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Glochidial development of the freshwater swan mussel (*Anodonta cygnea*, Linnaeus 1758) on native and invasive fish species

Verena Huber, Juergen Geist *

Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management, Technical University of Munich, Muehlenweg 22, D-85354 Freising, Germany

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ABSTRACT

The declining mussel *Anodonta cygnea* is an important keystone species in European freshwater systems. Information on the complex life cycle of *A. cygnea* regarding the attachment and metamorphosis of their larvae on suitable host fish species is lacking, yet important as a basis for conservation and fisheries management. Ten different fish species, including eight native and two non-native species from four different families, were simultaneously infested with the glochidia of *A. cygnea* in a standardized laboratory experiment. The results of this study confirmed the hypothesis that *Anodonta cygnea* can be considered a host generalist, as nine out of the ten tested fish species were suitable hosts, and different body parts were infested. Due to the observed differences in initial infestation rates and metamorphosis success, hosts were classed into "good hosts" (*Perca fluviatilis*, *Leuciscus idus*, *Salmo trutta*, *Gasterosteus aculeatus*, *Ctenopharyngodon idella*), "poor hosts" (*Leucaspius delineatus*, *Gobio gobio*, *Rutilus rutilus*, *Pseudorasbora parva*) and "not hosts" (*Rhodeus amarus*). The larval development differed strongly between the single host fish species with regard to success and duration of metamorphosis, as well as timing and synchronization in larval drop-off, suggesting evolutionary consequences of the use of different host fish species. The finding that two non-native fish species were identified as suitable hosts for the glochidia of *A. cygnea* illustrates that the generalizations that non-native species are a threat to native mussel communities and that co-evolutionary patterns between hosts and mussels determine host suitability do not always hold true.

1. Introduction

Freshwater bivalves play a key role in the functioning of the ecosystems in which they occur (Boeker et al., 2016; Lummer et al., 2016; Vaughn and Hakenkamp, 2001). As ecosystem engineers they act as connective link between the pelagic and benthic zones of a water body (Gutierrez et al., 2003; Lopes-Lima et al., 2016; Nobles and Zhang, 2011). Their ecosystem functions include the transfer of matter and energy from the water column to the benthos with strong effects on primary and secondary production, biogeochemical cycles, sedimentation rates and water clarity (Lummer et al., 2016; Strayer et al., 1999). Despite their important roles in freshwater ecosystems, there is a worldwide decline of freshwater mussel populations (Bogan, 2008; Geist, 2010; Lopes-Lima et al., 2016; Lydeard et al., 2004). Currently, 12 out of 16 European freshwater mussel species are listed as threatened or near-threatened on the IUCN Red List (Lopes-Lima et al., 2016).

The swan mussel (*Anodonta cygnea*) was originally considered a widespread species throughout Europe, Russia and the Middle East (Lopes-Lima, 2014). It occurs in a diversity of habitats with slow or no flow current, including small ponds as well as lakes and lowland rivers (Zettler et al., 2006). Despite the swan mussel still being listed as a species of "least concern" in the IUCN Red List of Threatened Species, global population trends are still unknown and there is a strong indication of currently underestimated declines in abundance on local scales (Lopes-Lima, 2014). *A. cygnea* in Europe has a conservation status of "near threatened" according to the IUCN European Red List of non-marine molluscs (Cuttelod et al., 2011). In the study region of this work, Germany, the species is considered "threatened" (Jungbluth and von Knorre, 2009). It is even listed as "highly endangered" according to federal German legislation (Bundesartenschutzverordnung (BArtSchV), 2005; Lopes-Lima, 2014; Zettler et al., 2006) as well as on state-specific lists such as the Bavarian Red List of endangered species (*A. cygnea* with a status of "endangered") (StMUV, 2005).

Like all other mussel species of the order Unionoida, *A. cygnea* has a complex life-cycle with an obligate parasitic phase of the larvae on a suitable host fish (e.g. Bauer, 1994; Lefevre and Curtis, 1910; Lopes-Lima et al., 2016; Weber, 2005). Gravid mussels eject mature glochidia into the water column usually during the time period between late winter and spring (Lopes-Lima et al., 2016; Niemeyer, 1993). During the parasitic phase on the fish, glochidia metamorphose into juvenile mussels. The larvae of the swan mussel usually attach to multiple body parts of a fish host, for example fins, opercula and gills, which is in contrast to other mussel species of the order Unionoida which often exclusively attach to the fish gills (e.g., *Margaritifera margaritifera*: Bauer, 2001; Blažek and Gelnar, 2006; Waechtler et al., 2001). After

* Corresponding author.
E-mail address: geist@wzw.tum.de (J. Geist).

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completion of the parasitic phase, the fully developed juvenile mussels of *A. cygnea* drop off the fish host and bury into the lake bed substratum until they start their adult life as filter feeders (Waechtler et al., 2001).

Since the attachment to, and metamorphosis on a suitable host, can be an important bottleneck in the life cycle of endangered freshwater mussel species (Stoeckl et al., 2015; Taeubert et al., 2012a; Taeubert et al., 2012b), such information is urgently needed as a basis for conservation, fisheries management and supportive breeding (Gum et al., 2011). In contrast to the very host-specialized species *M. margaritifera*, the larvae of *A. cygnea* are considered to be host fish generalists with a wide range of host fish species (Bauer, 2001; Waechtler et al., 2001). However, to date, research and conservation strategies into host suitabilities of European freshwater mussels have mostly focused on the thick-shelled river mussel (*Unio crassus*) and the freshwater pearl mussel (*M. margaritifera*). A recent review on the conservation of European freshwater mussels has thus suggested the need of identifying host fishes for other species (Lopes-Lima et al., 2016), including *A. cygnea*.

The general aim of this study was to characterize the host relationships of *A. cygnea*. Specific objectives included (1) identifying suitable host fish species for *A. cygnea* from the native fish community in central Europe, (2) assessing host suitability and thus possible competition with non-native fish species, (3) characterizing and comparing the duration of the larval phase on different fish hosts, and (4) determining glochidial infestation at different body parts of a host. Specifically, the following hypotheses were tested: (1) Glochidia of *A. cygnea* are generalists concerning their host choice (following the suggestion by Bauer (2001) and Waechtler et al. (2001)) and suitable hosts are equally infested, showing similar metamorphosis success. (2) Non-native fish species are non-suitable hosts for the glochidia of *A. cygnea* and thus reduce overall metamorphosis success by competing with native hosts. (3) Development times of *A. cygnea* larvae differ between host fish species as previously found in other mussel-host fish relationships (Taeubert et al., 2012a). (4) The numbers of attached glochidia strongly vary between different types of tissue of a single host fish, with gills showing highest infestation rates as suggested by Jansen et al. (2001) and Schneidt (1998).

2. Materials and methods

2.1. Collection of glochidia

Seven gravid *A. cygnea* individuals from the Neusee near Bernried (Bavaria, Germany) were collected at the 23th of October 2014 and brought to the laboratories of the Aquatic Systems Biology Unit at Technical University of Munich, Germany where they were held until glochidia release. Because of the high morphological plasticity of the Anodontines, all specimens were genetically validated following the method in Zieritz et al. (2012). The seven adult mussels were kept in aquaria and maturity status of the glochidia inside the marsupia was checked once a week from February to the beginning of May 2015 in order to identify the ideal time point for glochidial collection. On the 13th of May 2015 fully developed glochidia were detected in all seven specimens. Thus, marsupia of the seven specimens were flushed with a squirt bottle to collect the glochidia for the following infestation process. Glochidia from each specimen were individually stored in 1 L beakers at 4.0 °C for <24 h. Before host fish infestation, the viability of the larvae was assessed by checking for an active clamping mechanism after addition of NaCl to a small amount of glochidia (Taeubert et al., 2012b). In total, a number of ~300,000 larvae from all seven adult *A. cygnea* was harvested.

2.2. Infestation

Ten different fish species were infested with the larvae of seven adult *A. cygnea* on 14th of May 2015. The selection of tested fish species was based on broad taxonomic representation of different fish families and species that naturally co-occur with *A. cygnea*. In addition, two non-native species (*Ctenopharyngodon idella*, Valenciennes 1844; *Pseudorasbora parva*, Temminck & Schlegel 1846), which currently spread within the *A. cygnea*-distribution area, were also included to test their suitability as hosts. Native fish species from the families Salmonidae (*Salmo trutta*, Linnaeus 1758), Cyprinidae (*Leuciscus idus*, Linnaeus 1758; *Gobio gobio*, Linnaeus 1758; *Rhodeus amarus*, Bloch 1782; *Rutilus rutilus*, Linnaeus 1758; *Leucaspius delineatus*, Heckel 1843), Percidae (*Perca fluviatilis*, Linnaeus 1758) and Gasterosteidae (*Gasterosteus aculeatus*, Linnaeus 1758) as well as the introduced Cyprinidae *C. idella* and *P. parva* were tested. *C. idella* was primarily introduced to European waterbodies for weed control (Cross, 1969), whereas *P. parva* was primarily introduced accidentally (Copp et al., 2010). Juvenile fish (<1 year, trout <2 years) with no previous contact to unionid mussels were used to exclude a possible immune-response due to previous contact with glochidia. Information about the tested fish species, their origin and number of individuals per species used for the experiment are given in Table 1. Before starting the infestation process, the glochidia from all seven parents were gently mixed to a homogenous suspension and acclimatized over 2 h to the temperature of the laboratory water (bank filtrate, river Moosach, ~12.0 °C) used for the infestation bath. Infestation procedures followed the previously described standard protocol by Taeubert et al. (2012b). The glochidial concentration in the infestation bath was ~8500 larvae per liter. Overall, 397 specimens of the ten different fish species were simultaneously infested in the same infestation bath with the larvae of *A. cygnea* to ensure identical starting conditions (Taeubert et al., 2013a). During the infestation, the water was mixed continuously to ensure a homogenous suspension of glochidia and equal attachment conditions. After 30–45 min within the infestation bath, the fish were transferred into a second water bath without glochidia for 15 min to remove non-attached larvae. In addition, 127 individuals from all species not exposed to the glochidia were used as a control. The control fish were treated in the same way as the infested ones to check if influences like stress due to handling or holding conditions affect the mortality during the experiment.

2.3. Post-infestation procedure

After the common infestation bath, specimens were sorted and different species kept in separate holding units (three replicates per species). In addition, in every species, one tank with control fish was maintained under identical conditions. The custom-made, funnel-shaped holding units (Fig. 1) contained a volume of ~40 L water at a mean temperature of 15.8 °C. This system was applied to ensure that all dropped-off glochidia accumulate at the bottom end where they could be collected by opening a lid. Within each holding unit, nets were placed ~10 cm above the bottom of the tanks to protect the glochidia and excysted juvenile mussels from possible predation by the fish (Fig. 1). To account for differences in fish sizes among different species and to ensure optimal holding conditions, the number of specimens per tank was adjusted accordingly. Holding units containing brown trout were provided with constant flow of water at a colder temperature (12.8 °C) due to requirements of this species. The water temperature in the fish holding units was measured with temperature loggers (Lascar Electronics Limited, Salisbury, UK) every 30 min. Fish were not fed during the experiment.

2.4. Glochidial development and excystment

Five liters of water from every tank (12.5%) were changed daily. The 5 L water-outflow from all holding units with infested fish were afterwards checked for excysted glochidia and juvenile mussels under a stereomicroscope (Stemi DV4, Zeiss, Munich, Germany, 12.8× amplification) by filtering the water through a sieve (mesh size 200 μm). Criteria for the occurrence of living juvenile mussels were