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Habitat traits, population structure and host specificity of the freshwater pearl mussel *Margaritifera margaritifera* in the Waldaist River (Upper Austria)

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Abstract: In European streams and rivers, the freshwater pearl mussel (*Margaritifera margaritifera* L., 1758) faces extinction. This is also true for the Waldaist River, with 20,000 specimens recorded in the early 1990s then Austria's most important pearl mussel river. Nowadays, there is only a single 320 m stretch with noteworthy mussel densities. During an in-depth survey of this river stretch in 2010, we detected a total of 2,774 specimens. Mussel microhabitats were confined to patches of sand and fine gravel (psammal and akal) at run sections of the river, stabilized by large boulders. Pearl mussels avoided large accumulations of fine sediments. Typically situated at undercut slopes, preferred microhabitats were 0.25–0.50 m deep at baseflow with current velocities (at 40% depth) of 0.2–0.6 m s^{-1}. A comparison of the present stock with data from 1997 revealed a rapid decline in mussel density down to 27%. We also noticed strongly reduced growth and a high mortality of medium age classes. Juvenile mussels were completely lacking. With respect to host specificity in terms of glochidia survival, the brook char *Salvelinus fontinalis* (Mitchill, 1814), a suitable host in North America, shed glochidia within eight days. In the brown trout *Salmo trutta* L., 1758, two strains were investigated. Glochidia survival, growth and prevalence were significantly higher in the Danish than in the Austrian hatchery strain.

Key words: freshwater pearl mussel; *Margaritifera margaritifera*; habitat; population structure; host specificity

Introduction

The distribution area of the freshwater pearl mussel (*Margaritifera margaritifera* L., 1758) ranges from Europe to eastern North America; the species is critically endangered throughout this area (Mollusc Specialist Group 1996; Young et al. 2001). This is also true for Austria, where this species has always been restricted to the Bohemian Massif (Gumpinger et al. 2002). Here, the once very large mussel beds have strongly declined and are presently only represented by tiny relict populations in which old age classes dominate (Moog et al. 1993). Due to its complex life cycle that includes parasitic larvae (= glochidia) spending up to eleven months in the gills of mostly young Salmonid fish (Hastie & Young 2003; Hruska 1992) followed by a juvenile stage in the hyporheic interstices, the pearl mussel is particularly vulnerable to anthropogenic interference (Wächtler et al. 2001). It therefore represents a very sensitive indicator species of oligotrophic, calcium-poor streams and rivers (Geist 2010). In Central Europe the pearl mussel inhabits patches of fine sediments that are stabilized by larger substrates in canopy-shaded streams and rivers of second to fifth stream order (Hastie et al. 2000, 2003; Moog et al. 1993; Skinner et al. 2003). The abundance of fine sediments is of crucial importance for the successful development of young mussels (grain size < 1 mm) as an interface between stream bed and hyporheic interstices (Altmüller 2002; Altmüller & Dettmer 2006; Geist & Auerswald 2007; Sachteleben et al., 2004).

In the present study we investigated microhabitat requirements, habitat traits and the population structure of adult mussels in the field. The parameters were assessed in the largest still-existing population in Austria, which is restricted to a 320 m long section of the Waldaist River in Upper Austria. The second part of the study focussed on the parasitic larval stage which, in Central Europe, begins in August / September when females release their larvae (glochidia) into the surrounding water (Moog et al. 1993). In the laboratory we investigated glochidia prevalence and host choice by exposing three fish taxa originating from different geographical regions to mussel larvae. By selecting non-native hosts for the experiments we aim to address the effects of replacing indigenous fish populations by Atlantic hatchery strains (Weiss et al. 2001). The aim of

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Table 1. Limnochemical parameters of the Waldaist River (arithmetic mean with range). Data were provided by the Provincial Government of Upper Austria, Surface Water Management, and are based on 30 samples taken between 2007 and 2009 at the river stretch investigated (Kapfer et al. 2012).

Parameter	Mean	Minimum	Maximum
pH	7.2	6.3	7.8
Conductivity ($\mu S\ cm^{-1}$)	100.7	70	150
BOD_5 ($mg\ L^{-1}$)	1.23	0.1	2.3
DOC ($mg\ L^{-1}$)	6.8	2.8	20.0
Total hardness	1.7	1.3	1.9
NH_4-N ($mg\ L^{-1}$)	0.016	0.009	0.034
NO_2-N ($mg\ L^{-1}$)	< 0.003	< 0.003	0.007
NO_3-N ($mg\ L^{-1}$)	1.1	0.7	1.7
P_{tot} ($mg\ L^{-1}$)	0.036	0.018	0.070
o-PO_4-P ($mg\ L^{-1}$)	0.015	0.008	0.022
Ca ($mg\ L^{-1}$)	9.3	7.5	10.0
Mg ($mg\ L^{-1}$)	1.6	1.1	2.0
Na ($mg\ L^{-1}$)	6.7	5.1	11.0
K ($mg\ L^{-1}$)	1.2	0.9	1.5
SO_4 ($mg\ L^{-1}$)	9.1	7.2	10.0
Cl ($mg\ L^{-1}$)	9.3	6.6	17.0

the present study was to enlarge our present knowledge of the microhabitat preferences and the parasite-host relationships which provide a basis for both the protection of remaining stocks and artificial breeding programs of the freshwater pearl mussel.

Material and methods

Study area

As the mussel bed investigated in the present study is one of the last remaining populations of *Margaritifera margaritifera* in Austria, its exact geographical position is not given here for conservation reasons. The bed is situated in the Waldaist River, located in the district of Freistadt, Upper Austria. The Name "Waldaist" is composed of the prefix "Wald-", meaning "forest", and the proper name "Aist" that can be derived from the Slavonian name "Agasta", roughly translated to "rapidly flowing river". Its two headwaters are called "Schwarze Aist" and "Weiße Aist", their names referring to distinct differences in water colour: the former – meaning "black rapidly flowing river" – drains a large marsh and shows a dark red to dark brown water colour resulting from dissolved humic substances and ferrous compounds, whereas the latter – translated to "white rapidly flowing river" – appears totally transparent. After the confluence of the two headwaters their colours mix, thereby creating the typical amber colour that the Waldaist is known for. The total catchment area of the Waldaist River equals 647 km^2, making the river system the largest of the Mühlviertel region, the crystalline part of Upper Austria north of the Danube River. Moog et al. (1993) described the Waldaist River as "the best remaining freshwater pearl mussel stream in Austria with a population of highest relevance and worthiness of protection". At that time the Waldaist supported a population of $\sim$ 20.000 specimens. Since then, numbers have declined drastically.

A summary of limnochemical parameters of the Waldaist River is given in Table 1.

Sampling in the field

At the beginning of the investigation we mapped the shorelines and the largest rocks of the 320 m long stretch of the Waldaist River where the mussel bed is situated, using a Leica GS 20 GPS device. Based on these data and an aerial photo, a GIS map of the study site was created (ArcGis 9.3 program). At the mesohabitat scale, we divided the investigated river stretch (stream width = 9–12 m; mean water depth = 0.5 m; baseflow discharge = 2.2–3.0 $m^3\ s^{-1}$) into pools, riffles and runs. In addition, bank vegetation and sediment types (choriotopes) were mapped using the typology of Graf et al. (2008). We identified the following choriotopes for characterising the microhabitats of the mussels: pelal (mud; grain size < 0.063 mm), psammal (sand; 0.063–2 mm), akal (fine to medium-sized gravel; 0.2–2 cm), microlithal (coarse gravel; 2–6.3 cm), mesolithal (cobbles; 6.3–20 cm), macrolithal (large stones; 20–40 cm), megalithal (boulders > 40 cm), xylal (woody debris < 10 cm). We used a glassbottom frame to map choriotopes; sediment patches with homogenous grain size were identified and grouped into choritope categories, and their respective areas were measured. In order to test these groupings, sediment samples from each choriotope were taken and analysed in the laboratory. For this, the samples were dried to constant weight in an oven at 50 °C and the grain sizes were separated by using a Retsch VS 1000 (Retsch GmbH, Germany) machine. In addition to sieve mesh sizes defined by DIN 4022 we also separated the grain size of 1 mm, because the proportion of grain sizes < 1 mm are thought to be the most important substrate for pearl mussel habitats (Sachteleben et al. 2004). In the small grain sizes (< 0.063 mm and 0.063–0.2 mm) we measured the ash-free dry weight at 450 °C in order to quantify the organic content.

Mussels were mapped from 22 July to 28 October, 2010. For the mapping we constructed a measuring grid across the river stretch and used a glassbottom frame. Hidden locations (undercut slopes, roots of the bank canopy and space beneath large boulders) were hand-scanned. At patches with high mussel densities, we hand-sieved sediment samples to a depth of 40 cm for detecting buried juvenile and adult mussels. The following biometric parameters were taken in the field by using a sliding gauge: maximum shell length, maximum shell height and maximum shell width at umbo level. The animals were then immediately reset to their original habitats. After this, we linked the exact location of each specimen with the mapped choriotopes and the abiotic parameters at meso- and microscale (= sediment grain size in the immediate vicinity of the mussel). Current velocity (at 40% depth and near-bottom velocity at 2 cm above sediment surface) was measured at mussel sites using a propeller meter (Ott Z 200). In addition, we also obtained velocity data from a number of randomly-chosen sites without mussels in order to define velocity thresholds.

Laboratory experiments

In the laboratory, empty shells were prepared for age determination by immersion in hot (75 °C) potassium hydroxide solution for 1–2 hours. Subsequently, the softened periostracum was gently removed with a brush, thereby exposing the yearly growth increment layers of the ostracum. The layers were counted and used for length-age regressions which, in turn, could be used for age determination of living mussels in the field (Bauer 1992). As young mussels were lacking in our samples, additional data of juveniles published by Ofenböck (1998, unpublished report) were used for supplementing our data.

The laboratory tests for host specificity and glochidia survival were performed in a local fish farm. We performed two experimental runs (low and high glochidia density) in three fish taxa, two strains of brown trout (*Salmo trutta* morpha *fario* L., 1758) and brook char (*Salvelinus fontinalis* Mitchill, 1814). Brown trout strain A and the brook trout were obtained directly from the local fish farm, the second, triploid brown trout strain D originated from a German fish farm which used eggs from the Atlantic line of *S. trutta* imported from Denmark. A subsequent genetic analysis conducted by Steven Weiss (Institute of Zoology, University of Graz, Austria) showed that strain A was in fact a domesticated Atlantic fish strain which was close to autochthonous Austrian fish farm populations. All fish were of age class 0+ with mean body lengths (± SD) of 9.2 ± 1.5 cm (brown trout strain A), 9.6 ± 1.2 cm (brown trout strain D) and 9.1 ± 0.9 cm (brook char), respectively. Body length did not differ significantly between the three taxa (ANOVA; $P = 0.1$). For identification during the experiments, strain D was marked by removal of the adipose fin. After anesthetizing the fish with clove oil (six drops per 10 litres of water) the fin was removed with nail scissors. The wound was disinfected with potassium permanganate solution (approx. 1 g per litre).

Glochidia were obtained from adult mussels from the Waldaist River, using the methodology of Wellmann (1943). For an exact timing of the highly synchronised glochidia release (Young & Williams 1984) mussels were regularly checked by carefully opening the shell approximately 1 cm wide by means of specially adapted pliers. This enabled us to check the gills for already existing marsupia (= breeding chambers). When gills were found to be thickened in a characteristic manner, the breeding chambers were punctured with a hypodermic syringe. These samples were immediately checked using a field microscope (Enhelion Micron pro) and attributed to one of five possible developmental stages (Scheder et al. 2011):

Stage 1: no visible differentiation, amorphous cell mass;

Stage 2: first constrictions on the surface visible;

Stage 3: complete differentiation of shells;

Stage 4: shells fully moveable, first snapping movements;

Stage 5: glochidium already hatched from the egg, frequent snapping movements.

Artificial infection of host fish is possible from stage 4 onwards. We took ten mussels with glochidia ready for infection, placed them in a bucket with water barely covering the animals and exposed them to sunlight, thereby forcing an emergency release of glochidia due to respiratory stress (Wellmann 1943; Scheder & Gumpinger 2008). The infection of the host fish was performed in two large, strongly aerated basins by mixing the glochidia suspension into the basin water. Two successive experiments were conducted, each with 150 fish from each taxon. For experiment 1 we added two doses of glochidia suspension to the fish tank, for experiment 2 one dose. After 45 minutes of exposure to the glochidia, the fish were transferred to two flow channels where they remained for the rest of the experimental period. The limnochemical properties of the water used in the experiments were not significantly different from the Waldaist River water. The fish were fed daily with commercial trout food. After one, eight, 29 and 49 days post infection 20 fish per taxon and experimental run were removed, killed with an overdose of clove oil and either analyzed immediately or frozen. Because brook char had already shed all glochidia after eight days, only ten specimens per experimental run were sampled after 29 days and then the experiment closed in this taxon. Freshly killed fish were dissected, gills removed, gill filaments were separated from the gill arches; glochidia were counted (410 fish) and measured under a microscope (20 glochidia each from 131 fish).

Data analysis

Data were tested for agreement with a Poisson series by using the Kolmogorov-Smirnov test statistics. For the statistical analysis of normally distributed data we used the Student *t*-test, an analysis of variance and the Scheffe post hoc test, for non-normally distributed data the $\chi\check{n}$-test, Mann-Whitney *U*-test, Kruskal-Wallis- and Friedman-tests, Nemenyi post-hoc test and Spearman rank correlation coefficients.

To obtain information on habitat preferences (choriotope types, current velocity ranges) of pearl mussels we used Ivlev's electivity index, which was originally developed for dietary preferences. According to Krebs (1989), Ivlev electivity E_i is given by:

$$E_i = r_i - n_i / r_i + n_i \quad (1)$$

where r_i is the percentage of habitat type i chosen by pearl mussels and n_i is the percentage of that habitat type present in the study area. Electivity varies from –1.0 to +1.0.

In order to quantify the condition of the fish used in the laboratory experiments, the Fulton condition factor K was calculated which is given by:

$$K = (G * 100)/L^3 \quad (1)$$

where L is the total fish length (cm) and G is the fish weight (g) (Ricker, 1975).

We used Microsoft Excel (Version 2003), SPSS 16.0 and SigmaPlot 10.0 for the statistical analysis and to generate graphics.

Results

Sediment structure and mapping of freshwater pearl mussels in the Waldaist River

The sediments of the investigated reach of the Waldaist River mainly consisted of cobbles (27%; grain size 6.3–20 cm) and coarse gravel (26%; 2.0–6.3 cm), both predominating in fast-flowing riffles, whereas the most abundant sediment type in run sections was fine to medium-sized gravel (24%; 0.2–2.0 cm). Undercut slopes were dominated by boulders and large stones (9%; < 20 cm). Sand patches (8% of the total area; 0.063–2.0 mm) were situated mainly in sheltered positions near the banks or between coarse sediment texture which stabilized these fine sediment accumulations. The remaining areas of the investigated reach were covered by medium stones, mud patches and woody debris.

In total, 2,774 living pearl mussels were detected in the study reach, with all animals located at the substrate surface. Despite sieving of a high number of sediment samples down to 0.4 m depth, no specimens were detected in the hyporheic interstices. Mussel densities were highest in two areas at the undercut slopes along the southern and northern bend (Fig. 1); at these locations which were heavily shaded by riparian canopy, we

Fig. 1. Density of *Margaritifera margaritifera* within the investigated 320 m stretch of the Waldaist River. Densities were highest at sandy patches between large boulders (black ovals) near undercut slopes. Upper right insert: catchment of the Waldaist within the borders of Austria.

mapped 405 and 1,798 specimens, respectively. At the other stream sections, 0–200 specimens were counted (Fig. 1).

Habitat selection

At the mesohabitat scale, pearl mussels significantly ($P < 0.001$) preferred runs (1.868 specimens) over riffles (879) over pools (27). This was also reflected by the preferred water depths of 0.25–0.5 m (Figs 2A, B).

Near-bottom flow velocities (v_b) at the study reach ranged from 0 to 0.67 m s^{-1}, current velocities at 40% depth (v_{40}) from 0.00 to 1.00 m s^{-1}. For the freshwater pearl mussel v_b seems to be more important, because this parameter is relevant for the level of the breathing siphos of the animals. At this near-bed level, flow speeds of 0.00–0.10 m s^{-1} were highly significantly ($P < 0.001$) preferred by the mussels, whereas the favoured v_{40} current speed was in the 0.20–0.55 m s^{-1} range (Figs 2C, D). Sites with higher flow velocities were colonized only occasionally, although single animals were also found in the strongest flow, reflecting the ecological plasticity of the species. Preferred low-velocity sites were almost exclusively situated within deadwater zones downstream of large stones and boulders; this is why such sites were frequently correlated with v_{40} current speeds of 0.30–0.60 m s^{-1} measured already above those roughness elements (Figs 2C, D). A significant relationship between near-bed flow velocity at the site and the shell length of the animals was proven: larger animals were more abundant within strong, smaller animals within low current speeds (Spearman's rank correlation coefficient = 0.133; $P < 0.01$).

With respect to choriotope types, boulder-stabilized macrolithal (large stones) was highly significantly ($P < 0.001$) chosen by *M. margaritifera*, although only 9% of the streambed area were covered by this sediment fraction. Other stabilized sediment types with high mussel densities were, in decreasing order, akal and mesolithal, whereas mussels were completely lacking within large uniform areas of fine sediments (psammal, pelal; Fig. 2E).

When looking at the microhabitat – which was defined as the substrate within a 5 cm radius of a pearl mussel – fine sediments which enabled burrowing were significantly preferred (Fig. 2F; psammal: 49% of the total, akal: 33% of the total). Only 3% of the mussels were collected in mesolithal sediments.

Population structure and shell morphology

Of the 2,774 mapped mussels 2,194 individuals were measured. The average shell length of the Waldaist River population was 93 mm (range: 47–144 mm). The most abundant length classes (= 41.5% of the total)

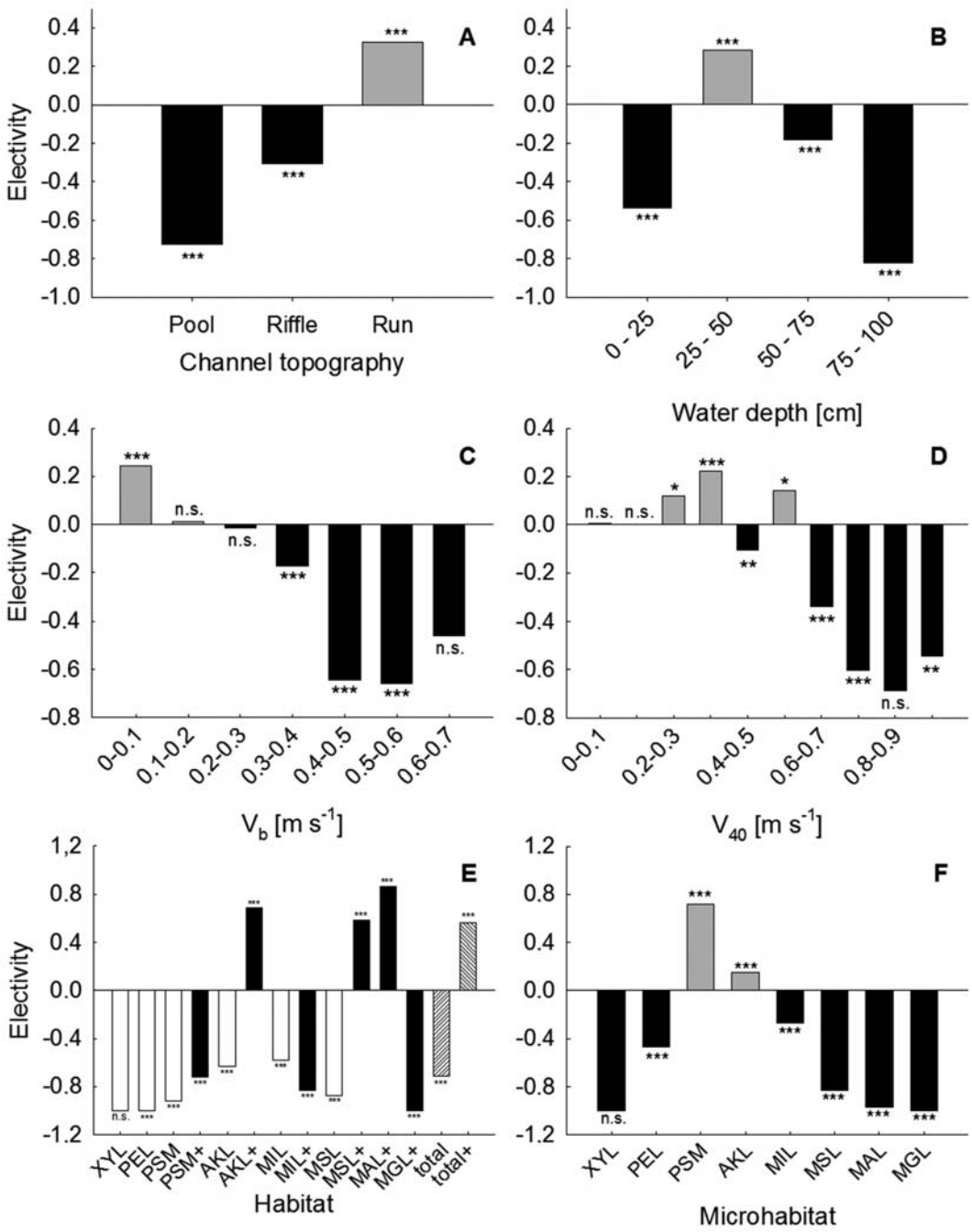

Fig. 2. Habitat preferences of *Margaritifera margaritifera* in the Waldaist River, given as Ivlev electivity (Ivlev 1961). The electivity index varies from –1 to +1, with values between 0 and +1 indicating preference and values between 0 and –1 indicating avoidance (Krebs 1989). Asterisks indicate significant choices (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. $P > 0.05$). A: channel topography; B: water depths (cm); C: bottom current velocity (v_b); D: current velocity at 40% depth (v_{40}); E: mesoscale sediment grain size (+: substrate stabilized by boulders); F: microscale sediment grain size (XYL – Xylal, PEL – Pelal, PSM – Psammal, AKL – Akal, MIL – Microlithal, MSL – Mesolithal, MAL – Macrolithal, MGL – Megalithal).

were, in decreasing order, 80–84 mm, 75–79 mm and 85–89 mm (Fig. 3). When comparing our data with a previous survey (Ofenböck 1998, unpublished report), it becomes obvious that the population structure has not changed significantly ($P > 0.05$; Fig. 3). In both studies, no recent reproduction was detected, i.e. specimens younger than 10 years were lacking in the samples. The length class distributions of empty shells and live mussels was not significantly different (χ^2 test; $P > 0.05$), suggesting a constant mortality rate over the entire adult stage of *M. margaritifera* in the Waldaist River. In total, 76 empty shells were collected, but only 18 shells were usable for age determination, the rest being shell fragments or unsuitable due to heavy corrosion. Therefore, additional data from Ofenböck (1998, unpublished report) were used for calculating length-age regressions. Although logarithmic or Bertalanffy models have been frequently used (e.g., Bauer 1992; Hastie et al. 2000), we found a second-order logarithmic regression as best fit for our data set ($y = 92.9 - 55.2 * \ln(x) + 14.9 * \ln(x)^2$;

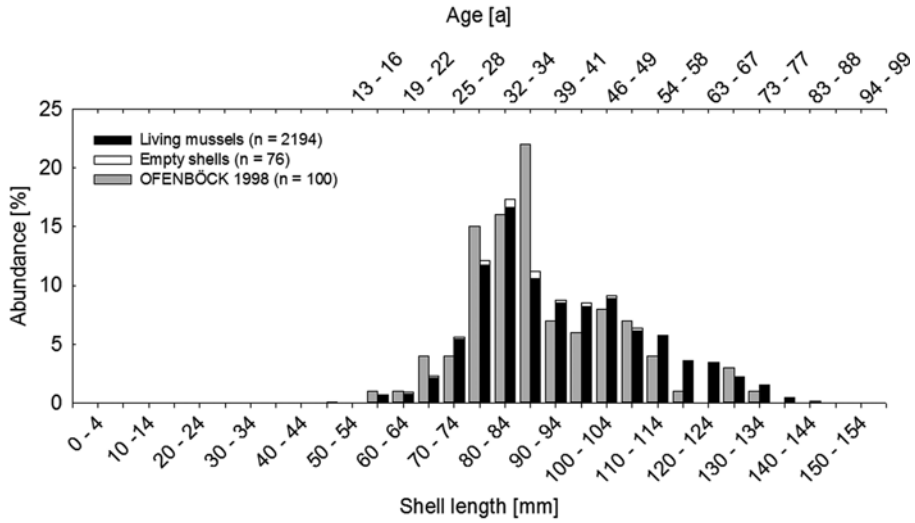

Fig. 3. Population structure of *Margaritifera margaritifera* at the investigated reach of the Waldaist River in 1997 and 2010 (black and white bars). For age determination, annuli of 18 intact shells where counted after removing the periostracum with KOH. Additionally, data from Ofenböck (1998, unpublished report) were used ($n = 106$; grey bars) to calculate length-age-regressions ($y = 92.9 - 55.2 \ln(x) + 14.9 \ln(x)^2$; $P < 0.01$; $r^2 = 0.75$) for defining the age shown in the upper abscissa.

$r^2 = 0.75$; $P < 0.001$). According to this model, pearl mussels of 45–60 mm length are 10 to 19 years old, whereas the largest shells (137–145 mm length) are 80 to 89 years old (Fig. 3, upper abscissa). When comparing the age groups of our samples with the data of Ofenböck (1998, unpublished report), the proportion of mussels 10–19 years old is 0.8% *versus* 1.0%, of 20–29 year old specimens 15.6% *versus* 14.0% and of 30–39 year old mussels 53% *versus* 37.4%. Generally, the proportion of older age classes today is higher than it was 13 years before, although these differences were not significant (χ^2 test; $P > 0.05$).

With respect to shell morphology, the outline of younger specimens was always ovoid with a convex ventral side. This is also true for about half the proportion of older specimens, the remainder showing a kidney-shaped outline with a concave ventral side. Shell height was always in the range of 38–57% of shell length (arithmetic mean = 46%), whereas shell thickness ranged from 19 to 39% of the shell length (arithmetic mean = 28%). In addition, we observed a (non-significant; $P > 0.05$) trend of increased shell heights in pearl mussels inhabiting low-current microhabitats.

Host specificity, infection rates and growth of glochidia
The initial rate of infection 24 h after glochidia addition was strongly influenced by the size of the infected fish; fresh weight was found to be a better predictor for infection rate than total length. For this, we standardized the number of glochidia per fish and took glochidia abundance times 10 divided by fish fresh weight. In brook char, the initial infection rate was significantly lower ($P < 0.001$; arithmetic means ± 95% CL in experiment 1: 16 ± 13, in experiment 2: 10 ± 7 glochidia) than in brown trout. Within the latter, the corresponding figures were 534 ± 153 and 430 ± 102 in strain A (Austrian hatchery fish) and 560 ± 138 and 266 ± 57 in Atlantic strain D from Denmark, respectively. Between brown trout strains, differences were not significant ($P > 0.05$). Within individual fish, glochidia infection decreased significantly from the anterior (first and second) to the posterior (third and fourth) gill arches ($P < 0.001$; Friedmann test).

The brook char used in the experiments were no suitable host fish for the pearl mussels of the Waldaist River. Just eight days after infection, gills of this species were free of glochidia; this was confirmed by another control 29 days after infection. In brown trout, the results of the two experiments were different.

In experiment 1, glochidia abundance of strain A decreased from 534 ± 153 (arithmetic mean ± 95% CL) to 87 ± 49 glochidia per fish within 49 days (Fig. 4A), with the largest decrease recorded in the first eight days. In strain D this decrease was significantly less severe ($P < 0.001$; 561 ± 138 to 361 ± 81 glochidia; Fig. 4A); the apparent decrease in numbers of surviving glochidia after eight days and the increase after 29 days are conspicuous, but non-significant and most likely a methodic artifact.

In experiment 2, glochidia abundance 49 days after infection was not significantly different between the two strains ($P > 0.05$; 430 ± 102 to 60 ± 34 and 266 ± 57 to 95 ± 37 glochidia, respectively). However, in this experiment the initial infection rates were different (Fig. 4B). When expressing glochidia decrease over time in terms of percentage of initial infection (Figs 4C, D), we observed a decrease down to 16% (experiment 1) and 14% (experiment 2) in brown trout strain A, and a decrease to 64% (experiment 1) and 36% (experiment 2) in strain D. With respect to initial infection, these differences between strain A and D were significant (*U*-test; $P < 0.001$ for experiment 1, $P < 0.05$ for experiment 2).

The proportion of infected individuals with respect to the total of fish used in the experiments (= prevalence) reflected our results of glochidia abundance in

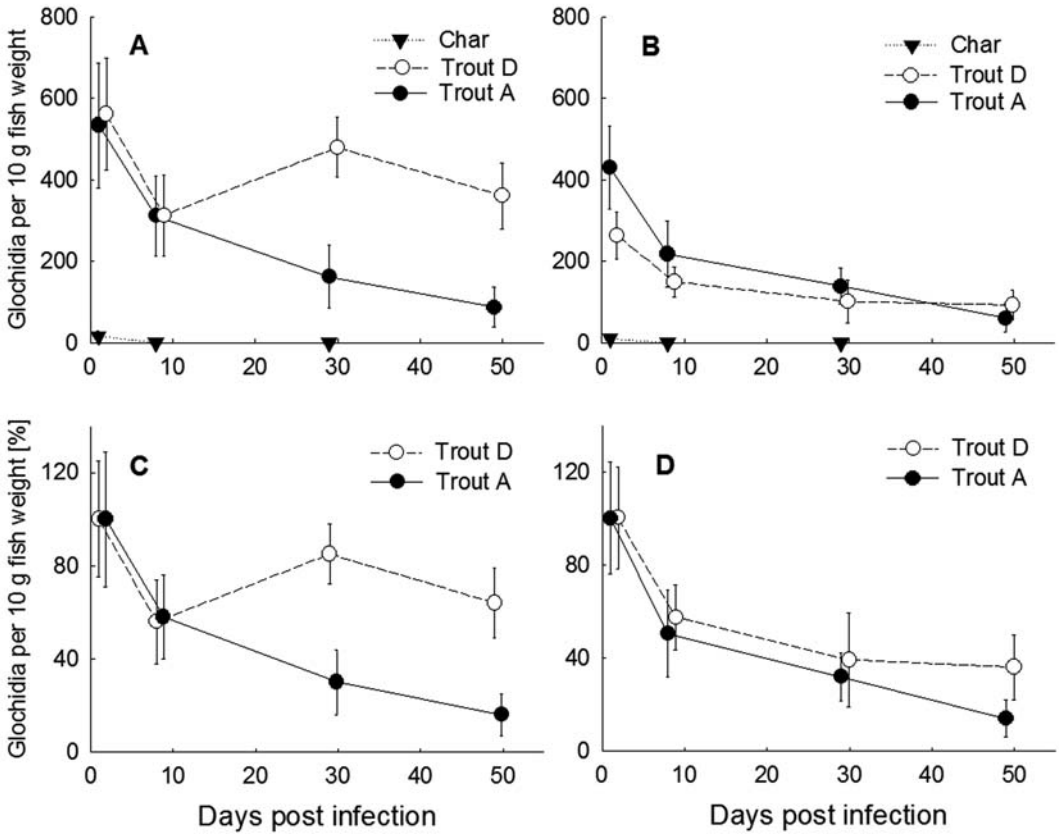

Fig. 4. Glochidia survival (n per 10 g of fish weight; arithmetic means ± 95% CL) given as function of time after infection. A, B: Survival rate of glochidia within the gill tissue of three fish species/strains in experiment 1 (A) and 2 (B); C, D: Survival rate of glochidia (n per 10 g of fish weight) within the gill tissue of the two trout strains given as percentages of the initial infection in experiment 1 (C) and 2 (D).

Table 2. Prevalence (percentage of infected individuals) as a function of time, given for the three fish species/strains in experiments 1 and 2.

Days post infection		1	8	29	49
Exp. 1	Brook char	68%	0%	0%	–
	Brown trout D	100%	100%	100%	100%
	Brown trout A	100%	100%	65%	55%
Exp. 2	Brook char	62%	0%	0%	–
	Brown trout D	100%	100%	60%	67%
	Brown trout A	100%	100%	80%	68%

the gills. Just 24 h after infection, 32% (experiment 1) and 38% (experiment 2) brook char were already completely devoid of glochidia, whilst in both strains of brown trout all individuals remained fully infected till eight days after infection. In experiments 1 and 2, the proportion of infected individuals of strain A decreased to 55% and 68%, in strain D it remained high in experiment 1 (100%) and decreased to 67% in experiment 2 (Table 2). Generally, correlations between condition factor and glochidia density proved to be not significant ($P < 0.05$) except for brown trout (strain D) in experiment 1.

Glochidia growth over the study period was generally continuous, but somewhat lower during the first days after infection (Fig. 5). In experiment 1, glochidia size increased from 75 ± 0 to 200 ± 13 µm (arithmetic mean ± 95% CL) in the gills of brown trout strain D and to 217 ± 3 µm in strain A during the 49 days of the study with differences being not significant ($P > 0.05$). In Experiment 2, glochidia size was significantly ($P < 0.05$) higher in strain D (175 ± 10 µm) than in strain A (158 ± 14 µm). Correlations between glochidia size (49 days after infection) and glochidia abundance were not significant in experiment 1; however, in experiment 2 glochidia were significantly larger in brown trout with higher glochidia densities (Spearman's rank correlation coefficient = 0.57; $P < 0.01$).

Discussion

Habitat selection

The study highlights and defines specific habitat preferences of the freshwater pearl mussel population in the Waldaist River. Stretches with macrolithal, flow velocities of 0.2–0. 6 m s^{-1} and water depths of 0.25–0.5 m were most densely colonized. In several comparable studies in Scotland, Austria and Germany similar preferences of *Margaritifera margaritifera* were reported (Foeckler 1990; Hastie et al. 2000, 2003; Moog et al. 1993). However, there are European populations that show totally contrary habitat preferences (Degerman et al. 2009; Baer 1995; Jung 2011). For instance, fresh-

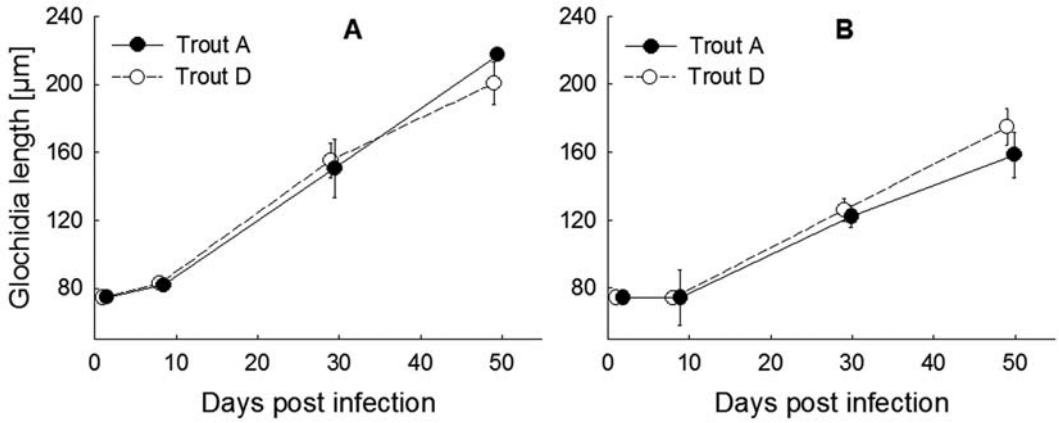

Fig. 5. Glochidia growth within the gill tissue of the two trout strains A and D given as glochidia length (mm; arithmetric means ± 95% CL) as a function of time (A: experiment 1, B: experiment 2).

water pearl mussels in a millrace of the Gießenbach brook in Upper Austria are exclusively found in sandy substrates which completely lack coarser sediment particles (Jung 2011). According to Strayer (2008), preferred sediment grain sizes of mussels may vary widely in different breeding waters because the crucial factor is substrate stability during flood events. In natural rivers the substrate stability is highest in sections where large boulders prevent finer sediments from being washed away, hence the mussels in the Waldaist River were detected preferably in those, though rare, habitats. In the Gießenbach millrace, such stabilizing elements of coarse particles are not necessary for the mussels, because flood events are restricted to the natural streambed, whereas discharge of the millrace is regulated at a uniform level.

Population structure

A striking observation was the fact that juvenile mussels (total length < 45 mm) were missing in the Waldaist population. Juveniles are often underrepresented in population studies, because they are regularly buried in the streambed (Hastie et al. 2000); in the present study, however, juveniles were not only lacking near the substrate surface, but also in sieved sediments down to a depth of 40 cm. The absence of juvenile freshwater pearl mussels has been described from a wide range of sites (Bauer 1988; Moog et al. 1993; Scheder & Gumpinger 2008; Geist 2010) and is typical of populations in watercourses with significant anthropogenic influence, since adult mussels tolerate deteriorating environmental conditions more easily than the highly sensitive juveniles (Strecker et al. 1990; Moog et al. 1993; Baer 1995).

When comparing the population structure of the mussel population analyzed by Ofenböck back in 1998 (unpublished report) with the results of the present study, we noticed a high concordance of the data set. The outer growth rings of the measured juvenile shells turned out to be considerably narrower than the corresponding rings of adult shells. This suggests that the growth rate of juveniles has slowed down. Our results corroborate the findings of Ofenböck (1998, unpublished report), who had already detected reduced growth rates in the Waldaist when compared with data obtained in previous decades. A series of environmental factors can influence mussel growth, among them food quality and quantity, temperature, oxygen content, salinity, NH_3, pH (Wootton 1998), Ca^{2+} (Strayer 2008), organic load or biochemical oxygen demand (Bauer 1992). Moog et al. (1993) indicated critical limits for most of the aforesaid parameters for Austrian mussel waters; in the Waldaist, all values are within those limits. As other naiad species or *Dreissena polymorpha* (Pallas, 1771) are missing in the Waldaist, food competition can be excluded. The temperature regime has remained constant for the past thirty years (Jung 2011). An alternative explanation for the reduced growth rates is a deterioration of the food quality. According to Hruska (1995, 1998), the reduced connectivity of watercourses and their adjacent landscapes, the elimination of autochthonous riverside woodlands in favour of spruce monocultures, and the drainage of marsh areas directly affect the quality of the washed-in detritus and its Ca^{2+} content, resulting in the malnutrition of mussels. This theory has been questioned (Sachteleben et al. 2004); however, Schreckenbach (1995) proved that among five freshwater pearl mussel populations the one with the highest mortality rates was distinctly undernourished, and Wahlström (2006) demonstrated that the organic content of sediments < 0.063 mm was higher in shellfish waters with successful reproduction than in overaged populations. Ofenböck et al. (2001) assume that the nutritional quality in the Waldaist has decreased due to the massive afforestation with spruce trees, which would support Hruska's (1995) hypothesis. The results of the present study also take the same line, as reduced growth rates are very likely to be associated with deteriorated food conditions – provided that all other relevant parameters have remained unchanged, as is the case with the Waldaist River.

The age distribution of the collected empty shells also refers to degraded living conditions. According to Bauer (2001), in unimpaired populations mortality rates are relatively low as soon as the interstitial

stage has been completed; hence most empty shells are the remains from old specimens at the end of their life span. In the Waldaist, however, there is no difference in the age-class distribution of living mussels and empty shells, demonstrating a significantly increased mortality rate in young individuals. Anthropogenic activities like impoundment flushings in the upper reaches or the immission of unidentified toxic substances (Scheder & Gumpinger 2008) have at least contributed to the current situation.

Host fish specificity, infection rates and growth of glochidia

Infection rates were significantly higher in brown trout than in brook char; this applies both for the initial infection and subsequent encystment. In brook char, all glochidia were repelled after eight days post infection. Bauer (1987) also detected high mortality rates in glochidia that used brook char as host fish, but was able to find living larvae as well; therefore, he regarded this species as clearly suboptimal, but nevertheless not completely resistant. The fish used in the present study, however, turned out to be unsuitable hosts; the reason might be an especially narrow host spectrum on the part of the observed mussel population, or an increased resistance of the chosen brook char strain to glochidia.

One full day after infection, survival rates of glochidia were higher in brown trout strain D than in strain A. Furthermore, prevalence in experiment 1 as well as growth rates in experiment 2 were distinctly higher in strain D. The apparent decrease in numbers of surviving glochidia after eight days and the following increase after 29 days are conspicuous. The phenomenon is very likely to be a methodic artifact; the variance of the infection rates was very high eight days after infection, and the difference between the two sampling dates is non-significant: 95% confidence intervals overlap. A possible methodic error source might be the fact that glochidia were still so small during the first two samplings, that especially in frozen fish gills the actual number of encysted larvae might have been underestimated – this phenomenon did not occur during later samplings anymore.

On the whole, the results suggest that strain D has to be regarded as more suitable than strain A. As both samples represent domesticated Atlantic strains, this significant difference is remarkable. One possible explanation could be linked to the immune response of the different host strains. One can assume since strain D fish were more heavily infected they might show a weaker constitution than strain A fish. Indeed, in strain D the Fulton condition factor was significantly lower (Scheffe post hoc, $P = **$), resulting in 0.97 ± 0.13, compared to strain A with a condition factor of 1.10 ± 0.14. Those results support the above mentioned hypothesis. Another approach is the occurrence or absence of *Margaritifera margaritifera* in the river system the host fish originated from. Assuming that only the strain A fish originated from a system where the freshwater pearl mussel occurs, the immunological system of strain A fish is likely to cope more successfully with glochidia infection than strain D.

The only significant correlation with respect to glochidia growth was detected in experiment 2 where glochidia were significantly larger in host fish with higher infection rates. Bauer & Vogel (1987) assume that in suitable host fish the mortality of glochidia is regarded to be lower while growth rates are expected to be higher at the same time. On the other hand, in high-resistant fish glochidia are more likely to be rejected, and successfully encysted larvae grow significantly lower.

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