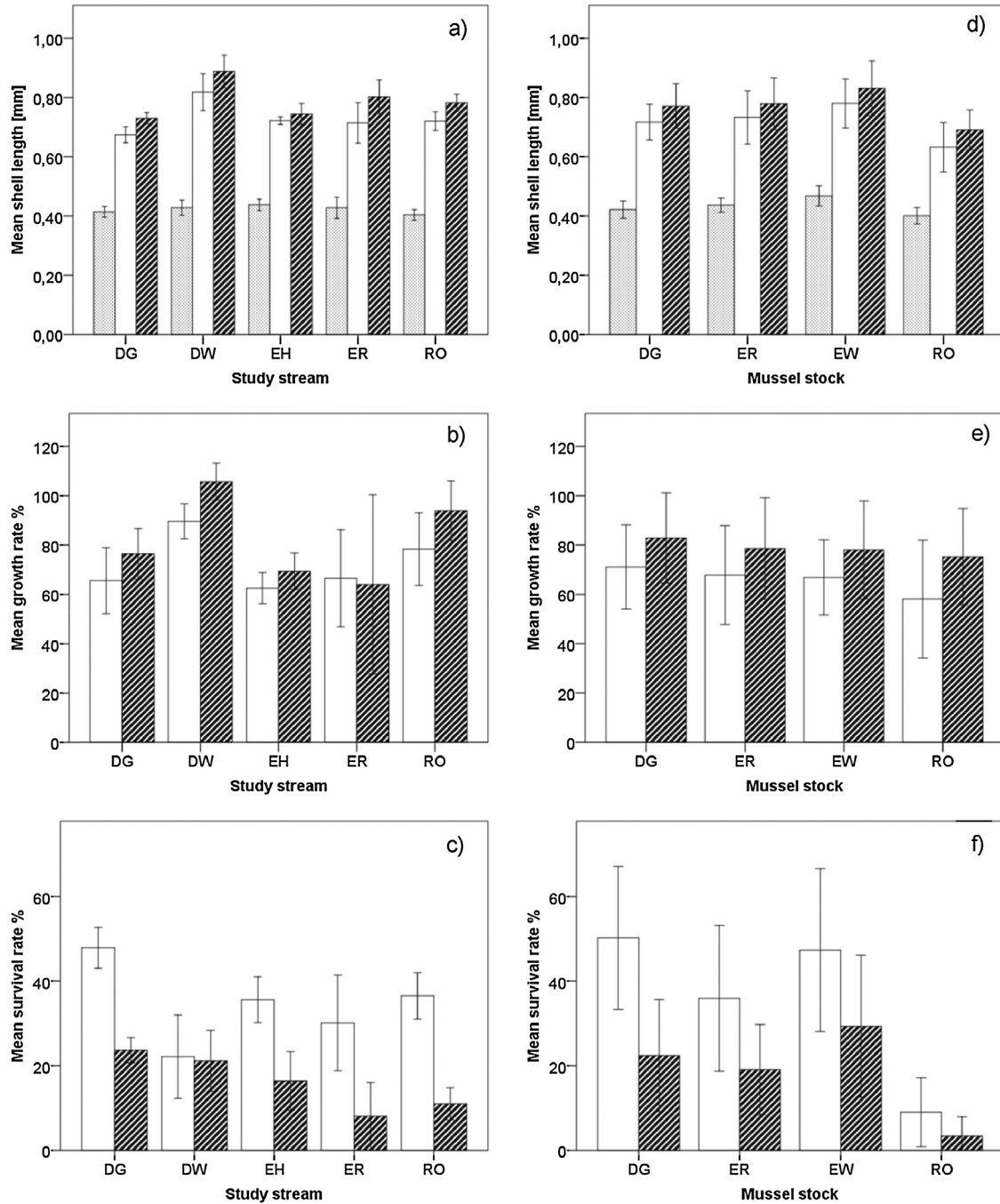

Fig. 2. Study stream specific (a–c) and mussel stock-specific (d–f) initial (dotted bars), before winter (white bars) and total (shaded bars) mean shell lengths, growth rates and survival rates with standard deviations.
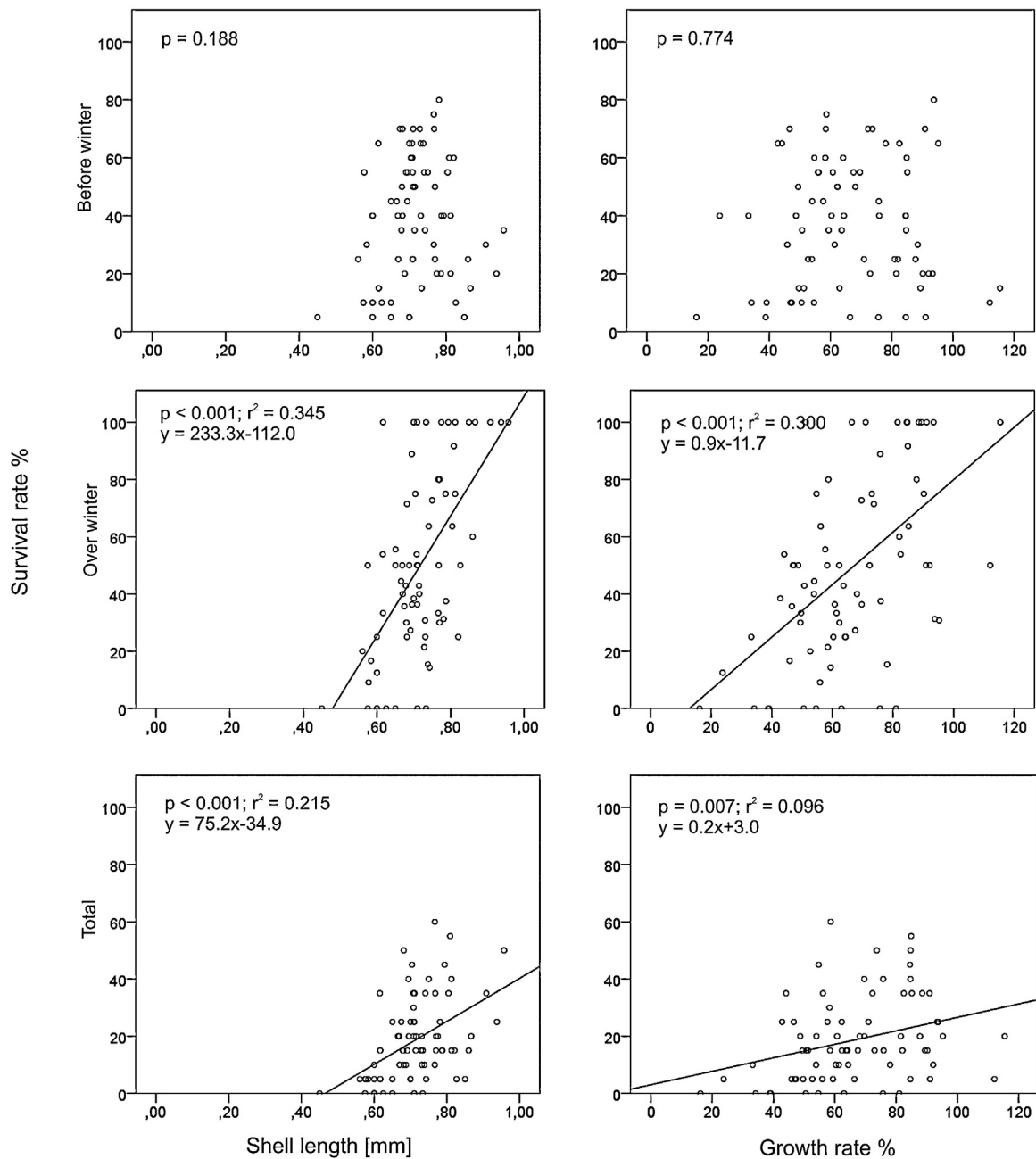
Fig. 3. Before winter, over winter and total survival rates as a function of juvenile mussel shell length and growth rate.

and Tamhane-T2 test, $p < 0.001$). This result remained the same, no matter if streams were analyzed simultaneously or separately. Stock-specific total survival did not differ between study streams (one-way ANOVA, $p \geq 0.133$).

Strongest indications of local adaptation were found in the stock DG. This stock revealed highest total survival rates of 33% in its native environment compared to 13–27% in the other streams (one-way ANOVA, $p = 0.135$). Total growth rates were highest in DW with 101%, but were not significantly lower in the native stream DG (81%, Tukey-HSD, $p = 0.119$). Furthermore, non-native stocks did not perform better in the DG than the native stock, though performance of DG was not significantly increased, except for the comparison of total survival to RO (Tukey-HSD, $p = 0.025$). In contrast, stock RO rather showed tendencies of maladaptation, as in study stream RO total survival of RO was significantly lower than of DG with 3% compared to 19%, respectively (Student's t-test, $p = 0.001$). Total growth

rate was also lower with 76% compared to 96%, though not significantly (Student's t-test, $p = 0.245$). It is important to note that time can substantially influence the relative performance of study stocks (Fig. 4). For instance, before winter survival rates of stock DG were significantly lower in stream DW than in stream DG (29% compared to 60%; Tukey-HSD, $p = 0.009$), but total survival rates became similar to each other with 27% and 33% (Tukey-HSD, $p = 0.935$).

Discussion

This study tested the influence of freshwater pearl mussel stock and study stream conditions on juvenile mussel performance with a special focus on local adaptation and the use of juvenile *M. margaritifera* as bioindicators for stream habitat quality. The results indicate a significant influence of both, mussel stock and study stream conditions, on juvenile performance and point to a variable
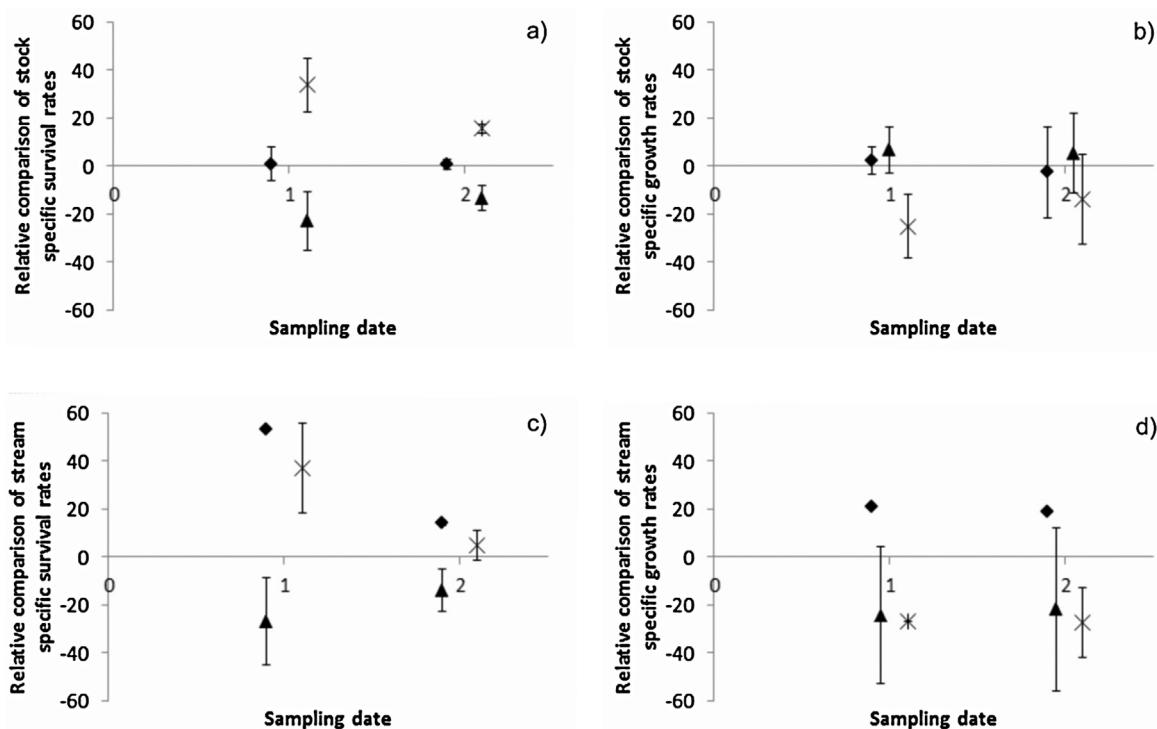
Fig. 4. Relative comparison of mussel stock specific (a) survival and (b) growth rates (*i.e.* differences between survival and growth of stocks in native versus non-native study streams) as well as study stream specific (c) survival and (d) growth rates (*i.e.* differences of survival and growth rates between native versus non-native mussel stocks in a study stream) after three and nine months of exposure (sampling dates 1 and 2). Quadrats = RO; Triangles = DG; Stars = ER. Note that positive values indicate higher growth or survival of non-native versus native stocks, or of stocks in non-native versus native streams (*i.e.* where mussel stocks originated from).

amount of local adaptation among pearl mussel stocks. This specificity makes a careful consideration of mussel stock obligatory to avoid bias in bioindication studies.

Stream specific influences on juvenile mussel performance

Several studies indicate that key habitat parameters determine survival and growth rates of juvenile freshwater pearl mussels regardless of mussel stock. For instance, Buddensiek (1995) and Hruska (1992) found a correlation between juvenile growth and water temperature during the summer growing season. Shell length is considered as an indicator for juvenile mussel fitness and higher shell lengths are believed to increase the probability of winter survival (Lange and Selheim, 2011). These results were confirmed in this study, as juveniles exposed in the warmest study streams grew fastest over summer and a correlation between shell length and winter survival was detected. In addition to water temperature, food quality (*e.g.* detritus composition) is important for the performance of juveniles. In our study, organic carbon and C/N ratio tended to be higher in streams with higher survival and growth rates, though differences were not significant. In contrast, Geist and Auerswald (2007) did not observe any separation between functional and non-functional pearl mussel streams in terms of detritus composition on a European scale, indicating that this factor may only locally differentiate high and low quality streams. Organic carbon content and C/N ratios were generally lower in their study with mean values of 4.01% and 11.28, respectively. The $\delta^{13}C$ values of approximately −28 ‰ indicated that all streams are heterotrophic systems and detritus originated mainly from terrestrial material, as this signature is typical for C_3 plants (Troughton et al., 1974). The $\delta^{15}N$ values of about 3 ‰ cannot be as clearly matched, as ^{15}N compounds are processed in various transformation pathways and can therefore deviate from the signatures of the source materials (Kellman and Hillaire-Marcel,

2003). Furthermore, signatures of different nitrogen compounds derived from the same source material can vary (Bedard-Haughn et al., 2003). Nevertheless, low $\delta^{15}N$ values, as found in this study, are usually associated with forested catchments and low human influence (Harrington et al., 1998). Agricultural catchments, especially with intensive livestock farming (due to manure and septic waste production) are characterized by elevated levels of $\delta^{15}N$ values of 6–20 ‰ (Harrington et al., 1998; Lefebvre et al., 2007; Ohte, 2013; Peterson and Fry, 1987) and are unlikely to be of high importance for the river stretches analyzed herein. Yet, nitrate input from artificial fertilizers is known to have $\delta^{15}N$ signatures corresponding to the values in this study (Bedard-Haughn et al., 2003; Ohte, 2013) and therefore cannot be excluded to play a role in some of the investigated catchments.

Stock-specific influences on juvenile mussel performance

In contrast to these obviously generally valid correlations between abiotic conditions and juvenile performance, univariate GLM indicated a significant influence of mussel stock as well, particularly on survival rates. This observation points to local adaptation of mussel stocks and is in accordance with previous observations of strong genetic population structuring and host fish specificity down to the subpopulation level (Geist and Kuehn, 2005; Geist et al., 2010; Karlsson et al., 2014). However, the relative comparison of stock and stream specific survival and growth rates suggests generally low levels of local adaptation as well as different adaptation levels of the study stocks. There are various explanations for these inconsistencies in adaptation levels. It was shown that local adaptation is scale-dependent and in some species can be detected on the microscale, whereas in others at least a regional scale has to be considered (Kaltz and Shykoff, 1998). Furthermore, population size and intra-population genetic variability influence the ability to adapt to specific or changing environmental conditions. Due to

the small population sizes of the mussel stocks used in this study, it cannot be excluded that this hypothesis only applies to some of the study stocks. Jones (2013) further proposed that species can only show local adaptation to intact, but not to degraded habitats. Nearly all Central European freshwater pearl mussel streams are presently considered more or less degraded as evident from a lack of natural recruitment in most of them (Geist, 2010; Sousa et al., 2014). Additionally, differences during the parasitic stage may also contribute to variation, not only on mussel stock but even on an individual level, as unionid juveniles receive nutrients from their host fish (Fritts et al., 2013). Depending on the fitness of host fish specimen and the intensity of their immune response, excysting juvenile mussels may start at different energetic levels. In addition, there are indications that excystment timing may influence size and fitness of juvenile mussels (Jung et al., 2013). Some experiments also found a correlation between glochidial densities on the host fish and their size after excystment, though the majority of studies did not confirm this result (Jung et al., 2013; Taeubert et al., 2010). To reduce individual differences as far as possible, locally infested host fish were kept under identical conditions for juvenile mussel excystment and juveniles were further kept under identical conditions until start of the experiment. Nevertheless, the stock-specific results should be interpreted with caution until they are being confirmed in a greater dataset.

Conclusions for bioindication and conservation

Freshwater mussels are considered target species for conservation, at the same time matching the concepts of flagship, keystone, umbrella and indicator species (Geist, 2010). Suitable bioindicators are characterized by their relevance, reliability, robustness, responsiveness and reproducibility. The freshwater pearl mussel fulfils all of these criteria, but bioindication experiments with endangered juvenile pearl mussels appear only useful in potential habitats (*i.e.* oligotrophic, silicate streams) and in situations in which the success of restoration measures or chances of reintroduction are to be assessed. Generally, the freshwater pearl mussel is notably one of the most sensitive freshwater organisms, reacting highly sensitive to changes in abiotic habitat conditions (Bauer, 1988; Geist, 2010; Geist and Auerswald, 2007; Hastie et al., 2000; Oesterling et al., 2008; Taskinen et al., 2011). Due to the high conservation status and mostly small population sizes, the availability of adult individuals is low. However, the availability of juveniles is continuously increasing due to intensive breeding efforts throughout Europe (Gum et al., 2011), during which juveniles are already most often exposed in Buddensiek cages in streams for rearing purposes, as was the case in our study. Consequently, bioindication would just increase the benefit of a system already in use, without sacrificing an additional number of juvenile mussels. The rearing success of juvenile mussels in Buddensiek cages was previously controversially discussed in the literature (Gum et al., 2011; Schmidt and Vandre, 2010). Based on the obvious suitability of the exposure setup of our study as well as results from Gum et al. (2011) and Spisar (pers. comm.), this system appears appropriate for use in bioindication studies that aim at testing water and nutrient quality of streams. The survival rates in our experiment were comparable to or higher than survival under natural conditions, which is estimated to range around 5% during the juvenile phase (Young and Williams, 1984) and revealed stream specific variation with better juvenile performance in study streams hosting the most intact mussel populations. However, the fact that significant stock-specific differences in juvenile performance were detected, demands a careful consideration of this parameter in the interpretation of bioindication results. The direct comparison between bioindication results using different mussel stocks should be avoided. Furthermore, cages were exposed to the free-flowing water in this experiment. Exposure to the substratum

will alter bioindication results with probably further reduction of survival rates.

Different study stocks showed different levels of local adaptation, but the specific cause for this observation remains unclear. Here, further research including completely intact habitats and mussel stocks may help to further clarify the picture. Comparisons of stock-specific survival in native versus non-native streams implicate that exposure of juveniles outside of native habitats is able to increase success of breeding programs, although rearing of juveniles in the native stream is still the preferable method. Consequently, rearing of juveniles in non-native habitats is recommendable in case of low breeding success in native habitats to bridge the time which is needed to restore the stream and for the purpose of risk spreading.

Acknowledgements

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