*Practical experience in the rearing of freshwater pearl mussels (*Margaritifera margaritifera*): advantages of a work-saving infection approach, survival, and growth of early life stages*

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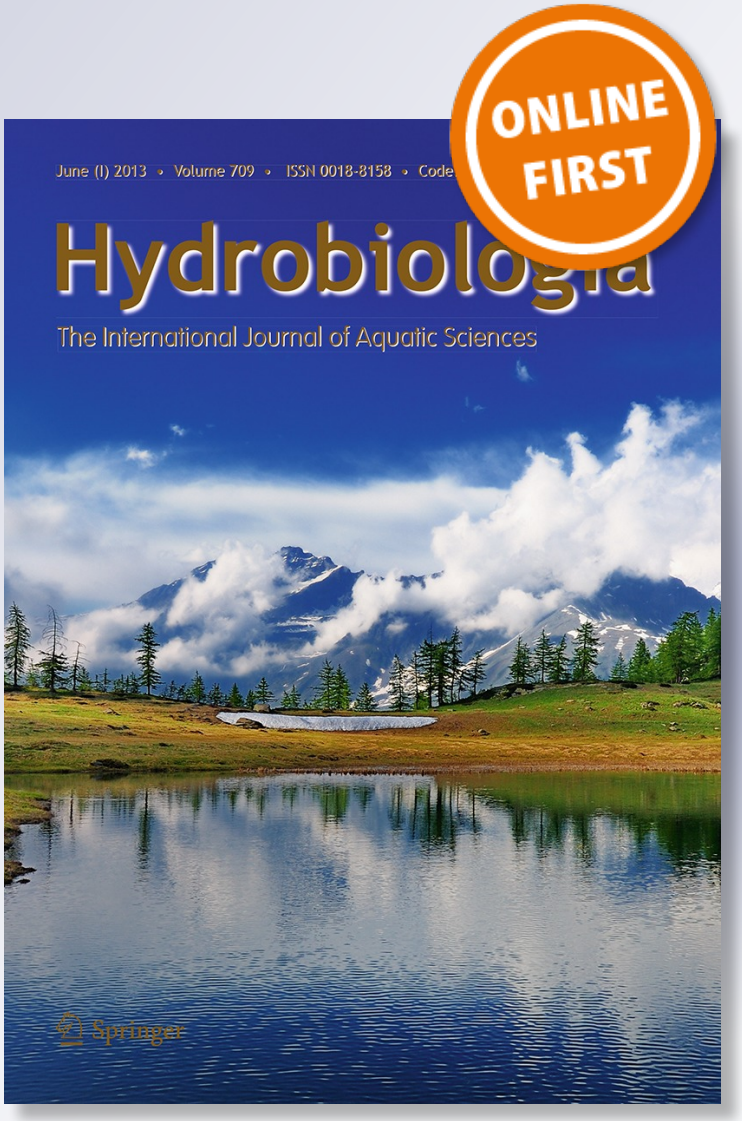

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FRESHWATER BIVALVES

Practical experience in the rearing of freshwater pearl mussels (*Margaritifera margaritifera*): advantages of a work-saving infection approach, survival, and growth of early life stages

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Abstract The critically endangered freshwater pearl mussel (*Margaritifera margaritifera* Linnaeus 1758) is the target species of an Austrian conservation project that involves captive breeding. In order to optimize the operational procedure, controls were conducted at several decisive stages, including infection of host fish (for which a time- and work-saving enclosure approach was tested), larval growth during the parasitic stage, growth of juvenile mussels in climate chambers at different temperatures, and growth and survival of re-introduced juveniles in field cages. High infection rates could be attained under near natural conditions. Distinctive patterns in the way the gill arches of the host fish were infected could be detected. Encysted glochidia showed significantly different successive growth stages, related to water temperature. In all, five distinctive growth stages could be detected in the course of the first 562 days of observation. The stages are described and the respective daily increments given. Very high survival rates were achieved during hibernation in the field as well as at the laboratory. The study suggests a way for saving time in the infection procedure that can more effectively be invested in an intensive maintenance of juveniles at the laboratory and during hibernation in the field.

Keywords Freshwater pearl mussel · *Margaritifera margaritifera* · Captive breeding · Larval growth · Survival rates · Water temperature

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Introduction

The freshwater pearl mussel (*Margaritifera margaritifera* Linnaeus 1758) is considered one of the most endangered mollusc species in Europe (Young et al., 2001; Hastie et al., 2003) and is listed both on Appendix III of the Bern Convention (Council of Europe, 1979) and on Annexes II and V of the EU Habitats Directive (Council of the European Union, 2006). Populations have declined throughout its range for at least 100 years due to a variety of reasons, most important of which are industrial and agricultural pollution as well as habitat degradation caused by hydropower exploitation and river management (Hastie et al., 2003). In the last quarter of the past century various central-European authors reported a dramatic reduction to just 2–3% of previous estimates (Baer, 1970; Bauer, 1979; Jungbluth, 1988; Moog et al. 1993; Gumpinger et al. 2002). Since then, the decline has progressed unabated, resulting in an alarmingly small number of remaining populations. Many conservation

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projects, with various approaches, have since been conducted all over Europe (Geist 2010, Gum et al. 2011). In Austria, all of the remaining populations are over-aged and lack juveniles (Scheder & Gumpinger, 2008), which will inevitably lead to extinction unless comprehensive protection measures are taken immediately. The Department for Nature Conservation of the Government of Upper Austria has launched a long-term species conservation project, dealing both with captive breeding and habitat restoration. In the course of a preliminary design study the relevant parameters of glochidial growth and survival on host fish gills as well as survival and growth of juvenile mussels in climate chambers and field cages were examined.

As proposed by Gum et al. (2011) in a recent review paper, the publication of results concerning the rearing and culturing of freshwater pearl mussels is necessary to improve the efficiency of the applied methods and to understand conservation measures on wild populations. This study tries to meet these requirements by contributing detailed data to the existing body of experience. It also emphasizes the advantages of a near-natural, time- and work-saving infection approach by means of an enclosure in a mussel-inhabited millrace.

Materials and methods

The field study was carried out in a millrace of the Gießenbach brook, Upper Austria, Austria, where a formerly unknown freshwater pearl mussel population had been discovered only shortly before the present study was run (Scheder & Gumpinger, 2007). The millrace consists of two distinctly different, directly adjoining stretches: a 400 m long semi-natural section colonized by freshwater pearl mussels and a 20-m long (and 2 m wide) concreted box section via which the water is discharged through the former mill yard before it is fed back into its main watercourse. The box section is disconnected from the semi-natural upstream section by an impassable 3.5 m high dam—the former mill weir—and can be detached from the downstream reach by means of transversally inserted metal bars. It also represents an enclosed flow-through system in which fish can be kept under natural flow conditions.

In August 2010, 255 juvenile brown trout (*Salmo trutta* Linnaeus 1758), previously uninfected yearlings, 14–16 cm in length, obtained from a local fish farmer, were released into the enclosed millrace stretch. Infection with freshwater pearl mussel glochidia took place naturally without any further interference when the mature mussels in the upstream reach released glochidia which were transported downstream by the water flow. The infected host fish were kept in the enclosed stretch all winter long.

From October 2010 to April 2011 infection controls were performed once a month (62, 96, 136, 165, 207, and 244 days after the introduction of the host fish into the enclosure). Five fish at a time were dispatched, measured and weighed, and their gills were dissected. Glochidia were counted separately on each gill arch, the total number was documented and 20 glochidia per fish were measured to an accuracy of 1 µm.

In May 2011 (261 days after the introduction of the host fish into the enclosure) 25 of the remaining infected fish were transferred to a rearing facility. The facility consisted of a large 2,000 l fish tank with a conical bottom and a hole in its centre that was connected to a 250-l water butt by means of a plastic hose. The water within the system was continuously pumped in a circuit between the two vessels, passing through a 100 µm mesh when flowing from the fish tank into the water butt. After undergoing metamorphosis, the juvenile mussels dropped from their hosts, sank to the centre of the conical bottom and were drawn through the hole and hose into the mesh. The mesh was rinsed out once a day and the contents were checked for juvenile mussels as described by Thomas et al. (2010).

The mussels were then transported to the laboratory where they were put into 0.5 l plastic boxes, filled with water and detritus collected from the Gießenbach brook. A separate plastic box was used for each collecting date. The mussels were fed an algal and rotifer suspension according to Eybe & Thielen (2010), consisting of 120 µl Shellfish Diet 1800™ (mixed diet of *Isochrysis* sp., *Pavlova* sp., *Thalassiosira weissflogi*, *Tetraselmis* sp.; cell size 5–20 µm, 2 billion cells per ml) and four drops of Nanno 3600™ (*Nannochloropsis*), both suspended in 10 l stream water. Food concentrations were doubled after six weeks (according to Eybe & Thielen, 2010) and tripled after ten months (as recommended by Eybe & Thielen (2010) for the rearing of mussels larger than 1 mm). The boxes were stored in climate chambers at a constant temperature of 18°C/64.4°F from June to

September and of 6°C/42.8°F from October to April. Water and detritus changes were carried out once a week during periods of high temperature, and once in a fortnight when the temperature was low. The contents of each box were drained through a 180 µm mesh and rinsed thoroughly, until the detritus was washed away completely and only the mussels remained in the sieve; the boxes were cleaned and filled with fresh water and detritus. In the course of the water changes the mussels were checked for possible fungal infection or other afflictions. Dead or infected specimens were removed; each live mussel was measured by means of a dissecting microscope (Leica S8APO) and a measuring eyepiece to an accuracy of 12.5 µm.

In October 2011, 213 mussels (all but 50 juveniles that remained in the climate chamber in two groups of 25 specimens each) were transferred back to the Gießenbach millrace. Five field cages (according to Buddensiek, 1995; slightly modified) were made for this purpose, consisting of acrylic glass panes into each of which 48 holes were drilled. Into each of the holes one juvenile mussel was placed after a preceding measuring procedure. Juvenile mussels were only put into the cages when they had reached at least 1 mm in length (according to Lange & Selheim, 2011). The perforated panes were then sealed on the outsides with a 360 µm mesh and fixed in the brook by means of iron rods. During the winter months the cages were attended to once a week in order to avoid debris accumulation; biofilm was removed from the mesh by means of a toothbrush. In order to minimize adverse effects on the juvenile mussels, the cages were not opened until May 2012; then, each mussel was measured again individually. The specimens that remained in the climate chamber for hibernation were attended to and measured once in a fortnight as described above.

All statistical analyses were performed by means of the programme SPSS 8.0.0. Data were tested for normal distribution using the Kolmogorov–Smirnov test; in normally distributed data, levels of significance were calculated using the *t* test according to Student; otherwise the Friedman test was applied as non-parametric test.

Results

The natural infection in the millrace was successful, as 85.7% or 24 of the 28 fish dispatched for the hibernal infection control had been infected. Infection intensity varied markedly between specimens; four fish were not infected at all, whereas the maximum infection amounted to 1,524 glochidia per fish. On average, 430 larvae were counted on each trout. No correlation between body length or body weight and the total number of glochidia per fish was found.

Separate gill arches were infected with significantly different intensities (Friedman test; $P \leq 0.001$), with the fourth arches on either side significantly showing the lowest numbers of encysted glochidia ($P \leq 0.001$ when compared to 2nd and 3rd arches; $P \leq 0.01$ when compared to 1st arches). The two median gill arches statistically showed the heaviest infections, both on the right- and on the left-hand side (Fig. 1).

Glochidial growth during encapsulation in the host fish gills was observed over a period of 182 days. During this period, the mean total length of the measured glochidia increased by 82 µm from 216 to 298 µm (Fig. 2). Different growth stages were observed: In late autumn and winter glochidial growth was comparably low with a mean total increment of only 32 µm (from 216 to 248 µm) within 103 days, equalling a mean daily increment of 0.31 µm. From February to late April, growth increased perceptibly, with the mean body length increasing by 50 µm from 248 to 298 µm in only 79 days, giving a mean daily increment of 0.63 µm. This significant increase ($P \leq 0.001$) correlated with rising water temperatures and occurred as soon as maximum daily water temperatures exceeded 8°C/46.4°F.

Infected fish was transferred to the rearing facility in late April 2011, when water temperatures in the

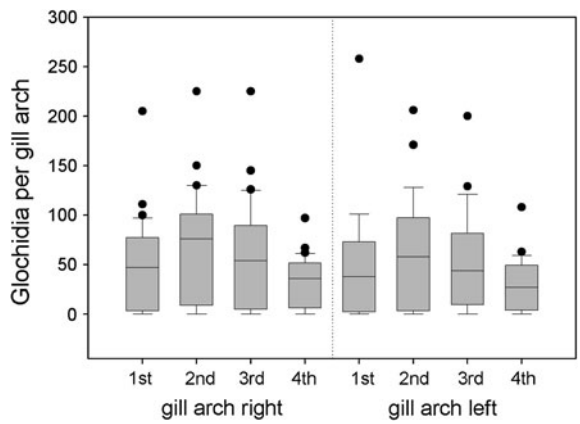

Fig. 1 Glochidial distribution on fish gill arches

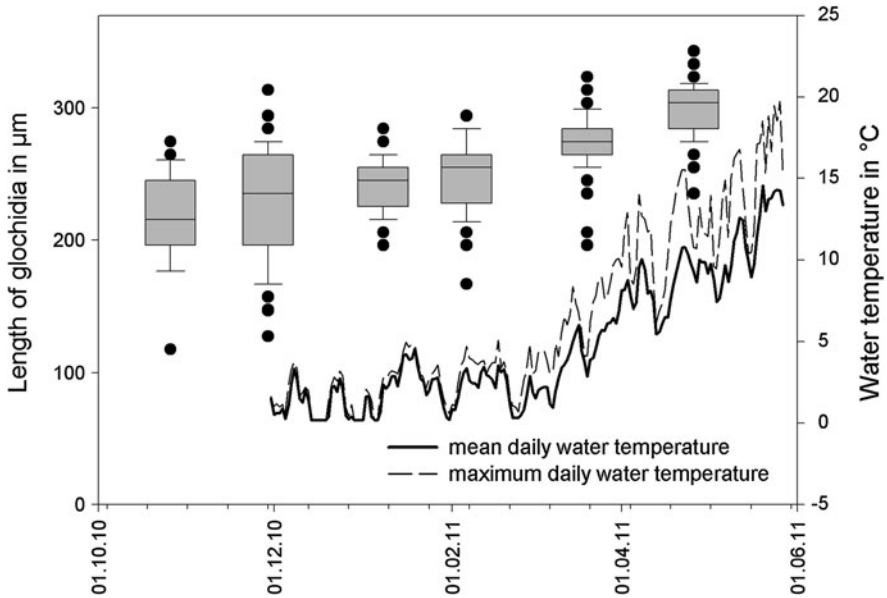

Fig. 2 Glochidia growth on host fish gills in relation to water temperature

millrace had risen above 10°C/50°F. The first juveniles excysted from their hosts when the temperature in the facility first reached 18°C/64.4°F, and when temperatures had exceeded 15°C/59°F for 18 consecutive days, respectively (Fig. 3).

No measurements of encysted larvae were carried out while the host fish were being kept in the rearing facility. Measurements of juvenile mussels were thus not accomplished until the collecting phase had been completed. During this period of 69 days, the mean body size increased by 311 µm—from 298 µm in late encysted larval stages to 609 µm in early juvenile mussels—resulting in a mean daily increment of 4.5 µm.

Mussels were then transferred to a climate chamber and kept at a constant water temperature of 18°C/64.4°F from June to October 2011. During that 91 day warm water period the mean body length increased by

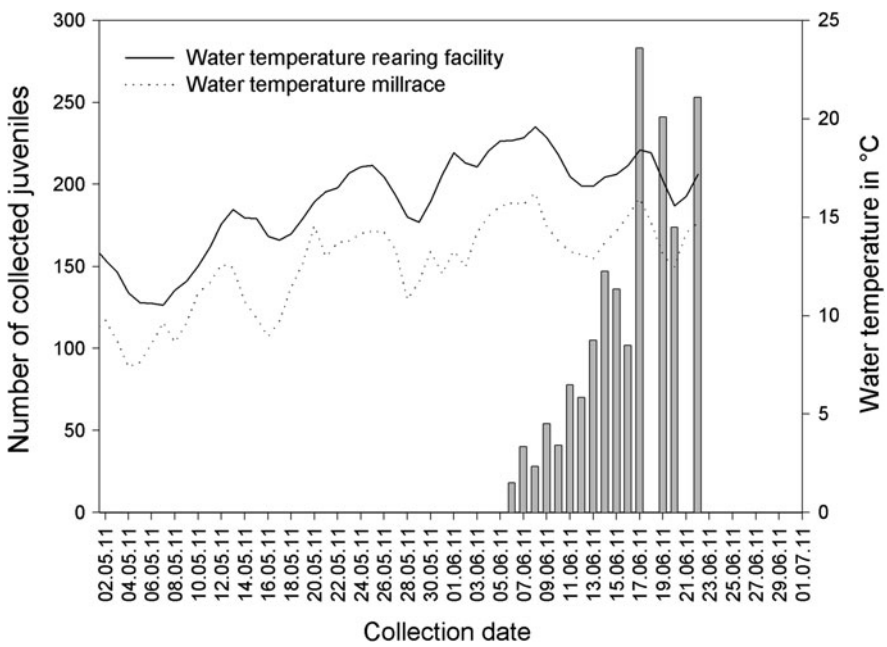

Fig. 3 Juvenile excystment in relation to water temperature

almost two-and-a-half times from 609 µm to 1,456 µm, making this time span the most productive with a mean daily increment of 9.3 µm. After the warm water period, the larger part of the mussels (213 specimens) was reintroduced to the Gießenbach millrace, whereas 50 specimens remained in the climate chamber where the temperature was lowered to 6°C/42.8°F for hibernation. Growth in these specimens ceased immediately: the hibernation period lasted for 151 days, during which only a negligible mean length increment of 72 µm (from 1,456 to 1,528 µm)—or a mean daily increment of 0.48 µm—could be detected.

When temperatures were raised again after 151 days in spring 2012, growth slowly started increasing as well. In those final 69 days at the laboratory the mean body length reached 1,646 µm, the mean daily increment during this period was 1.7 µm. Finally, the mussels that hibernated in the climate chamber were reintroduced into the Gießenbach millrace like their conspecifics.

All in all, five distinctly different growth stages were determined in the freshwater pearl mussel population that was reared at the laboratory (Fig. 4a, b). While the larvae were still attached to the host fish, they showed a mean daily increment of 0.45 µm that rose markedly to a tenfold during metamorphosis and more than doubled again when the juveniles were kept at warm water conditions. Hardly any growth took place during hibernation, until the water temperature was increased again in early spring.

The mussels that hibernated in field cages were measured before they were reintroduced into the millrace and again after 206 days of hibernation. The mean body length had risen by just 35 µm from 1,578 to 1,613 µm, equalling a mean daily increment of 0.17 µm. As the chambers in the field cages held one single specimen each, length measurement at individual level was possible. More than a third of the hibernating mussels had not grown at all, 57.8% of the individuals had grown by 25 to 100 µm, but also increments of up to 300 µm were registered sporadically (Fig. 5).

Survival rates were not significantly different in climate chambers and in field cages, respectively (t test; $P = 0.262$). In the mussels that were kept in climate chambers and attended to regularly, the median of survival rates was 96.3%; in the field cages that were largely left to their own resources, it was not significantly lower with 87.6% (Fig. 6). In both experiments there were samples with a survival rate of 100%.

Discussion

Glochidia harvesting is usually a rather time-consuming task. Gum et al. (2011) describe two different strategies: in one approach, mussels must be inspected regularly in order to determine the best time for the infection of the host fish; in the other, artificial flow-through systems into which adult mussels and host fish are transferred must be constructed and maintained. In the approach presented in this study, time and effort can be reduced to a minimum. There is no need to handle adult mussels at all, as they are let alone in their self-chosen habitats and undergo their reproduction cycle without any human interference. The special situation of the enclosed box section minimizes the maintenance effort: As a mill weir is used to keep the fish from escaping instead of an upstream grate, there is no risk of a log jam or debris congestion, as there would be in a conventional enclosure. Maintenance is limited to cleaning the downstream grate on occasion.

The infection rates that were achieved in this study were comparatively high, especially when taking into account that no human intervention had taken place; they averaged 430 glochidia per fish, with a maximum of 1,524. Below, those numbers are compared to infection rates observed previously both in the wild and in hatcheries. Such a comparison involves the risk of disregarding certain factors that might influence the infection rates, such as host fish densities, age, and possible previous exposure of host fish towards glochidia, or the suitability of different fish strains as hosts. As far as possible, those factors are taken account of in the following discussion.

Natural infection rates are highly variable in different watercourses. In the River Waldaist—the river with the largest remaining freshwater pearl mussel population in Austria—Haunschmid & Kozak (1998) investigated juvenile host fish in stretches with different mussel densities. Only young-of-the-year fish were examined, as they are known to be most important in pearl mussel reproduction, considering that older fish usually show a lower susceptibility toward glochidiosis, most likely due to an acquired immunity response resulting from previous exposures

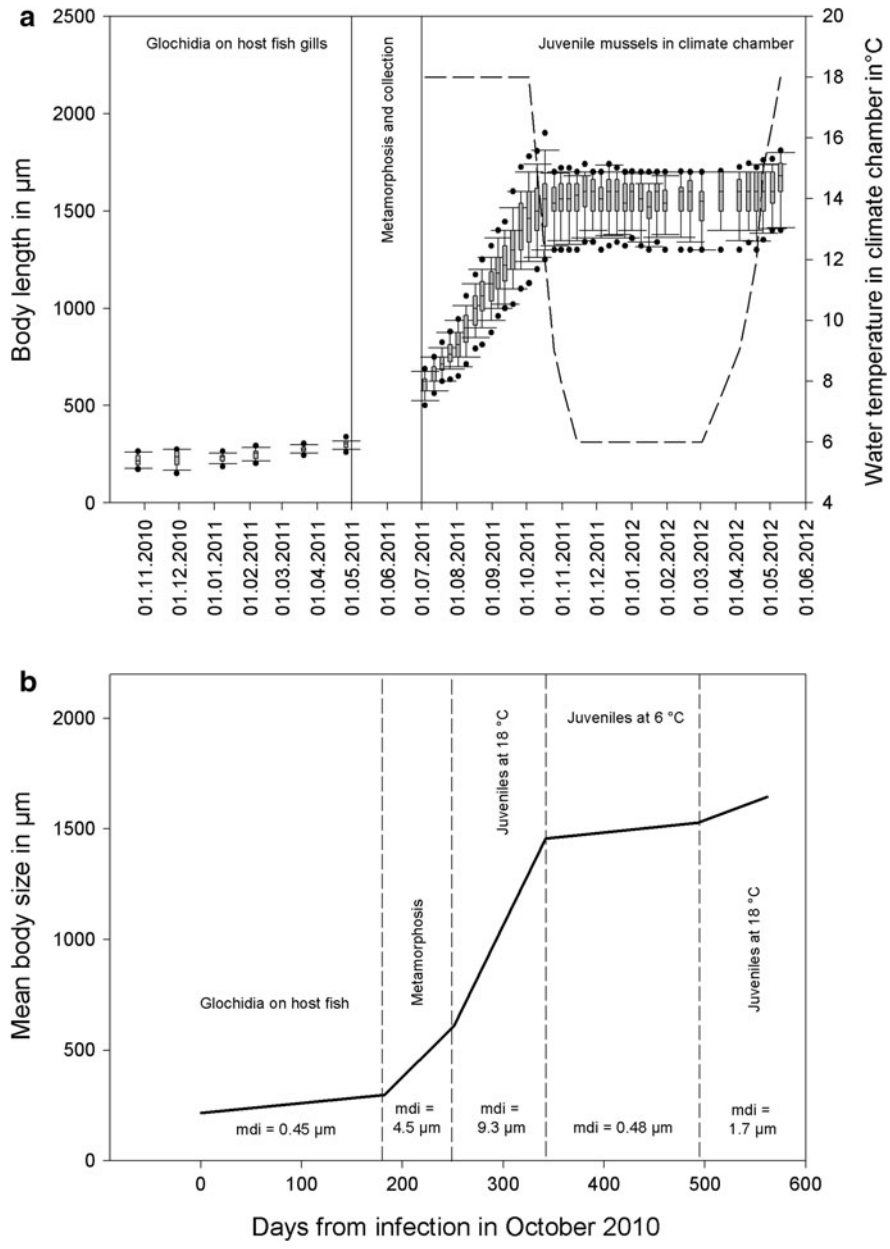

Fig. 4 Growth stages of glochidia and juvenile mussels of *Margaritifera margaritifera*

(Bauer, 1979). As only fish without any previous infection were examined both in this study and in the study performed by Haunschmid & Kozak (1998), the results can be considered comparable on that score. Even in the most densely colonized stretch, infection rates in the River Waldaist only came up to an average of 125 glochidia per fish, (which is only 29% of the figures in the Gießenbach millrace), the most heavily infected fish bore 351 glochidia (compared to 1,524 in the Gießenbach millrace). Those noticeable differences cannot easily be interpreted, as several conditions varied between the two studies: First, in the River Waldaist fish were free to move unrestrictedly, which means they were also free to resort to the mussel bed when glochidia were released in order to actively feed on them, whereas they were kept in a 20 m long enclosure in the Gießenbach millrace without any chance to approach the adult mussels. Second, fish densities accounted for 100 fish per 100 m in the River Waldaist, whereas 255 trout were kept in a 20 m

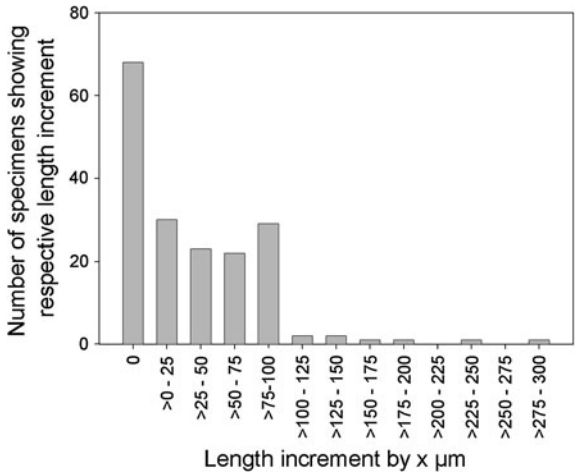

Fig. 5 Length increment in mussels hibernating in field cages ($n = 180$)

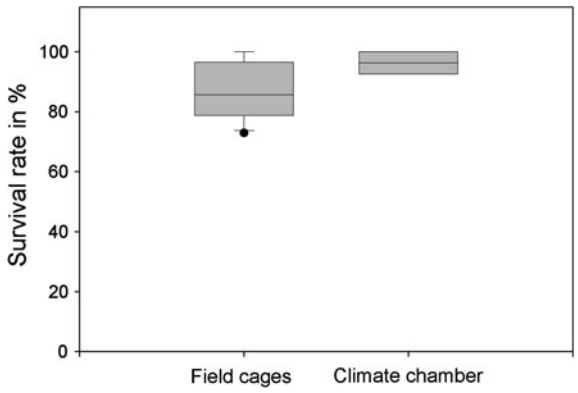

Fig. 6 Survival rates of juvenile mussels during hibernation (median survival rates in field cages: 87.6%; in climate chambers: 96.3%)

stretch of the Gießenbach millrace. Third, mussel densities in the River Waldaist amounted to several thousand specimens, whereas they only reached 195 in the Gießenbach millrace. In addition, one special aspect must necessarily be discussed at this point: the suitability of different strains as host fish. Täubert et al. (2010) proved that from three different strains of brown trout the one originating from the natural pearl mussel distribution range was the most suitable host, concerning both infection rates and glochidial growth rates. In the River Waldaist, the studied fish sprang from natural recruitment, whereas they were obtained from a local fish farmer in the Gießenbach millrace. Accordingly, most of the differences mentioned would rather suggest lower infection rates in the Gießenbach millrace, which is in clear contrast to the actual results. It might be assumed that the enclosure has determined the higher infection rates, as all the released glochidia were directed through a confined space which the fish were not able to leave, and might therefore have been exposed to larger a quantity of glochidia than they would have in a natural river.

Young & Williams (1984a) state an average of 452.6 larvae per wild host fish in the Stac Burn in Western Scotland, and a maximum of 1,602, so their numbers closely correspond with the infection rates attained during the present study. It could be assumed that infection rates in the wild might be likely to correlate with the density of parent stocks. However, the total number of freshwater pearl mussels in the Gießenbach millrace accounts for only 195 specimens (Scheder & Gumpinger, 2007), which is equivalent to 0.375 mussels per m^2. In the relevant stretch of the River Waldaist, Ofenböck (1998) quantified the mussel density at more than 100 specimens per m^2. In the Scottish Stac Burn the densities accounted for 28.8 mussels per m^2 on average and for 124 maximum (Young & Williams, 1984a). Those figures do not suggest a correlation between mussel densities and infection rates—indeed, the host fish in the Gießenbach millrace (with low numbers of mussels) were as heavily infected as the ones in the densely populated Stac Burn and showed considerably higher infection rates than the ones in the densely populated River Waldaist.

The observed infection rates in this study are comparable with rates attained in experiments dealing with artificial infection; Wellmann (1943) stated an average of 500 glochidia per host fish that were infected artificially in a glochidia suspension. Likewise, Jung (2011) carried out an infection experiment in which host fish were infected in differently concentrated glochidia suspensions to which the fish were exposed for 45 min. Infection rates in suspensions with maximum glochidia concentrations (containing larvae from ten gravid mussels in ten litres of river water) were up to 560 ± 138 glochidia per fish; maximum infection equalled 1,217 larvae per trout. The infection rates achieved in this study showed numbers comparable to artificial infection experiments, although fish densities and glochidia densities were lower; in artificial infection, fish and larval material are crowded together in a confined space in

order to augment the probability of infection to a maximum. In this study, mussels and fish were kept in a near natural environment and at comparably low densities. The experiment therefore shows that high infection rates do not necessarily require an artificial infection, but can also be achieved in near natural situations—on the condition that the fish cannot flee from glochidia exposure.

This study shows that different gill arches were infected at significantly different intensities. The second and third gill arches were more heavily infected than the first and fourth, with the smallest numbers being observed on the fourth gill arch. Blažek & Gelnar (2006) found the same pattern for *Unio* and *Anodonta* species, which are both closely related to *Margaritifera margaritifera*. Similar results were attained by Jung (2011) with the heaviest infections found on the second gill arches on both sides, whereas the fourth arches were infected least heavily by *Margaritifera margaritifera* larvae; (aberrant from the pattern described above, the first arches in that experiment were infected significantly more heavily than the third arches). However, Young & Williams (1984b) found higher numbers of freshwater pearl mussel glochidia on the first and fourth gill arches than on the second and third, which completely opposes the pattern mentioned above. As the above studies each give coherent results, the interpretation of the distinct differences is intricate. It might be assumed that—considering the studies were carried out in different watercourses at different times—infection patterns might differ between catchment areas and years, but remain constant within them.

The frequently found pattern of the second gill arches being most heavily infected might be explained by the fact that larvae of the freshwater pearl mussel show a strong susceptibility to salt concentrations in the surrounding water; as in all unionoid mussels, they snap their valves shut as soon as they sense high salt concentrations (Ziuganov et al., 1994; Kotpal, 2010). This predisposition enables them to find an appropriate site to attach themselves to, as fish accomplish their salt metabolism via their gills and salt concentrations are therefore highest in the direct vicinity of the gill filaments (Penzlin, 1996). It seems reasonable to assume that, in flowing water passing through the host's gill cavity, the salt concentration does not increase perceptibly until the first gill arch is being passed. When the mussel larvae start reacting to that increase, they might have already passed the first with the water flow and therefore attach to the second gill arch.

The observed pattern of glochidial growth during hibernation in the host fish gills confirms the findings of Young & Williams (1984b) who report only a slight growth in autumn, cessation of growth in winter and a rapid growth in spring and early summer, and of Schmidt & Vandré (2010) who did not detect any growth in encysted larvae from early November to late March when mean water temperatures were around 2°C. Water temperature is very likely to account for that general growth pattern; in the Gießenbach millrace, a clear increase in growth in springtime could be detected as soon as water temperatures reached 8°C/46.4°F. Schmidt & Vandré (2010) state a resumed growth after hibernation when water temperatures reached an average of 5°C. Ziuganov et al. (1994) point out the temperature response of glochidia with an example in which experimentally infested fish that were kept at 14°C/57.2°F carried glochidia with lengths of 400 µm, whereas larvae on fish kept at 0°C/32°F only reached 240 µm at the same time.

Water temperature is an important factor in the timing of metamorphosing juvenile excystment. Hruška (1992) states the necessity of a continuous period of 14–16 days with average water temperatures of 15°C/59°F and a sum of between 1,300–1,860 day degrees for juvenile metamorphosis. This study confirms this premise as, in fact, juvenile mussels started dropping from the infected trout exactly 18 days after the first time that the average daily temperature had risen above 15°C/59°F and stayed at that level.

The survival rates attained during hibernation in the field cages turned out to be comparably high in this study. Survival rates in the field cages ranged from 73 to 100%, the median amounting to 87.6%. The cages were kept in a millrace where the discharge was more or less constant over time, hence no flood events accounted for fine sediment peaks. The mesh was cleaned at least three times a month (or more often during fall of leaves or rain periods) in order to maintain a constant water flow through the cells; debris accumulations were removed at shorter intervals. The high effort of attending and feeding the juveniles at the laboratory unto survivability and of regular maintenance in the field is very likely to have contributed to the unusually high survival rates. This implies Buddensiek cages can be an appropriate

means of keeping juvenile mussels alive during their hibernation period, but constant attendance and care are essential if reasonable survival rates are intended, as it is stated by Gum et al. (2011).

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