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Variable development and excystment of freshwater pearl mussel (*Margaritifera margaritifera* L.) at constant temperature

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ABSTRACT

The highly endangered freshwater pearl mussel (*Margaritifera margaritifera* L.) has strongly declined throughout Europe and is a priority species in aquatic conservation. The complex life cycle of *M. margaritifera* includes an obligate development phase of glochidia larvae on a suitable host fish. Knowledge on the progression of the parasitic phase and on the factors governing excystment of juvenile mussels are particularly crucial for artificial breeding and conservation measures. The core objective of this study was to study excystment of *M. margaritifera* after maintaining the infested hosts under constant water temperatures between 11 and 12 °C and to determine the sum of daily water temperatures (day degrees) required by *M. margaritifera* for completion of metamorphosis. In a standardized laboratory experiment, excystment of juvenile mussels from brown trout (*Salmo trutta*) was found between 1700 and 3400 day degrees post infestation, indicating highly variable development times of individual glochidia and the absence of a previously postulated threshold temperature of ≥15 °C for successful excystment of living juveniles. Consequently, the parasitic phase does not seem to limit the current distribution range and reintroduction of the species into cool headwater areas, as well as the culturing under constant water temperature conditions in typical salmonid fish hatchery setups. The concept of day degrees of development may also be useful to test the ecological implications of observed genetic differences among different populations.

Introduction

The freshwater pearl mussel (*Margaritifera margaritifera* L.) is highly endangered in Central Europe and has become a priority species in aquatic conservation (Bauer 1991; Young 1991; Geist 2010, 2011). The complex life cycle of *M. margaritifera* includes an obligate parasitic phase on suitable host fish species such as brown trout (*Salmo trutta* L.) or Atlantic salmon (*Salmo salar* L.) (Young and Williams 1984a; Hastie and Young 2001). In summer, the freshwater pearl mussel (FPM) larvae (glochidia) are released by gravid females and reach their fish hosts passively with the water current. After inhalation, the glochidia attach to the gills and become encysted by epithelial cells of the host (Young and Williams 1984b; Hastie and Young 2003). During encystment, glochidia grow approximately 6–10 fold in size and metamorphose into juvenile mussels (Ziuganov et al. 1994) before they excyst and bury into the river substratum. After about five years, the juvenile mussels appear at the substratum surface and live as filter feeders (e.g. Young and Williams 1984a; Bauer 1991, 1997; Hastie and Young 2000). Although several studies investigated the suitability of different host fish species as well as the post-parasitic phase (Young and Williams 1984a,b; Taeubert et al. 2010), there is only limited knowledge concerning the factors which govern excystment. Hruška (1992) found that *M. margaritifera* require a mean water temperature ≥15 °C for at least 14 days at the end of the parasitic phase for successful excystment which would restrict the geographic distribution of this endangered mussel species to streams with higher temperature conditions during summer. To our knowledge, this observation has never been empirically verified. Information of the influence of temperature on excystment is also crucial for the assessment of pearl mussel habitat quality and is essential for identifying suitable areas for reintroduction. Since artificial breeding efforts in *M. margaritifera* increased in recent years (Hastie and Young 2003; Preston et al. 2007; McIvor and Aldridge 2008; Thomas et al. 2010; Gum et al. 2011), detailed knowledge of the timing of excystment can be helpful for effective temperature management during mussel propagation and culturing (Hastie and Young 2003).

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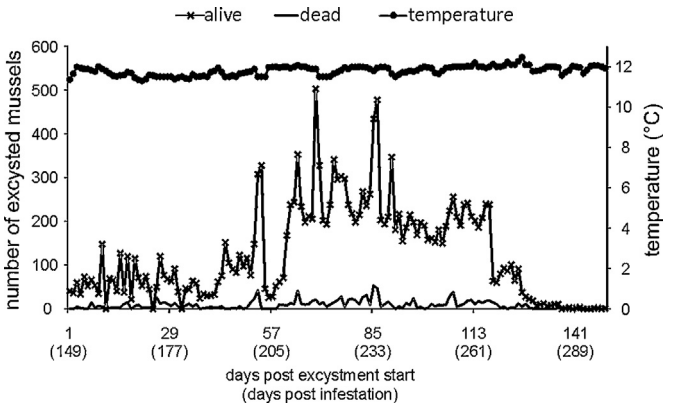

Fig. 1. Excystment chronology of juvenile *M. margaritifera*, subdivided into counts of living and dead specimens.

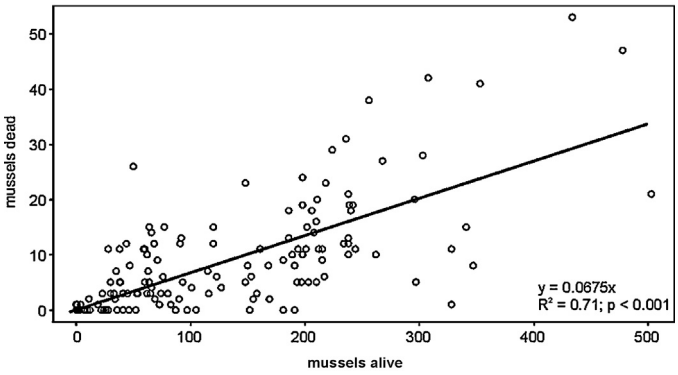

Fig. 2. Relationship between the excystment of living juvenile mussels and dead juveniles.

The core objective of this study was to determine the sum of daily water temperatures (day degrees) which *M. margaritifera* require for completion of metamorphosis under constant water temperatures between 11 and 12 °C which are typical for most groundwater-fed salmonid hatcheries. Obtained results were compared with available literature from different countries and can help deduce strategies for facilitating artificial breeding of this endangered mussel species.

Materials and methods

Glochidia collection from mussels of the Zinnbach population in Germany, and infection procedures were carried out on September 8th, 2010 following the protocol described in Taeubert et al. (2010). For standardization and in order to avoid any bias due to an acquired resistance to glochidia of unionid mussels in the host fish (Dodd et al. 2005), only hatchery reared fish with no previous contact to glochidia were used in this experiment. Mean infestation rate was ~1500 glochidia per fish. In the laboratory of the Aquatic Systems Biology Unit, 100 infested brown trout (*Salmo trutta*) were maintained in a 300 L flow-through circular tank with ground water supply (0.5 L s^{-1}). Initially, fish were fed 2% of body weight twice a week with commercial trout chow (1.8 mm Aqua Pro, Skretting). In order to reduce feces and to facilitate the recovery of excysted juvenile mussels, fish were only sparsely fed once a week after the start of excystment (3rd February 2011). To collect the excysted juvenile mussels, the tank outflow was filtered through a bucket with an incorporated 200 μm screen and the recovered material was checked for the presence of juvenile mussels under a binocular (SZX10, Olympus, Hamburg, Germany) using 6.3× magnification. Viability of juvenile mussels was assessed based on two criteria: Active contraction of the adductor muscle, and active movement (evident from movement of the foot). In contrast, dead juveniles could be easily identified by wide gaping of the valves and by the absence of any reaction. During the experimental period, water temperature was recorded every hour by temperature loggers (Lascar Electronics Limited, Salisbury, UK). No changes in mean water temperature before and during excystment were found. The mean temperature between infestation and the start of excystment (September–February) was 11.5 °C (SD = 0.2 °C). During the excystment period (February–July), a mean water temperature of 11.8 °C (SD = 0.2 °C) was recorded (Fig. 1).

Results

The excystment of living juvenile *M. margaritifera* started on February 3rd and continued up to July 1st. Over this period of 149 days, a total of 19,891 juvenile mussels were collected, including 18,595 living juvenile mussels and 1296 dead mussels (7% of all collected mussels). A positive linear relationship between the excystment rates of living juvenile mussels and dead juveniles was found (Fig. 2). On average, ~185 viable mussels $fish^{-1}$ were recovered, following initial infestation rates of 1500 glochidia $fish^{-1}$. This indicates a ~88% loss of glochidia during metamorphosis. It was not possible to observe active foot movement for every single mussel with closed valves due to high number of excysted juveniles. However, for every day of the excystment period, active foot movement of several juvenile mussels was recorded. The host fish mortality between the infestation and the start of excystment was 5%, while 42% of the host fish died until the end of excystment phase.

The sum of daily water temperature (day degrees) from infestation to completion of metamorphosis ranged between ~1700 day degrees and ~3440 day degrees (dd). However, at the beginning and the end of the excystment period only few mussels were found, while more than 80% of all juvenile mussels excysted over a 73-day period between 2220 and 3080 dd. The peak daily excystment rate with 503 mussels (2.6% of total excysted mussels) was found 2530 dd (217 days) post infestation (Fig. 1).

Discussion

In order to prevent extinction of priority *M. margaritifera* populations, several European countries have initiated conservation measures and breeding programs. These programs often use artificial infestation approaches and semi-natural cultivation of juvenile freshwater pearl mussels (Gum et al. 2011). After successful excystment from suitable hosts, juvenile mussels are collected and pre-cultured in small containers before they are transferred into cages and placed in natural rivers or semi-natural flow channels (Hruška 1999, 2001; Thomas et al. 2010; Gum et al. 2011). One critical step during artificial propagation is the timing of the excystment process since the small size of excysted mussels impedes their collection. Host fish were often not fed before and during excystment to reduce the amount of fish feces and facilitate mussel collection. Detailed knowledge of the timing of the excystment process is crucial to minimize holding of infested fish and time-consuming control for the presence of juvenile mussels. In addition, this knowledge helps to optimize and standardize artificial breeding of *M. margaritifera* and to avoid the loss of juvenile mussels due to delayed collection (Hastie and Young 2003). However, according to the available literature, the start of excystment of *M. margaritifera* appears highly variable. For example Ziuganov et al. (1994) described the start of excystment between 18 days post infestation and 11 months post infestation under natural temperature conditions (Table 1). Hruška (1992) established the concept of