Czech University of Life Sciences Prague

Faculty of Environmental Sciences

Department of Zoology and Fisheries

Faculty of Agrobiology, Food, and Natural Resources

Study the effects of invasive mussel from E. Asia on the native mussel species in the Czech Republic

Diploma Thesis

Supervisor: Ing. Karel Douda, Ph.D.
Masters Student: Seth William Donrovich

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DIPLOMA THESIS ASSIGNMENT

Seth Donrovich

Nature Conservation

Thesis title

Study the effects of invasive mussel from E.Asia on the native mussel species in the Czech Republic

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Objectives of thesis

1) To prepare a literature review on the potential impacts of invasive bivalve A. woodiana on native European mussels
2) To investigate the role of cross-resistance in parasitic success of native mussel species.

Methodology

analysis of literature resources, experimental testing of host-parasite compatibility, data analysis, synthesis.

The proposed extent of the thesis

30 pages

Keywords

Unionidae, host, parasite, invasive species, freshwater bivalves

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Recommended information sources

Douda, K., M. Vrtílek, O. Slavík, and M. Reichard. (2012). The role of host specificity in explaining the invasion succes of the freshwater musel Anodonta woodiana in Europe. Biological Invasions 14, 127-137.

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The Diploma Thesis Supervisor

Ing. Karel Douda, Ph.D.

Supervising department

Department of Zoology and Fisheries

Electronic approval: 30. 3. 2016

prof. Ing. Iva Langrová, CSc.

Head of department

Electronic approval: 31. 3. 2016

prof. RNDr. Vladimír Bejček, CSc.

Dean

Prague on 10. 04. 2016

Author’s Declaration

I hereby declare that I have written this diploma thesis independently under the direction and guidance of my supervisor, Ing. Karel Douda, Ph.D. I have properly cited all literature and publications from which I have acquired information for the diploma thesis. All figures, including photographs, and tables within the diploma thesis are my original works or that of my supervisor.

Seth William Donrovich

In Prague, 15.04.2016 ……….………………………………..

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Abstract

Freshwater mussels are vital to the health and stability of ecosystems, but are unfortunately one of the most highly endangered orders worldwide. These delicate organisms are facing threats of overharvesting, habitat destruction, and competition from invasive freshwater mussel species for both resources and host fish. Since its introduction, the invasive *Anodonta woodiana* (Lea 1834) (Chinese pond mussel) has become a dominant species throughout freshwater ecosystems in Europe. This study presents a literature review on the potential negative impacts of *A. woodiana* on native freshwater mussels. In addition, the ability for the freshwater fish species *Squalius cephalus* (Linnaeus 1758) (European chub) to host native *Anodonta anatina* (Linnaeus 1758) (duck mussel) after primary infections from *A. woodiana* was examined to test the impact this invasive species had on the development of native freshwater mussel species. The results indicated that juvenile *A. anatina* mussels were nearly twice as successful developing on naïve host fish than on primed host fish, which have built up an acquired immunity. The highly tolerant and adaptive *A. woodiana* is continuing to expand its range and the experimental conclusions confirm the impact this invasive species has on the growth and development of native freshwater mussels. Persistent awareness, education, and action are needed in order to increase conservation efforts and protect these keystone species.

Keywords: Unionidae, host, parasite, invasive species, freshwater bivalve

Abstrakt

Sladkovodní mlži mají zásadní význam pro funkci a stabilitu vodních ekosystémů, bohužel jsou ale celosvětově velmi ohroženou skupinou organismů. Tyto zranitelné organismy čelí riziku nadměrného lovu, ničení stanovišť a konkurenci s invazními druhy mlžů o zdroje a hostitelské ryby. Invazní *Anodonta woodiana* (Lea 1834) (škeblice asijská) se po svém příchodu do Evropy stala na řadě lokalit dominantním druhem. V této práci je zpracována literární rešerši potenciálních vlivů invazního druhu *A. woodiana* na domácí mlže a dále byla testována schopnost sladkovodní ryby *Squalius cephalus* (Linnaeus 1758) (jelec tloušť) být hostitelem původního druhu *Anodonta anatina* (Linnaeus 1758) (škeble říční) po předchozí infikaci *A. woodiana* z hlediska úspěšného vývoje parazitických larev. Výsledky naznačují, že vývoj v juvenilní jedince byl téměř dvakrát tak úspěšný na naivních rybách než na rybách, které byly již infikovány invazním druhem a vytvořily si imunitu. Velmi tolerantní a přizpůsobivá *A. woodiana* pokračuje ve svém šíření a experimentální studie ukazuje, že to může mít negativní dopad na růst a vývoj domácích druhů mlžů. Zvýšený zájem, osvěta a také aktivní opatření jsou potřeba, aby mohla být zajištěna ochrana této skupiny klíčových druhů.

Klíčová slova: Unionidae, hostitel, parazit, invazní druhy, sladkovodní mlž

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Chapter 1: Introduction

Freshwater mussels, which are of the order Unionoida (alternatively Unionida) constitute approximately 217 genera and 900 species worldwide as of August 2015, according to the "Mussel Project" online database operated by Dan Graf and Kevin Cummings (http://www.mussel-project.net) (Walker et al. 2014, Graf and Cummings 2015). These aquatic invertebrates are native to all continents aside from Antarctica, with 16 native species being represented in Europe (Lopes-Lima et al. 2016).

Worldwide, freshwater mussels are drastically decreasing, with nearly half of the species threatened or worse, leading to profound consequences on all aspects of the surrounding environment and ecosystems (Bogan 1993, Burlakova et al. 2011, Lopes-Lima et al. 2014, Walker et al. 2014). The decline of freshwater mussels is of major concern for researchers, as they provide filtration, nutrient cycling, substrate stability, bioturbation, and control of suspended solids, while generally serving as bioindicators for overall ecosystem health and a link between the benthic and water zones (Bogan 1993, Williams et al. 1993, Bauer and Wachtler 2001a, Vaughn and Hakenkamp 2001, Lopes-Lima et al. 2014). Though the reasons of decline are numerous and varied, one of the main causes is from the introduction (advertent or inadvertent) of invasive freshwater mussels, which leads to competition with native species for space, nutrients, and host fish; the latter of which are necessary for the reproduction of freshwater mussels (Arey 1932, Dodd et al. 2006, Douda et al. 2011, 2013).

Currently, there has been little research done on the specific impact that invasive mussels have on native mussels with regards to competition for host fish. Freshwater mussels of the genus *Anodonta* are classified as host generalists, which concludes that their parasitic larvae (glochidia) are able to attach to a variety of host fish (Douda et al. 2011, 2013, Karplus 2014). Two species of interest are discussed in detail: the European native *Anodonta anatina* (Linnaeus 1785) (duck mussel) and the invasive *Anodonta woodiana* (Lea 1834) (Chinese pond mussel), from East Asia. These species have different reproductive cycles, with *A. woodiana* releasing their glochidia throughout the summer (Sarkany-Kiss et al. 2000, Afanasjev et al. 2001) and *A. anatina* releasing their glochidia in autumn or spring (Hinzmann et al. 2013, Karel Douda 2016 pers. comm.).

The aim of the research is to determine whether host fish have the capability of harboring multiple freshwater mussel species in succession, or whether prior glochidial infections will limit the capability of the fish to host additional species. More specifically, native *Squalius cephalus* (Linnaeus 1758) (European chub) will be artificially infected with glochidia from *A. woodiana* 0, 1, or 3 times. In other words, there will be a control group of naïve fish, a group that had been primed once by the glochidia of *A. woodiana*, and a third group that had been primed with the glochidia of *A. woodiana* on three occasions. Then, the same three groups host fish will be artificially infected with glochidia from *A. anatina* to determine the success rate of juvenile mussel metamorphosis. In addition, the status and trends of freshwater mussel conservation will be examined thoroughly throughout the diploma thesis.

In addition to Chapters 1 and 2 (Introduction and Aims), Chapter 3 is comprised of an in depth literature review detailing the biology, ecology, and current conservation status of freshwater mussels, an overview of invasive species, a description of *A. woodiana*, host-parasite relationships, the fish and freshwater mussel relationship, and finally an examination of fish immunology. Chapter 4 describes the experiment, including a thorough description of materials and methods, composed of site-description, transport, infection, monitoring, and data analysis; experimental results; and a discussion of the results. Chapter 5 summarizes the research, the impacts of *A. woodiana*, and an assessment for freshwater mussel conservation moving forward.

Chapter 2: Aims

There are two primary aims of the diploma thesis. The first is to serve as a comprehensive review about the potential threats resulting from the invasion of *A. woodiana* to the current status and conservation of native freshwater mussels. The ecology of freshwater mussels, invasive species, and specifically, *A. woodiana* are discussed in detail. In addition, the host-parasite relationship is explored with the focus on the relationship between fish and freshwater mussels, including fish immunology.

The second aim is to determine if there is an effect of primary glochidial infections of *A. woodiana* on secondary infections from *A. anatina* on host fish *S. cephalus*. Two equal groups of the native host fish – *S. cephalus* were first infected with glochidia of *A. woodiana*, either once or on multiple occasions. The same groups of host fish, in addition to a third group of naïve fish, are then infected by *A. anatina* glochidia. The transformation success rates are compared between the three groups of *S. cephalus* to determine if prior infections from *A. woodiana* impact the growth and development of the native freshwater mussel *A. anatina*.

It is expected that the results of this diploma thesis will contribute to the research regarding the potential effects that invasive freshwater mussels have on native freshwater mussels. This will be accomplished by realizing the role that adaptive immunity has on successive glochidial infections and determining whether and to what degree native host fish are capable of hosting multiple species in succession. This research, as part of the "Impact of non-native species on host-parasite relationships: importance of interpopulation variability" study funded by the Czech Science Foundation can potentially serve to accelerate conservation efforts of native freshwater mussels in Europe and help protect against invasive species.

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Chapter 3: Literature Review

The aim of the literature review is to give detailed background information on freshwater mussels and their relationships with host fish. In addition, the invasive *A. woodiana* and its impact are explored in detail and current conservation efforts, trends, and overall outlook are discussed based on existing published scientific literature.

Chapter 3.1 Freshwater Mussels

There are currently five orders of bivalves that are present in freshwater ecosystems: Arcoida, Myoida, Mytiloida, Veneroida, and Unionida (alternatively Unioniforms), which is the group that is composed of unionid bivalves (unionids), or freshwater mussels (Bogan 2008, Graf and Cummings 2015). The order of freshwater mussels can first be divided into two superfamilies: Etheriodea and Unionoidea (Walker et al. 2014), with the distinction being based on the type of larvae produced - Etheriodea producing lasidia and Unionoidea producing glochidia (Bauer 2001a).

The two superfamilies can be broken further down into three families each: the Etheriodea superfamily includes Etheriidae, Iridinidae, and Mycetopodidae; and the Unionoidea superfamily includes the Hyriidae, Margaritiferidae, and Unionidae families, with *A. anatina* and *A. woodiana* both belonging to the family Unionidae (Bogan and Roe 2008, Walker et al. 2014). Overall, within the families, there are 217 genera and approximately 900 species (http://www.mussel-project.net) (Walker et al. 2014, Graf and Cummings 2015). Native to Europe are two species from the Margaritiferidae and 14 from the Unionidae families (Lopes-Lima et al. 2016).

3.1.1 Biology and Ecology

Anatomy

Exteriorly, the shells of freshwater mussels are composed of an outer prismatic layer that is covered by periostracum, a protein-like substance that varies according to environment, and prevents the shell from dissolving, while the interior layer is nacreous, or pearly (Bauer 2001b). The left and right (or top and bottom) shell valves of freshwater mussels are approximately the same dimensions (Gosling 2008). The shell halves are connected to each other at the hinge, and may show a variety of teeth that are interlocked (Smith 2001). The shell morphology includes rings that can determine age and growth rate, similar to rings on a tree (Haag and Rypel 2011).

The mussel is attached to these shells by the posterior adductor and interior adductor muscles, which also control the movement of the foot (Gosling 2008). The foot is a tongue-like organ, which is used for digging into the substrate, or in some species, secreting protein threads for attachment of the mussel to the substrate, mussels, or other objects (Bauer 2001a, Gosling 2008).

On each side of the foot, there are two large gills, which aid in oxygen uptake, contain cilia that create a water current and transport mucus into the mouth as a form of nutrition, and are used as a marsupium for the development of eggs for reproduction (Bauer 2001a). Also included in the interior anatomy is the mantel cavity which is controlled by two external tissue flaps, called the mantel (Gosling 2008). Within the mantel cavity, there also exists two siphons, – an inhalant and exhalant, which aid in collecting both food and oxygen and eliminating waste, respectively (Stankovic and Jovic 2013).

Feeding

Freshwater mussels are planktivores, meaning their diet is composed primarily of zooplankton and phytoplankton (Williams et al. 1993). Moreover, they are filter feeders that feed via a process that includes water retention, ingestion, and assimilation (Dillon 2000). To put in in simpler terms, essentially, freshwater mussels feed via water filtration. Water is retained as the bivalve opens its shell, which can vary to up to twenty partial openings per hour, or, oppositely the mussel may be completely closed for hours at a time (Dillon 2000). During feeding, the mantel cavity is filled and wanted food is ingested, while unwanted food is sent to the palps and gills; it is here where the unwanted food is converted into mucus and expelled as a pseudofeces via the exhalent siphon (Dionisio Pires et al. 2005, Bontes et al. 2007).

Habitat

Unionids inhabit freshwater ecosystems on all continents except for Antarctica, with southeastern North American and Southeast Asia having the highest biodiversity (Bogan 2008). Throughout Europe, freshwater mussels may occupy a wide range of habitats, dispersed from Ireland and the United Kingdom in the west to Russia in the east (Lopes-Lima et al. 2016).

Freshwater mussels typically inhabit slow rivers, canals, drainage ditches, ponds, lakes, and reservoirs, and, though they prefer river ecosystems, any of these freshwater bodies are suitable as long as there is the presence of fish and a muddy substrate for which the mussels to lodge themselves (Boycott 1936). In Europe, it was found that native *Anodonta* species prefer large streams and standing waters of a high trophic level (Patzner and Muller 2001). The habitat for freshwater mussels is primarily determined by three factors: biogeographical features, such as mountain ranges; availability of host fish for reproduction and dispersal; and environmental and biotic factors, such as water quality and sediment, or competition (Vaughn and Taylor 1998). The habitat of juvenile mussels is slightly different than that of adults. Freshwater mussels that are not fully developed tend to prefer a sandy substrate, in areas hidden behind boulders, for their habitat (Dillon 2000).

Mussel densities are highest in ranges from 0.5 to 2 meters in depth, as conditions including temperature, flow, availability of food, and light decrease with depth (Dillon 2000). However, this is not always the case. For example, in extremely clear lakes, high densities may occur at increased depths, while fluctuations in water level and predation can limit the density of mussels at shallower depths (Dillon 2000). As an example, generally, the largest density of *A. anatina* occurs at a depth of one meter (Haukioja and Hakala 1974).

Reproduction and Lifespan

Freshwater mussel populations are generally equal in terms of males and females present (Dillon 2000), and regarding species that are dioecious, sexual dimorphism is common, though it may be difficult to detect (Bauer 2001b). In some studies, it was found that a number of freshwater mussel species could be classified as hermaphroditic (either dominantly, partially, or occasionally) (van der Schalie 1970). In these cases, it is stated that facultative hermaphroditism occurs due to stressful conditions in the environment (Gates et al. 2015). To further illustrate this point, it was found in experiments with *Margaritifera margaritifera* (Linnaeus 1785) (freshwater pearl mussel) that when populations are absent of males, most females will become hermaphroditic and reproduce by self-fertilization to sustain populations (Bauer 1987). The nature of the habitat may also influence or even dictate the presence of hermaphrodites. As an example, *A. anatina* was found to display hermaphroditism in standing waters, but were dioecious in streams and other lotic waters, where there were likely larger populations (Hinzmann et al. 2013).

In cases where there are both males and females present in an ecosystem, males release sperm into the water where it collected by females, leading to fertilization, and the newly formed larvae are held within the gills of female mussels until they are released into the surrounding water (Dillon 2000, Strayer 2008). In more detail, males cast sperm into the water and females release eggs into suprabranchial chambers, where the fertilization eventually takes place (Gates et al. 2015). To initiate the fertilization, the sperm enters the female mantel cavity through the inhalant current (Lopes-Lima et al. 2016).

For Unionoidea mussels, the enlarged gill, as a result of harboring glochidia, of the female is referred to as a marsupium, and remains enlarged for the brooding time (Dillon 2000). Though most freshwater mussel species in the Northern hemisphere release their glochida in the spring or summer in the same year that fertilization takes place (referred to as short-term brooding), with metamorphosis occurring later that same year (usually 2-6 weeks later) some species of mussels fertilize their eggs in late summer or autumn and store the glochida over winter to release them the following spring (referred to as long-term brooding) (Watters and Dee 1999, Gates et al. 2015). During this brooding time, there is a nutrient and physiological exchange between the parent mussel and the larvae along with protection against the unfavorable environmental conditions (Schwartz and Dimock 2001). In addition to exhibiting a swollen appearance, the parent mussel exhibits increased oxygen consumption and reduced filtration rates (Wachtler et al. 2001 ex. Tankersley and Dimock 1993).

Glochidia are triangular, spherical or hatch-shaped, having diameters of 60 to 400 μm, and contain anatomical features such as a shell, adductor muscles, sensory hairs, ciliary fields, a mantle, and in some cases a larval thread (Wachtler et al. 2001). Once mature, the glochidia are released from the marsupium and travel through the suprabranchial canal and excurrent syphon (Dillon 2000). Studies indicate that the number of glochidia produced by female freshwater mussels can range from several thousand to several million annually, depending on both the size of the female and the size of the glochidia (Neves et al. 1985, Wachtler et al. 2001). For instance, female mussels of the species *Anodonta cygnea* (Linnaeus 1758) (swan mussel) are able to produce, on average, a half a million glochidia (Boycott 1936). The released glochidia then attach as parasites to host fish in order to complete their development into juveniles (Dillon 2000). However, the mortality rate of glochidia is extremely high, due to their immobility and difficulty in finding suitable hosts (Jansen et al. 2001). This phenomenon will be discussed in more detail in Chapter 3.5. Freshwater mussel reproduction is complex and arduous from gamete production to fertilization to brooding to glochidia release. To put it in perspective, the entire reproductive cycle for *A. anatina* takes approximately 10 months to complete (Hinzmann et al. 2013).

Longevity of freshwater mussels can span from 4 years to nearly 200 years (Haag and Rypel 2011). There are differences amongst the lifespans of Unionoidea families. Unionidae mussels (such as *Anodonta*) are characterized by a shorter lifespan and fast growth, while Margaritiferidae are characterized by a longer lifespan and slow growth (Haag and Rypel 2011). *Anodonta* typically live for less than 30 years, reaching sexual maturity from 1-4 years (Lopes-Lima et al. 2016), whereas *M. margaritifera* can typically live for 132 years (Bauer 1992).

3.1.2 Conservation Status

Worldwide, out of the 511 freshwater mussel species that are listed in the 2015 *IUCN Red List of Threatened Species*, 224 (44%) are designated as Threatened or Near Threatened (Lopes-Lima et al. 2016). Though there are a large number of cases in Europe, the majority of these species reside in the USA (Christian and Harris 2008). Specifically in Europe, of the 16 native species present, 12 are Threatened or Near Threatened (including three being Critically Endangered, two Endangered, two Vulnerable, and five Near Threatened); three are of Least Concern; and one species has yet to be assessed (Lopes-Lima et al. 2016).

Recent extinctions of unionids are higher than the combination of all other taxa throughout the world combined (Burlakova et al. 2011). Worldwide, freshwater mussel biodiversity is on the steady decline, attributed to a multitude of factors, summarized as including overharvesting, water pollution, habitat degradation, changes to freshwater regimes, and invasive species (Walker, et al., 2014). Invasive species and a thorough study of *A. woodiana*, including its role as an invasive species will be discussed in Chapters 3.2 and 3.3, respectively.

The long lifespan of freshwater mussels, which can trivialize impacts when considering individuals, lead to detrimental effects over larger populations and longer time periods (Bogan 1993). Freshwater mussels are considered keystone species and if they are eliminated from an ecosystem, there are severe implications for the entire aquatic environment (Bauer and Wachtler 2001a). It is generally considered that target species for conservation, such as umbrella, keystone, indicator, or flagship species will positively impact the conservation efforts of other species in the ecosystem (Geist and Kuehn 2008).

Importance of Freshwater Bivalves

The filtering behavior of freshwater mussels, along with their sessile lifestyle and need for high water quality make them good indicators to the overall health of an ecosystem (Williams et al. 1993). In fact, freshwater mussels are known for the removal of seston concentration (floating matter) from bodies of water, even up to 15% of the seston in eutrophic rivers between the months of May and October (Pusch et al. 2001). Various toxic substances can permeate the mussel, and, when these specimens are studied, can give a signal to the overall health and stress of the environment (Bogan 1993). In fact, mussel filtration can even be used as a bioindicator to quantify the effects of pollution (Hartmann et al. 2016). Due to the long life span of some freshwater mussels, the changes in environmental conditions of a body of water can be studied for time periods of over a century (Bauer and Wachtler 2001a).

Furthermore, freshwater mussel communities help stabilize the substrate, transfer biomass from the water column to the benthos, recycle nutrients for the ecosystem, control the concentration and composition of suspended particles, and aid in bioturbation

(Christian and Harris 2008, Hinzmann et al. 2013, Lopes-Lima et al. 2014). Freshwater mussels can serve as habitats for other invertebrates and assist in securing aquatic plants to the substrate (Vaughn and Hakenkamp 2001). The role of freshwater mussels both as a predator and prey allow for an understanding and connection between the water and benthic sectors of the ecosystem (Lopes-Lima et al. 2014). Specifically, their role in nutrient cycling can be specified by linking benthic organisms with pelagic organisms and triggering production throughout the many trophic levels of the aquatic ecosystem (Vaughn and Hakenkamp 2001). Not only that, but they can facilitate algae growth and increase the amount of aquatic insects and their predators (Lopes-Lima et al. 2014 ex. Haag 2012). By excreting feces and pseudofeces, freshwater bivalves can add nutrients to the soil, which may increase sediment resources and abundance of macroinvertebrates (Vaughn and Hakenkamp 2001). The large size and density of mussels in some areas have led to their use as model organisms for research of cell thermal resistance, nervous cell structure, cell ciliary movements and other biochemical mechanisms, in addition to toxicological studies (Lopes-Lima et al. 2014).

Causes of Decline

The single greatest threat to freshwater mussels is the destruction of habitat (Williams et al. 1993). Freshwater mussels are eminently dependent on hydraulic conditions, requiring stable flow, water quality, and a suitable habitat (Gates et al. 2015). Periods of high flow can cause displacement, while periods of low flow reduce populations by stranding individuals or creating low dissolved oxygen levels (Lopes-Lima et al. 2016). That being said, fast growing species, such as *A. anatina* are capable of dealing with disadvantageous habitat conditions and respond quicker to change due to their quick regeneration time (Haag and Rypel 2011).

Modifications to the river regime such as damming or dredging can alter the flow, temperature, and substrate composition that is vital to the livelihood of freshwater mussels (Bogan 1993). Dams alter the physical, chemical, and biological components of the ecosystem, along with diminishing the availability of host fish by disallowing movement and migration throughout the channel (Williams et al. 1993). This habitat fragmentation prevents colonization after a severe disturbance (Lopes-Lima et al. 2016 ex. Haag 2012).

A number of pollutants, such as road salts, heavy metals, environmental pollutants (namely DDT and DDE), agricultural runoff, pharmaceutical compounds, and eutrophication can adversely affect freshwater mussel populations (Lopes-Lima et al. 2016). Pesticides, heavy metals, and untreated wastewater can be filtered by the bivalves and can have negative effects on their health and biology (Bogan 1993). Similarly, foreign substances can alter the filtration activity of freshwater mussels, as *A. anatina* has shown heightened sensitivity and limited filtration due to the presence of de-icing salts (Hartmann et al. 2016). Streams and other water bodies subject to chemical spills and coal mining have caused the extinction, or in the best case, a significantly reduced population of freshwater mussels which are now subjected to further disturbances (Haag and Williams 2014 ex. Haag 2012).

Erosion, as a result of poor agricultural practices, coupled with deforestation can significantly reduce populations by accumulation and shifting of the substrate (Williams et al. 1993). The addition of silt from activities such as head-cutting, coal and gravel washing, runoff, and destruction of riparian habitat can cause individual freshwater mussels to suffocate (Bogan 1993).

The nature of the fish and freshwater mussel relationship can also greatly contribute to the decline of freshwater bivalves, as changes to the water regime not only affect unionids, but also the fish prevalent throughout the ecosystem. With fish populations decreasing in these situations, the glochidia of some freshwater mussels have no host on which to attach and the local population will, in effect, become extinct (Bogan 1993). Many of the worldwide mussels can only parasitize either one (host specialists) or a small number of fish species, further cementing their dependence on the abundance of fish populations (Haag and Williams 2014 ex. Haag 2012). Additionally, fish may develop an immunity to infections over time as they age or due to previous infections from other mussel species (Jansen et al. 2001) (discussed in Chapter 3.6). The fact that freshwater mussels are dependent on host fish species for their reproduction makes them extra sensitive to disturbances in the ecosystem – these species have to withstand both direct and indirect impacts on the environment (Bogan 1993).

Other external organisms that can affect the status of freshwater mussels are alien unionids, which have been introduced and compete with native freshwater mussel species for all resources; or predators such as crayfish, some fish species, and even mammals (Williams et al. 1993, Zahner-Meike and Hanson 2001). In North America, and particularly in Europe during the last century, predation from the muskrat (Linnaeus 1766) (*Ondatra zibethicus*) has been of serious concern due to the rapid spread of the mammal throughout the continent and absence of natural predators (Zahner-Meike and Hanson 2001).

An increase in the harvesting of freshwater bivalves from diving or brailing, for use in the pearl industry, has rid populations of adult mussels, and in some cases, caused local extinctions (Bogan 1993, Williams et al. 1993). Besides, large mussels are also used as food source throughout their range by humans and other animals (Haag and Williams 2014). In a United States study, it was found that the number of threatened unionid species correlated negatively with human population density in general (Burlakova et al. 2011). All of the factors causing the decline of freshwater mussel populations would appear to be more detrimental to strictly dioecious species, as with any species there is a minimum effective population needed for sustainability and survival.

Future Outlook

Freshwater mussels are declining at a pace that conservation efforts cannot mimic during the last several decades (Walker et al. 2014). Current problems of habitat degradation, a lack of knowledge of freshwater mussel ecology and distribution, and their unique dependence on host fish are all contributing to the decline (Bogan 1993). By reducing unionids from the ecosystem, dramatic effects can happen to the ecological stability and environmental conditions of the aquatic environment, especially considering that each species has a particular niche (Vaughn and Hakenkamp 2001). To combat this problem, conservationists need to develop a plan with a set of objectives and create priorities with specific species in mind (Lopes-Lima et al. 2016).

Steps in the right direction have already been taken. Overall, the number of scientists dedicated to studying freshwater mussels has increased over the last few decades (Lopes-Lima et al. 2016). For instance, advances in genetics have permitted scientists and conservationists to determine with a high degree of accuracy the glochidia of different mussels which are present on different host fish, and this understanding can focus and streamline conservation efforts (Zieritz et al. 2012). The peculiar lifestyle of freshwater mussels makes them an interesting subject for studies concerning behavior and evolution (Douda et al. 2013, Lopes-Lima et al. 2014). In addition, an understanding of growth rates and lifespan can aid in predicting the effects of habitat change on mussel populations (Haag and Rypel 2011). Solutions can follow two directions: habitat restoration or aquaculture and the inclusion of freshwater mussels in fisheries for artificial propagation (Lopes-Lima et al. 2016).

Stakeholders met in 1995 to discuss the status of freshwater mussel conservation in North America and drafted a National Strategy for the Conservation of Native Freshwater Mussels (Christian and Harris 2008). Some of the primary objectives for the strategy focused on the need for increased coordination and communication between all parties involved in the study and management of mussels, development of knowledge on unionid ecology and impacts against freshwater mussels, importance of funding and public awareness, and finally, the incorporation of new technology and techniques for conservation efforts (Haag and Williams 2014).

To date, across Europe, there have been 28 projects within the LIFE program (with funding totaling over 64 million euros) for the restoration and protection of freshwater mussels and their habitats (Lopes-Lima et al. 2016). In Braganca, Portugal the first international meeting on the biology and conservation of unionids took place in 2012 (Lopes-Lima et al. 2014). Other organizations such as The Nature Conservancy (TNC) and the World Wildlife Fund for Nature (WWF) have proceeded to take action for conservation of freshwater mussels (Haag and Williams 2014). The European CEN (*Comit´e Europ´ een de Normalisation*) has set specific guidelines for conservation of these species *M. margaritifera* throughout Europe (Lopes-Lima et al. 2016). Further efforts are needed from other worldwide conservation agencies, NGOs, educational institutions, and the general public in order to raise awareness of these delicate species.

The only way to combat the decline of freshwater mussels is with a comprehensive understanding, awareness and education, and aggressive action.

Chapter 3.2 Invasive Species

It is estimated that for most countries the number of introduced species ranges between 10^2 and 10^4, though these estimates may be inaccurate as most cases of introduced species are undetected (Lodge 1993). Alien (exotic, non-indigenous) species refer to species which are introduced outside of their native range. They can occur either directly (climatic or tectonic causes) or indirectly (humans) (Vermeij 1996). Alien species are classified as not having any substantial negative impact on their new ecosystem, even if effective populations are established.

Invasive species, however, are alien species that negatively impact the environment, economy, or human health. Invasive species may be classified into four classes: competitors, predators, diseases and parasites, or modifiers of the environment (Strayer 1999). They flourish in their newfound ecosystem to the extent of being pests and reduce biodiversity or adversely affect humanity (Clay 2003). Though there are discrepancies in data, it can be concluded that between 10% and 35% of introduced species are able to establish sustainable populations in their new territory and between 2% and 40% of those species can be classified as invasive (Lodge 1993).

3.2.1 Causes of Invasion and Invasion Success

Throughout history, there has been a myriad of biological invasions that have occurred naturally, but human impact has both altered and accelerated the introduction of new species to foreign ecosystems (Lodge 1993). The systematic process for invasions is as follows: first, the introduction or arrival of the species to a foreign habitat; second, the initial colonization and establishment of the species; and finally, the dispersal and spread into secondary habitats, or integration into the new ecosystem (Vermeij 1996, Sakai et al. 2001).

Species are introduced to foreign ecosystems both directly and indirectly. Some direct causes for introduction are for use in the pet trade, as food sources, a means for pest control, or for general ecological control, while some indirect measures may be from attachment to other introduced species or inadvertent transport by humans (Watters

1997, Sakai et al. 2001). Most alien aquatic vertebrates are not deliberately introduced to a foreign environment, aside from a small number of isolated cases (Mack et al. 2000).

Invasive species tend to be the most successful in ecosystems that have gone through a disturbance (species tend not to dominate a disturbed habitat for long) or have otherwise been altered by humans (Moyle and Light 1996). In fact, humans, through cultivation or husbandry, actually increase the likelihood that introduced species, especially smaller ones who are protected from environmental hazards, become established and prosper in their new environment (Mack et al. 2000).

It is important for the new habitat to be accepting of an introduced species. Some qualities of habitats that are easily invaded are habitats that have climatically equal environments, are prone to early succession, have a low number of native species, are characterized by an absence of predators, incur frequent disturbances, or are susceptible to fragmentation (Lodge 1993). To summarize, invasive species are successful in new habitats if they share the same traits as native species, or, conversely, if they differ from native species and are able to fill an unoccupied niche in the ecosystem (Sakai et al. 2001 ex. Mack 1996).

Introduced species are not guaranteed to be successful in establishing populations in their new ecosystem. After initial introduction, there tends to be a lag time before the species fully implants in the new ecosystem or, alternatively, there is a need for multiple introductions, followed by rapid dispersal; organisms are also able to adapt and grow in their new ecosystem (Mack et al. 2000, Sakai et al. 2001). Assembly theory outlines that invasion success depends on species richness, a large number of invaders, resistance of communities, invasion order, and the shifting of communities to predictable states as a result of previous invasions and extinctions (Moyle and Light 1996).

3.2.2 Characteristics of Invasive Species

Once introduced to an ecosystem, there are notable characteristics of successful invasive species that contribute to sustainable establishments. First, characteristically, invasive species tend to form strong local populations and disperse rapidly (Sakai et al. 2001). A summary of the qualities of potential invasive species are: a high *r* (reproductive capacity), a high dispersal rate, single parent reproduction, genetic variability, phenotype plasticity, a large native range, eurytopic qualities, polyphagous behavior, and human commensalism (Lodge 1993). Other characters associated with highly successful invasive species are the ability to outcompete similarly niched organisms, likelihood of repeated introductions, and being highly valuable to humans (Sakai et al. 2001 ex. Moyle 1986). Newly introduced species might also have specific characteristics that are advantageous in the new ecosystem. For example, the invasive species may be resistant to local predation or a pathogen present in the ecosystem (Sakai et al. 2001).

However, not all introduced species are successful in invading their new ecosystem. Species that are sensitive to predation and climate variances are generally unsuccessful invaders (Lodge 1993). Furthermore, disturbance and disease can prohibit a species from successfully invading a new ecosystem, as summarized by Sakai et al. 2001. In summary, invasive species must have particular qualities, be present in large numbers, and enter the habitat when conditions are optimal (Moyle and Light 1996).

3.2.3 Effects of Invasive Species

Notable Consequences

Perhaps the gravest consequence of invasive species is the effect they have on native organisms and the overall biodiversity of the ecosystem. It was found that the presence of invasive species strongly decrease both the biodiversity and abundance in ecosystems (Gallardo et al. 2015). The effects on the ecosystem can be direct, such as predation, competition, parasitism, herbivory, and mutualism, or indirect, such as affecting the habitat or trophic interactions (Sakai et al. 2001). Other dire consequences can result from the introduction of a pathogen or interference competition (Mack et al. 2000).

Biological effects on the ecosystem can be intense, such as extinctions or severe pest problems, or more subtle like alterations to the food web, local extirpations, and behavioral changes to the native species (Power 1990). Invasive benthic filter-feeders were found to greatly reduce the abundance of planktonic communities, while increasing overall organic matter (Gallardo et al. 2015). A parasitic freshwater mussel species that depends on a native host fish, such as *A. anatina*, is negatively affected by invasive fish that come and outcompete potential host fish (Douda et al. 2013). Invasive species may also enter an ecosystem and mate with native species, effectively making them extinct (Mack et al. 2000). It is believed that extinctions from invasions are characteristic of species inhabiting islands or lakes, rather than seas or large land masses (Vermeij 1996). Due to the fact that some introductions have caused extinctions of native species, it can be assumed that a percentage of future introductions will lead to a further reductions of biodiversity (Lodge 1993).

A second consequence of invasive species is economic damage. The resulting alterations to the ecosystem can lend to severe economic hardships, such as losses in crops, fisheries, forestry, and grazing capacity (Mack et al. 2000). Globally, invasive species can cost in the range of millions and even up to billions of dollars, as researched by Sakai et al. 2001. It is reported that the invasive zebra mussels (*Dreissena polymorpha*) (Pallas 1771) have cost in the range of 2-4 billion dollars in biofouling mitigation costs in the United States Great Lakes region alone (Lodge 1993). The economic losses can come from reduced output or from money spent on control or eradication of the invasive species (Mack et al. 2000). Finally, invasive species may also adversely affect human health. For example, these species may alter ecological process or services such as gas and water vapor fluxes (Lodge 1993).

Future Action

To combat the effects of invasive species, governments can impose quarantines and restrictions on the transport of exotic organisms (Mack et al. 2000). This requires an understanding of the species in question along with prior knowledge of the proposed introduction. Furthermore, attempts can be made on species eradication, though there have mixed results to date and eradication can produce severe effects on the ecosystem (Mack et al. 2000). For example, eradication could lead to an increase in other organisms or otherwise damage the trophic interactions of species.

A last resort method of controlling invasive species is by directly removing the species. The use of chemicals, hunting, or trapping can help rid an ecosystem of invasive species, but can have negative impacts on the ecosystem (Mack et al. 2000). For example, the attempts to remove feral pigs (*Sus Scrofa*) (Linnaeus 1758) from the Hawaiian Islands included hunting, trapping, and poisoning; the latter of which induced vomiting and suffering in the pigs (Cruz et al. 2005). Moreover, the poisoning of rats (*Rattus rattus*) (Linnaeus 1758) as a means for control led to predators being exposed to the effects of secondary poisoning (Ruffino et al. 2015).

As with most poorly understood phenomena, it is important to continually research and provide education on the topic of invasive species. Understanding the traits of potential invaders and the vulnerability of communities to invasion can help predict and mitigate the effects of invasive species (Mack et al. 2000). Studies in phylogenic structure, genetic diversity, and evolution of the invasive species can provide some answers for understanding of the dynamics of invasive species and their communities (Sakai et al. 2001). In order to fully understand and create policies against biological invasions, we need to fully understand the epidemiology of invasions, estimate of the cost of such invasions, and finally, create public and government awareness of invasions and their consequences (Mack et al. 2000).

Chapter 3.3 *Anodonta woodiana*

A. woodiana, referred to commonly as the Chinese pond mussel, is a freshwater mussel species native to East and Southeast Asia (Watters 1997). Other common names of the species are the swan mussel or the Chinese huge mussel (Sarkany-Kiss et al. 2000). This large mussel is classified as part of the *Anodonta* genus, though some authors argue that according to the latest taxonomy, this species should more aptly be assigned to the genus *Sinanodonta* (Cappelletti et al. 2009), while others claim that the species should remain in the genus *Anodonta* (Nagel and Badino 2001). For the purposes of the diploma thesis research, the Chinese pond mussel will be referred to as *Anodonta woodiana* (*A. woodiana*) throughout the text.

3.3.1 Biology and Ecology

Anatomy and Reproduction

A. woodiana is one of the most sizable mussels of the unionid family, reaching a shell length 12 to 20 centimeters (Kraszewski and Zdanowski 2007). In Central Poland, it is believed that theoretically the shell can reach lengths up to 23 centimeters and weights of up to 850 grams (Afanasjev et al. 2001). As an example, during field experiments in Central Poland, the average size of observed individuals was 15.4 centimeters in length and 9.3 centimeters in width, while the average weight was 336 grams and 96 grams for total and soft tissue, respectively (Hliwa et al. 2015). Annual growth rates were found to vary between 2.5-3.4 cm in younger individuals, while rarely exceeding 2 cm in older mussels (Afanasjev et al. 2001). Biomass has reached 25 kg/m^2 containing 60 individuals in the Konin lakes system (Kraszewski and Zdanowski 2007). *A. woodiana* is anatomically and behaviorally similar to other freshwater mussels. For instance, it was observed that the filtration capabilities of *A. woodiana* do not differ to that of native freshwater mussel species in Korea (Kim, et al. 2011)

As with other members of the Unionoidea superfamily, *A. woodiana* reproduce by releasing glochidia, which then attach to a suitable fish host. It is inferred that *A. woodiana* can be classified as an accidental or abnormal hermaphrodite, as species sampled in Central Poland yielded a sex ratio close to 1:1 (Hliwa et al. 2015). For this species, reproduction occurs in the fall and the glochidia are released in summer to begin a stage of parasitism that varies due to environmental conditions and water temperature (Afanasjev, et al. 2001). The highest density of released glochidia occurs in the months of June and July and, in contrast to other freshwater mussel species, glochidia may be produced 2-3 times per year (Sarkany-Kiss et al. 2000).

There are varying reports as to how long the parasitic phase of *A. woodiana* lasts, though it is dependent on several factors such as temperature and other environmental conditions, and has been documented as little as a few days (Douda et al. 2011), 5-15 days (Sarkany-Kiss et al. 2000), or a wider range of 12-80 days (Afanasjev et al. 2001). Individuals live up to approximately 15 years in the wild, though most average between 12-14 years (Sarkany-Kiss et al. 2000).

Habitat

A. woodiana is native to two voluminous rivers in China – the Yangtze River and Amur River, where it was primarily located prior to its expansion in directions southward and westward (Kraszewski 2007). Characteristic to freshwater mussels, *A. woodiana* inhabit freshwater ecosystems, such as rivers, lakes, streams, pond, and reservoirs. It is most commonly seen in lowland freshwaters, such as ponds, oxbow lakes, canals located on floodplains, rivers which are slowly to moderately moving (0.05 to 0.3 m s^{-1}), and muddy riverbeds, commonly where other native unionids are present (Sarkany-Kiss et al. 2000, Kraszewski and Zdanowski 2007, Beran 2008). The species has been observed living concurrently with native mussel species, such as *Unio pictorum* (Linnaeus 1758) (painter's mussel), *Unio crassus* (Retzius 1788) (thick-shelled river mussel), *Unio tumidus* (Retzius 1788) (swollen river mussel), *Pseudanodonta complanata* (Rossmassler 1835) (depressed river mussel), *A. anatina*, and other mollusk species in silt-clay substrate at lowland areas of the Danube River (Paunovic et al. 2006). This is uniform with general freshwater mussel habitat requirements and overall preference for softer or fine-grained substrates for burrowing and lodging, with the ideal depth for *A. woodiana* being between 1.0 and 2.5 meters, where the species forms aggregations (Kraszewski and Zdanowski 2007).

A. woodiana live in temperatures between 10 and 30 degrees Celsius with a higher density occurring at higher temperatures, however, when the temperature increases above this threshold (30 degrees Celsius), the filtration process halts and eventually the mussel may perish (Kraszewski and Zdanowski 2007 ex. Afanasjev 1996). As the waters of Northern Europe are quite frigid, Kraszewski (2007) found that *A. woodiana* favor and perhaps thrive in heated water habitats, such as fishponds, where they were able to form viable populations. However, it is now believed that the thermal qualities of the water are not as influential as once thought regarding the sustainability of *A. woodiana* populations, with successful communities existing in colder bodies of water, such as those found in Sweden and subalpine Italy (Douda et al. 2011).

3.3.2 Role as an Invasive Species

Introduction to Europe

The first probable introduction of *A. woodiana* in Europe is hypothesized to have occurred at the Cefa Fishponds, Bihor County, Romania in 1959-1960, attached to the gills of young grass carp (*Ctenopharyn godonidella*) (Valenciennes 1844) (Watters 1997 ex. Sarkany-Kiss 1986, Sarkany-Kiss et al. 2000). However, the first *officially* recorded individual occurred in Romania in 1979 (Watters 1997, Beran 2008 ex. Sarkany-Kiss 1986). From that point, these invasive freshwater mussels spread throughout freshwater ecosystems on the continent, located in the countries of France, Hungary, Austria, Slovakia, Poland, Germany, the Czech Republic and the former Yugoslavia (Watters 1997; Kraszewski 2007). Moreover, it has been confirmed that this invasive species has penetrated freshwater ecosystems in Costa Rica, the Dominican Republic, and several islands in Indonesia, while it has been inferred that it is only time before *A. woodiana* penetrates freshwater ecosystems in the United States (Watters 1997).

The expansion of *A. woodiana* predominantly occurred through the introduction of alien fish species to European freshwaters, such as silver carp *(Hypophthalamichtlys molitrix)* (Valenciennes 1844), bighead carp *(Aristichthys nobilis)* (Richardson 1845), and grass carp to combat against the eutrophication in European freshwater ecosystems (Watters 1997, Paunovic et al. 2006, Kraszewski 2007, Cappelletti et al. 2009). The mussels were attached as glochidia to juvenile fish of these carp species, which were collected to be used as stock fish (Watters 1997). Therefore, the introduction of *A. woodiana* was not direct; it occurred accidently as an inadvertent companion to the imported fish species (Cappelletti et al. 2009). Other reasons for the introductions of these exotic fish species were for use as food, as a means to control either aquatic vegetation or mosquitos, for maintenance of aquaculture fish ponds, or as aquatic pets (Watters 1997). Currently *A. woodiana* is being exploited for its pearl production, which has also increased the spread of this invasive species (Beran 2008).

Success as an Invader

One reason as to why the introduction of *A. woodiana* to Europe has been so successful is its long gestation period (up to several weeks) which allows the infected fish to be transported long distances (Watters 1997). It is consequently visually undetectable during the time the alien fish are introduced and embedded into the foreign ecosystems. *A. woodiana* has been confirmed to be a host generalist, which means it has the ability to reproduce using a large variety of host fish (both native and invasive); and this has been instrumental to its success as an invader (Douda et al. 2011).

In addition, *A. woodiana* has been known to cope with a large range of environmental conditions, further cementing its success as an invasive species (Corsi et al. 2007, Douda et al. 2011) For instance, the species has been found to lack substrate-specificity, which is characteristic of other freshwater mussels (Corsi et al. 2007). Furthermore, the species is able to withstand and function in polluted or oxygen-deficient ecosystems, as holds true with the majority of the *Anodonta* habitat generalists (Sarkany-Kiss et al. 2000). *A. woodiana* has even shown a quick recovery time after exposure to insecticides and greater resistance against environmental contamination in general (Corsi et al. 2007). Though glochidia maturation occurred more intensely in warmer waters, the species is not limited to these ecosystems (Douda et al. 2011).

Due to its size, resistance, growth rate, and reproductive biology, *A. woodiana* has spread rapidly and overtaken the native mussel populations in many of the countries it has entered (Sarkany-Kiss et al. 2000, Corsi et al. 2007, Kraszewski 2007, Douda et al. 2011). In fact, this freshwater mussel species has become or has the potential to become a dominant species among all unionids; this warrants intensified efforts for conservation of native mussel species throughout its invaded territories (Watters 1997, Sarkany-Kiss et al. 2000, Pou-Rovira et al. 2009). For example, it has become the dominant species of the Cris/Koros river basin, located both in Romania and Hungary (Sarkany-Kiss 1997, Sarkany-Kiss et al. 2000). Since its first recording in Lake Balaton in Hungary in 2006, this invasive species has reached 50-80% of the total unionid biomass (Benkő-Kiss et al. 2013). In lowland rivers in Serbia, *A. woodiana* outnumbers native mussel species by a ratio of 2:1 (Paunovic et al. 2006). In Italy, among channels with soft substrate and a high trophic level, *A. woodiana* has become the dominant species and, in some cases, has competently replaced native mussels, such as *A. anatina* (Cappelletti et al. 2009). The species has already entered and has the ability to spread throughout inter-basin waterways in the Iberian peninsula (Pou-Rovira et al. 2009). Recently, a single individual of *A. woodiana* was found in Lake Sasko in Montenegro, making it the first of its species to invade the Central Mediterranean Subarea (according to FAO) or the southern Adriatic region (Tomović et al. 2013). Offering some optimism, ecosystems with temperature variations can lead to segregated mussel communities, due to the preference of warmer waters for *A. woodiana* (Kraszewski and Zdanowski 2007).

Effects on Native Mussels and Ecosystems

A. woodiana can directly compete with native mussels for resources, namely food and space, causing the native mussel populations to decline (Watters 1997). In addition to competing with native mussels for suitable hosts, *A. woodiana*, as a host generalist, can attach to most host fish prior to native mussels, causing a fish immune response and preventing the development of native juveniles (Douda et al. 2011). The ability of *A. woodiana* to attach to both invasive and native fish is problematic and can lead to extirpations of native freshwater mussel species (Watters 1997). Similarly, the presence of invasive fish species can lead to a competition to native fish populations, which are needed to host the glochidia of host-specific native mussels; in other words, in freshwater ecosystems containing a majority of invasive fish, the glochidia or *A. woodiana* can attach to the invasive fish, while native mussels now have only a limited native fish population on which to develop (Watters 1997, Pou-Rovira et al. 2009).

Due to its immense size and expanding biomass, the filtrating capacity of *A. woodiana* can have drastic effects on aquatic ecosystems (Vaughn and Hakenkamp 2001, Kraszewski and Zdanowski 2007). Furthermore, the presence of this species can drastically change the benthic community composition and affect the ecosystem equilibrium (Strayer 1999, Pou-Rovira et al. 2009).

It is speculated that through continued non-native fish introductions to water bodies or fish ponds, *A. woodiana* will be prevalent in additional ecosystems and continue to expand its already vast range (Kraszewski 2007). From current data, extrapolation signifies that *A. woodiana* will come to occupy the ranges of native species and become the dominant unionid species (Sarkany-Kiss et al. 2000). Due to the commercial importance of its host fish, its resilience, and limited detectability during transport, *A. woodiana* will likely continue spreading to additional countries throughout the world (Watters 1997). This offers a bleak outlook on native mussel species in the invaded ecosystems, creating a need for awareness and conservation.

3.3.3 Status in the Czech Republic

As mentioned, *A. woodiana* has penetrated European waters from the introduction of alien fish species and quickly spread throughout Europe (Watters 1997, Sarkany-Kiss et al. 2000, Beran 2008). The species was first recorded in the Czech Republic in 1996 in the Dyje River basin in southern Moravia (Beran 2008 ex. Beran 2007). This transpired in less than twenty years after the first recorded official occurrence in Romania, indicating the ability for rapid expansion of the species. From the first recorded individual in the Czech Republic in 1996, this species has become both more frequent and more widespread, reaching the Bohemia region in the western Czech Republic approximately eight years later in 2004 (Beran 2008).

Much as in other central European regions, *A. woodiana* prefers slow moving waters and muddy substrates in the Czech Republic at average altitudes of 158-205 meters (Beran 2008). This has led to competition with native mussel species in the Czech Republic. In terms of overall biomass, this species will almost surely become the dominant species throughout Central and Eastern Europe (Sarkany-Kiss et al. 2000). A conservative estimate is that *A. woodiana* will be able to encounter a suitable host fish in over 75 percent of freshwater habitats in the Czech Republic (Douda et al. 2011). The presence of *A. woodiana* puts native freshwater mussel species at risk for decline or possibly extirpation.

Chapter 3.4 Host-Parasite Relationships

A parasite is an organism living on or in another organism, known as a host. The parasite depends on the host for food, reproduction, or simply survival, and causes some degree of harm to the host. In some cases, when the parasite has the ability to cause death to the host, it is termed parasitoid.

3.4.1 Biology and Ecology

Parasites originated as independent species and evolved into parasitism over time by first coming into contact with a host and then developing some pre-adaptation, and eventually, a stronger fitness when attached to the host; the species can reproduce and flourish better with the host than independently (Poulin 2008). Once the bond (a relationship consisting of trophic, ethological, and immunological interactions) between host and parasite is formed, it is exceedingly difficult to break (Dujardin and Dei-Cas 1999). It is assumed that all hosts suffer some alteration to their biology and lifecycle, though the exact magnitude varies, depending on both the hosts and parasites (Anderson and May 1978).

The relationship of host and parasite needs to be understood in terms of a couple, a whole, which requires energy to separate (Dujardin and Dei-Cas 1999). This dynamic of the relationship is difficult to quantify, that for the duration of a given host-parasite relationship, the two species are grouped together as one complete organism. Perhaps surprisingly, in most cases an organism is a host to several parasitic species simultaneously (Poulin 2008).

The concept of reciprocal selection dictates that the strongest hosts would be the most resistant to parasites, while the strongest parasites would be able to infest the strongest hosts (Thompson 1994). Thusly, the relative fitness of both the parasite and the host are influential in the relationship and the strongest pairs would have the strongest bonds.

3.4.2 Host Specialists vs. Host Generalists (Host-Switching)

Host specificity means that a parasitic species is compatible with one specific host species. Therefore, theoretically, the parasite would not be able to infect any other species other than the host of which they have already established a relationship. Under this theory, when speciation of a host and parasite occurs simultaneously, once a host is absent (either extinct or locally extinct), the parasite will cease to exist (Hoberg and Brooks 2008).

However, current theories of host-parasite relationships are quite the contrary. Host generalists undergo a phenomenon termed host-switching, which is now not only accepted, but quite prevalent among parasitic species, occurring on a large range of temporal and spatial scales (Hoberg and Brooks 2008). For freshwater mussels, being a host generalist offers a competitive advantage over a host specialist, as the individuals can attach to a variety of host fish and are much more likely to survive into their adult stage (Bauer and Wachtler 2001b).

There are some specific attributes and underlying assumptions necessary for host-switching. Host-switching occurs in cyclic episodes, in particular when parasites colonize a new geographical area or there is a disruption to the ecosystem, parasites may favor different hosts (Hoberg and Brooks 2008). A large number of parasites are able to infect multiple hosts, depending on community composition and availability of a diverse number of hosts that come into contact with each other (Dunn and Hatcher 2015). It has been documented that expansion leads to a variety of host associations, as derived from the taxon pulse hypothesis (TPH), which basically states that organisms go through varying patterns of isolation and expansion (Hoberg and Brooks 2008).

3.4.3 Parasites and Invasive Species

Parasites can either accompany an invasive host species to the new ecosystem or release from their host prior to its invasion (Dunn and Hatcher 2015). If a release occurs, it allows invasive species to be successful in their new ecosystems as they are now devoid of parasites (Clay 2003). In fact, the number of parasites found on native species in an ecosystem is nearly double the amount of parasites found on introduced species (Torchin et al. 2003). This would seemingly allow the introduced hosts to flourish independently of their parasites and thrive in their new ecosystem.

However, as in the case with *A. woodiana*, it is possible for parasitic species to accompany their hosts to the new ecosystem (Clay 2003). Invasive parasites have highly impactful effects on the community or the ecosystem and can adversely affect tropic levels, biodiversity, the food web, interactions, and overall ecosystem function in their adopted habitat (Dunn and Hatcher 2015). Parasites who have invaded a new ecosystem with their hosts have been at least as successful in their new ecosystem as they had been in their native ecosystem (Torchin et al. 2003).

Chapter 3.5 Relationship of Fish and Freshwater Mussels

One of the most compelling host-parasite relationships is that of fish and freshwater mussels. The focus of the next two subchapters is on the relationship between host fish and parasitic freshwater mussels which produce glochidia (of the superfamily Unionoidea) and the role of fish immunology in this relationship. As freshwater mussels are practically immobile, it is believed that the need for host fish, and, thusly, this parasitic relationship was developed in order to disperse mussels throughout the freshwater ecosystem (Wachtler et al. 2001). While the host-parasite relationship brings about benefits to the developing freshwater mussel, generally, no disease or harm is inflicted upon the host fish (Dodd et al. 2006). Though, unique to this host-parasite relationship is that the generation time of the parasite exceeds that of the host by a factor of 10 or 20 (Bauer 2001c), a phenomenon that has been stable for at least 60 million years (Bauer and Wachtler 2001b ex. Bauer 1997).

3.5.1 Biology and Ecology

Dispersal

After an incubation period, or brooding time, within the outer gills of the mussel (the marsupium), glochidia are released into the surrounding water where they promptly attach to the gills or fins of host fish in order to be encysted, protected, and nourished (Karplus 2014). The dispersal of freshwater mussels is directly related to the presence of mobile host fish, meaning the more mobile host fish present in an ecosystem, the larger dispersal area of freshwater mussels (Schwalb et al. 2015).

As discussed, there is substantial variance between both the size and morphology of glochidia, and number of glochidia dispersed among different mussel species. As a rule, there tends to be a trade-off, in that the species with the smallest glochida disperse the most glochidia, while the species with the largest glochidia disperse the fewest (Bauer 1994). Once dispersed into the aquatic ecosystem, the parent mussel has the ability to attract potential hosts for their glochidia by exhibiting certain behaviors or placing lures to increase the likelihood of attachment to a host fish (Rogers-Lowery et al. 2007).

Host Availability

The pairing of host and parasite, in the case of freshwater mussels and fish species, is first determined by proximity and second, by immunological compatibility (Chapter 3.6) (Douda et al. 2014 ex. Rogers and Dimock 2004). It has been confirmed that in the USA, there exists a positive correlation between species richness of freshwater mussels and fish, thus both species thrive in densely populated ecosystems (Vaughn and Taylor 1998). Given a larger number of host fish, the likelihood for a dense freshwater mussel population increases.

Habitat and environmental conditions strongly influence the special distribution of both freshwater mussels and host fish (Vaughn and Taylor 1998). For instance, the distribution and movement patterns of host fish have directly affected the quantity of freshwater mussels, while the absence of host fish from, for example, the alteration of flow regimes by way of dams, have significantly deteriorated mussel populations (Watters 1996). However, it has been determined through induced infections, that cohabitation of species is not essential for a successful host-parasite relationship (Neves et al. 1985).

Host Selection

Freshwater mussels can either be host specialists or host generalists, with a capability to parasite a wide variety of hosts without preference (Karplus 2014). Freshwater mussels are compatible with a limited number of host fish species, ranging from one (for host-specific species) to 37 species in total (Dodd et al. 2006 ex. Watters 1994). A number of host generalists have been confirmed to infect over thirty different species (Strayer 2008, Douda et al. 2011). However, it is important to note that even host generalists, such as *A. anatina*, have a significantly lower success rate infecting alien or invasive fish species, compared to fish that are native to its home range (Douda et al. 2013).

Host generalists and host specialists have different methods for attracting host fish. Host specific species use specialized lures to attract and attach to their hosts: mussel flap lures, pulsating papillae of the mantle margins, or the superconglutinate (Karplus 2014). Host generalists detach their glochidia in mucus strands, which are then picked up by proximate compatible fish (Karplus 2014).

Traditionally, fish and freshwater mussel relationships are examined on the species level, however, there is existing evidence to support varying host compatibility among isolated populations within the same species (Douda et al. 2014). Furthermore, it is advisable to refer to host fish species as primary or marginal, rather than suitable or unsuitable due to the complexity of the host-fish relationships (Douda et al. 2012).

Attachment

There are specific qualities of the glochidial anatomy of freshwater mussels that makes the process of attaching to host fish possible. If the presence of gills or fins of host fish stimulate the tiny chimney-like hairs that occur between the shell valves, the glochidium, which may be hooked or hookless, will forcefully close, pinching the tissue of the host fish between the valves (Arey 1932).

Hookless glochidia of freshwater mussel species, which are elongated and round, can attach only to the gills of host fish, and are significantly smaller than their hooked counterparts (Arey 1932, Bauer 1994, Wachtler et al. 2001, Reis et al. 2014). For hookless glochidia attachment, the glochidia valves cut through the gill epithelium and connective tissue (stronger attachment indicates more tissue), cutting through softer tissue while pinching and constricting tougher tissue into an hourglass shape (Arey 1932).

Alternatively, the top and bottom shell valves of some glochidia are hooked (triangular shape) and contain a larval thread which functions as a tool for slowing the sinking action in the water, as well as for creating initial contact with the fish (Wachtler et al. 2001, Karplus 2014). The glochida use these aforementioned hooks to attach themselves to host fish gills much in the same manner as hookless glochidia, or due to the strength of the hooks, the fins may also be pierced and attachment can occur there (Arey 1932, Neves et al. 1985, Wachtler et al. 2001). Fin attachment may lie wholly within the epithelium, piercing host tissue and utilizing interlocked spines to confirm attachment (Arey 1932).

Once attached, each glochidium maintains its grasp using its two valves along with its adductor muscle - stimulation of chemosensory hairs located with the soft body of the glochidium causes vibrations of the adductor muscle, which allows the grip to become tighter and tighter (Wachtler et al. 2001, Karplus 2014). After attachment to the host fish, this adductor muscle remains contracted for the duration of the coupling period (Wachtler et al. 2001). This attachment process occurs immediately, with incision and compression occurring in less than a second (Arey 1932).

After initial attachment, there occurs a period when the contiguous cellular host fish tissue grows over the glochidium (Arey 1932). In other words, upon attachment, the glochidia are enveloped in an epithelial cyst, composed of the host fish tissue (Rogers-Lowery et al. 2007, Reis et al. 2014). The time for cyst formation takes a few hours, depending on conditions; for instance, lower temperatures slow down the encystation process (Arey 1932).

After a period of two of three days, the irregularly shaped cyst becomes smooth, thin, and uniform in shape (Arey 1932). The attachment commonly lasts for several weeks, though variables such as the host species, temperature, and aquatic conditions may significantly alter the length of this period (Watters and Dee 1999). As a generalization, the attachment can last from a period of three days up to ten months, with approximately twenty days being the most common length of attachment (Wachtler et al. 2001). For example, on salmonid species, it has been observed that the attachment period can range between one and ten months (Geist and Kuehn 2008). Glochidia attached to the tips of gill filaments are retained the longest, most likely due to the limited availability of nutrients and oxygen from that position (Arey 1932). As a rule, the glochidial size affects the length of time spent on the host, with the largest glochidia requiring the shortest time period and the smallest glochidia requiring the longest amount of time on their host fish (Wachtler et al. 2001). At the end of the attachment, the juvenile mussel pushes through the cyst, using its foot to break the cell wall until successfully liberated (Arey 1932).

Formed Relationships

In order to form a successful host fish and freshwater mussel relationship, three factors are required: initial contact between glochidia and host fish; suitability of the host for attachment and metamorphosis of the glochidia; and a strong resistance of the glochidia to host responses (Neves et al. 1985). Each year a highly voluminous amount of glochida are released, so the first factor is dependent on the ecosystem conditions, fish microhabitat preferences, fish behavior, and finally, number of fish present. If glochidia fail to initially attach to a host fish, they will eventually sink to the substrate, where opportunities for attachment at that point are highly unlikely and the glochidia perishes (Jansen et al. 2001). Regarding the second and third factors, unsuccessful infestations can be reduced to the premise that either the host fish contains insufficient chemical or nutritional requirements for the metamorphosis or from direct immune system rejection from the host fish (Neves et al. 1985). In these cases, glochidia may fail to be encysted or be sloughed off prior to transformation taking place (Jansen et al. 2001). Or, the failure may occur from the glochidia being abnormally encysted prior to detachment (Arey 1932, Rogers-Lowery et al. 2007).

Overall, the mortality of glochidia during the parasitic phase is exceedingly high, as estimates state that out of every billion glochidia produced, 10 (*M. margaritifera*) to

18,000 (*Pyganodon grandis* (giant floater mussel) (Say 1824)) survive to the age of 1 or 2 years (Jansen et al. 2001). Details regarding host fish immunity to glochidia will be expanded on in the subsequent subchapter (3.6). In summary, without the presence of host fish, compatibility of host and parasite, or lack of immunological effects, no fish and freshwater mussel relationship can be established.

3.5.2 Benefits

Benefits to Freshwater Mussels

Encystation on host fish provide protection of the glochida during the parasitic larva stage from predation and the elements of the aquatic ecosystem (Arey 1932, Wachtler et al. 2001). Additionally, during the period that the glochidia of freshwater mussels are encysted on their host fish (days to months), they obtain nutrients to aid in their metamorphosis (Arey 1932, Dodd et al. 2006) . The sustenance is absorbed from the blood of the host fish, likely via apical microvilli, which are located within the mantel and stored in granules throughout the parasitic phase of the mussel (O'Connell and Neves 1999, Wachtler et al. 2001). Moreover, together with the host tissue that is ingested during attachment, degenerating larval adductor mussel provides nourishment during transformation (Arey 1932). However, perhaps the greatest benefit to freshwater mussels is phoretic: the fact that it is able to be dispersed to locations upstream (Barnhart et al. 2008, Douda et al. 2011).

In general, the attached glochidia develop a complex internal structure, while externally exhibiting no change (Arey 1932). However, a small portion of glochidia display morphological growth during their parasitic stage, for instance, in *Margaritifera* (Wachtler et al. 2001). Moreover, there is a significant increase in carbon content (Wachtler et al. 2001) and lipid reserves in all freshwater mussels (Douda 2015). During this fixation time, glochidia develop their internal organs and begin the process of reaching their adult form (Douda et al. 2012). The primary changes that occur during the maturation are the replacement of adductor muscles used for attachment to the host fish with newly formed adductor muscles that remain with the mussel throughout its life, development of a mantel for food uptake and shell secretion, development of a digestive system, formation of the nervous system, appearance of the foot, and initial stages of paired gill apparatus (Wachtler et al. 2001).

Benefits to Fish

As a means to facilitate growth and development in fish, freshwater mussels may be able to provide a temporary shelter for some fish species when they are young or before they migrate to their adult spawning areas (Karplus 2014). This phenomenon serves to protect the fish species until they are developed enough to venture into the open water unprotected. In marine environments, the role of bivalves as a shelter provider may be prolonged, as some marine fish species may use the mussels as a habitat for their entire lives, from the juvenile to adult stages (Karplus 2014). Freshwater mussels in the substrate provide habitat heterogeneity in the form of cover and for foraging substrata for young fish (Ziuganov et al. 1994).

Bitterling fish (*Rhodeus amarus)* (Bloch 1782), introduced to Europe from East Asia, foster their eggs in mussel gills and deny hosting the glochidia from native European mussels (Reichard et al. 2012). Freshwater mussels serve as an incubator for the fish eggs, which eliminates the need for the fish parental care (Karplus 2014). This relationship is not exactly beneficial for the mussels. Though the bitterling embryos do not gain any nutritional advantages from the mussels, they can inhibit the growth of mussels by competing with them for oxygen, inflicting damage on their gills, and disrupting water circulation (Reichard et al. 2007).

However, the presence of *A. woodiana* once again contradicts matters. Bitterling are unable to use *A. woodiana* as hosts, but are only capable of the inverse - themselves hosting the glochidia of the invasive species (Reichard et al. 2012). This not only affects the status of the bitterling populations, but will also help to increase the presence of *A. woodiana* while causing stagnation of native freshwater mussel development.

Chapter 3.6 Fish Immunology

Fish immunology determines whether host fish are acceptant or resistant to parasitic attachment of glochidia from freshwater mussels (Arey 1932, O’Connell and Neves 1999). Fish immunology influences the reproductive success of a given freshwater mussel species with a potential host fish. In other words, if a glochidium is rejected by a host fish, it will not develop into a live juvenile mussel, and therefore not begin its adult life stage. Therefore, an understanding of the immunological response of host fish to glochidia is critical for freshwater mussel conservation.

3.6.1 Basic Fish Immunology

Fish immunology is the study of how the immune system of fish is protected from factors such as viruses, bacteria, and parasites (Lieschke and Trede 2009). Inherently, fish possess a mucosa that covers the entire external surface, gills, and digestive tract of fish and is immunologically active (Rogers-Lowery et al. 2007). The study of fish immunology includes both innate immunity (which is highly complex in comparison to mammals) and adaptive immunity (Lieschke and Trede 2009). Innate immunity refers to general defense mechanisms that develop immediately after the onset of a foreign substance and are continuously present on the fish. On the other hand, adaptive immunity arises as a response to a specific antigen. In order to successfully encyst on a host fish, glochidia must initially bypass the innate immune system of their potential hosts before adaptive immunity has the potential to take effect (Barnhart et al. 2008).

Innate Immunity

Innate immunity in host fish provides an initial boundary against infection from glochidia. The initial defense involves secretions composed of antimicrobial compounds, including lectins, complement proteins, lysozymes, and antimicrobial peptides (Lieschke and Trede 2009). It is the innate immunity that initially determines whether a fish is a suitable host for a particular glochidium (Barnhart et al. 2008). The inability of glochidia to maintain an initial attachment to a non-host, or incompatible fish, can be attributed to innate immunity, and is the reason why the potential parasites are sloughed within only a few days of their attempted attachment (Dodd et al. 2006, Douda et al. 2012). To illustrate this point, using an immunosuppressant, researchers were able to successfully infest non-host fish species with glochidia after innate immunity was bypassed (Kirk and Layzer 1997).

Adaptive Immunity

Adaptive immunity is generally developed after one or more infections, both in an artificial setting and in nature, and is ignited after an antigen evades the innate immune system (Dodd et al. 2006). Therefore, it is the adaptive immunity that inhibits or prevents secondary glochidial infections on host fish. The adaptive immune system is composed of lymphocytes in the form of cell-mediated immunity, T-cells, or humoral immunity, B-cells (Lieschke and Trede 2009). The defense factors of humoral B-cells are produced by fish in varying degrees after being infected with glochidia, and include antibodies, lysozymes, complements, interferons, C-reactive proteins, transferrins, and lectins, among other substances (O'Connell and Neves 1999 ex. Yano 1996). Cell-mediated T-cells are comparatively poorly understood, but also have a role as a potential defense mechanism against glochidia (Dodd et al. 2006).

3.6.2 Fish Resistance

Several studies have found that previously infected host fish contain specific antibodies against glochidial infection (Dodd et al. 2006). In other words, the serum of immune host fish species contains anti-glochidia antibodies, whereas the serum of naïve host fish species is absent of such antibodies, exemplifying the humoral immunity (Rogers-Lowery et al. 2007). The production of antibodies increases with both maturity, and with rising temperatures for certain fish species (O'Connell and Neves 1999 ex. Manning and Mughal 1985). It was observed that, while there is a positive correlation between fish size and number of glochidia hosted during the first infection, there exists a negative correlation between fish size and number of glochidia in each subsequent infection (Rogers and Dimock 2003). This strongly suggests that larger fish develop enhanced adaptive immunity after primary glochidial infections.

The response time of antibody formation for resistance varies among species of fish (Rogers-Lowery et al. 2007). To illustrate, several fish species, including salmon and trout, have developed specific antibodies after being infected with glochidia, which make subsequent infections difficult or highly unlikely (O'Connell and Neves 1999). Lymphocytes have been found around encysted glochidia in coho salmon (*Oncorhynchus kisutch*) (Walbaum 1792) and chinook salmon (*Oncorhynchus tshawytscha*) (Walbaum 1792) throughout their ranges (Meyers et al. 2016). Generally speaking, acquired immunity for fish against a specific parasite can persist for months or years, or in some cases, perhaps even permanently (Dodd et al. 2006).

3.6.3 Conservation Implications

The comprehensive understanding of fish immunology is mandatory for conservation efforts. Information of the immunological capabilities of host fish could benefit endangered or threatened freshwater mussel species (O'Connell and Neves 1999). Specifically, full awareness of the duration, frequency, and mechanisms of adaptive immunity could aid in the propagation of endangered freshwater mussel species worldwide (Dodd et al. 2006). Understanding these time frames can lead to efficient infections of host fish in laboratory settings and contribute to the flourish of freshwater mussel species.

The adaptive immunity of host fish on the other hand, is worrisome, as it could create competition amongst freshwater mussel species for naïve host fish (Dodd et al. 2005). Fish immunity does not stand alone as a hurdle for freshwater mussels to overcome. For instance, other factors, such as migration patterns and microhabitat preferences also influence the status of freshwater mussel species (Douda et al. 2012).

Chapter 4: Experiment

The purpose of the diploma thesis experiment is to test the effects of an invasive freshwater mussel on the reproduction of native freshwater mussels in the Czech Republic by examining the role of host fish immunity. The experimental null hypothesis states that there is no difference in the metamorphosis success rates of *A. anatina* on *S. cephalus* primed with *A. woodiana* compared to naïve host fish. Thusly, the number of live juveniles developed on host fish should be relatively equal between naïve and non-naïve individuals. In other words, host fish adaptive immunity will not alter the transformation success rate of *A. anatina* on *S. cephalus*.

Chapter 4.1 Methodology

4.1.1 Site Description

Collection of Anodonta woodiana

Collection of gravid *A. woodiana* occurred during the month of August 2015 from locations in the southern Moravian region of the Czech Republic, at the GPS coordinates: N 48º46’45”; E 17º00’60”. Skilled researchers, wearing dry suits, collected samples of the freshwater mussels by hand from the muddy substrate of slow-moving water bodies and placed them into collection buckets. Figure 1 shows collected *A. woodiana* on the banks of the water body where collection took place. The individuals were then gently opened to determine whether or not the individual was a gravid female. Suitable individuals were placed back into the collection bucket, while males and non-gravid females were returned to the substrate, ensuring that they were not harmed during the collection process.

Figure 1. Collection of *A. woodiana* on shore

Collection of Anodonta anatina

A. anatina specimens were collected from a slow-moving section of the Vltava River, on the outskirts of Prague in the Czech Republic at coordinates, 49.8565517 N and 14.70000211 E. The collection was completed utilizing the same process and equipment as the collection of *A. woodiana*. Figure 2 is a close-up photograph of an *A. anatina* individual in the laboratory at the onset of the laboratory portion of the experiment (after collection and transport to the facilities). All samples were collected on November 6, 2015, three days before the initialization of the experiment.

Figure 2. *A. anatina* sample in laboratory

4.1.2 Transfer and Storage

The selected individuals were transported from the collection site to the storage facility using large coolers. The coolers were filled with water from the collection site, so as to ensure the freshwater mussels were kept submerged during the duration of transport. These containers were sealed shut and carefully loaded into the transport vehicle to shield against spillage and prevent any damage to the samples. Both freshwater mussel samples were individually brought to the research site at the Czech University of Life Sciences (CULS) in Prague and placed into large 130x130x57 cm tubs containing aerated water until they were used in the experiment.

4.1.3 Infection Process

Squalius cephalus

Hatchery-reared *S. cephalus* were obtained from a local fish supplier (Czech Fishery Ltd., Rybarstvi Trebon, Czech Republic) and stored in the research lab for several weeks prior to the experiment. This fish species was chosen for the experimental trials because it is a known host for both *A. woodiana* and *A. anatina* (Douda et al. 2011, 2013). The age of the fish was 1+ years and all fish were determined healthy prior to the beginning of the experiment. For future identification throughout the experiment, the fish were tagged with a unique numerical code and returned to their holding tanks prior to infection.

Release of glochidia

For release of glochida, the shell valves of both *A. woodiana* and *A. anatina* were carefully opened using a shell opener and held in that position, while the glochidia were flushed out. This process was achieved by puncturing the marsupium with a syringe and injecting water to allow for the glochida to be expunged into a water-filled container. This process was repeated with several gravid females until a sufficient amount of glochidia were collected for use in the experiment. Figure 3 displays collected glochidia of *A. anatina*. After glochidial release, female mussels were then returned to their holding tanks.

Figure 3. Collection of released glochidia from *A. anatina*

To confirm the concentration and the viability of the *A. woodiana* and *A. anatina* glochida, 10 ml samples were chosen from the container and examined. First, the contents were placed on a gridded petri dish, and, using a stereoscopic microscope, the number of glochidia were counted and the overall density of the sample was determined. To test the infection potential of the glochida, sodium chloride was added to the petri dish, which was used to induce the glochidia shells to close (Reichard et al. 2012 ex. Wang et. al 2007). Then, the numbers of opened glochidia before and after the addition of sodium chloride were compared to determine viability.

Infection of Anodonta woodiana

Priming infections with *A. woodiana* were performed during the month of September in glochidia suspensions containing 2000-6000 glochidia liter $^{-1}$. Three distinct groups of 25 *S. cephalus* each were randomly selected for the experiment and their tag numbers were carefully recorded. Group A was primed once by *A. woodiana*, group B was primed three times, and group C consisted of individuals that were composed of naïve fish, not infected with the glochidia of *A. woodiana.*

Infection of Anodonta anatina

On November 9th, the three groups of 25 *S. cephalus* were recollected and identified by their tag number in order to be prepared for infections with *A. anatina*. The collected glochidia from *A. anatina* were distributed in a bath of dechlorinated tap water, containing a target concentration of 4000 glochidia liter^{-1} and poured into three inoculation 80 liter tanks. Figure 4 shows the distributed bath containing suspended glochidia prior to the induced infection.

Figure 4. *A. anatina* glochidia in bath prior to infection

The fish were then added randomly to the baths, in order to have the fish evenly distributed. The water was stirred manually and exchanged among the baths by the skilled researchers prior to and during the inoculation in order to keep the glochidia suspended in the water and to keep the suspension homogenous. This process was maintained continuously for 15 minutes in order to provide sufficient time for the glochidia to attach to the host fish. After the 15 minutes of inoculation time, the fish were transferred to another bath of dechlorinated tap water for 30 minutes to rinse off glochidia that had not attached for a (Reichard et al. 2012). Figure 5 shows a sample of *S. cephalus* in the glochidia bath.

Figure 5. *S. cephalus* with suspended glochidia in bath

4.1.4 Monitoring

The monitoring process took place from November 9, 2015 to December 1, 2015, a period of 23 days. With no recordings on the first day (November 9th), there were 22 recording days total. However, during the final few days, as the number of living juveniles and glochidia decreased, the monitoring process occurred every second day. Therefore, there was no count recorded on November 28th or November 30th. Due to the tediousness and time-consuming counting process, it was completed by a team of four skilled researches.

Fish Storage and Care

After initial glochidial infection, the *S. cephalus* individuals were transferred into 18 liter tanks, located in the aquatic laboratory of the Department of Zoology and Fisheries at the Czech University of Life Sciences in Prague. Each tank contained one fish, of an unknown group, chosen at random, filled with dechlorinated tap water, and maintained at ambient room temperature. The tanks were arranged in groups of ten, with four groups symmetrically on each side of the lab for a total of 80 tanks (75 were used for the experiment). The groups were arranged in four quadrants, with two groups stacked on each side of a divider. The fish tanks were part of a single recirculation system, being fed by a constant supply of oxygen and a slow flow of water from a tube, which was pinched tight to control flow. An example of the fish tanks is provided as photograph in Figure 6.

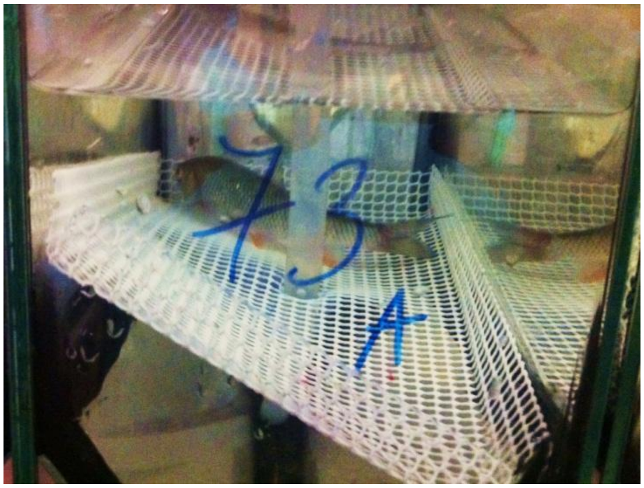

Figure 6. ***S. cephalus*** **in tank number 73A**

Daily, the fish were fed approximately one tweezer-sized pinch (several flakes) of commercial dried fish food. The feeding times varied by day, depending on the availability of the research team. During the experiment, it was determined that all the fish were in adequate health and there were no mortalities for the duration of the infection and monitoring process.

Collection of Samples

In order to collect all the suspended solids from the fish tanks, the system was flushed three times. In order to flush the systems, water was pumped from a storage tub, using a submersible pump, while simultaneously researches manually removed pins to increase flow in each tank. The seventy-five tanks were filled to nearly full capacity, before the pins were reinstated and the water retracted to the original level. This syphoning process was completed three times each day prior to the collection of samples.

This procedure was verified by previous experiments to be sufficient for flushing all detached glochidia and juveniles from holding tanks to filter cups (Reichard et al. 2012). Thus, the bottom mesh net filters were meticulously removed and placed into carefully labeled petri dishes, which were then stacked, placed in a carrying vessel, and transported to the laboratory for content analysis. The filtered samples after collection and placement into the protective containers from tank 57C are shown in Figure 7.

Figure 7. Filtered sample from tank 57C

Counting

Using a wash bottle, the mesh filters were rinsed into a petri dish, which was divided into 20 quadrants (5x4) in advance to aid the counting procedure. The petri dish was placed under a stereoscopic microscope (40x magnification) and the contents were scrupulously counted and recorded. The contents of interest were living juveniles (both active and inactive) and glochidia (both open and closed). Live juveniles were determined by foot or valve movement (Reichard et al. 2012). Among the sample contents were also debris or waste, which varied in quantity, depending on the tank and day. The tank number; numbers of living active, living inactive, opened glochidium, and closed glochidium; name of researcher; and date were recorded by hand on the designated sheet. After the count was sufficiently completed, the petri dish was thoroughly rinsed and the process was repeated for the next tank filter. After data from all the filters was recorded, they were rinsed in hot water and returned to the laboratory, where they were reattached to the bottom of the tanks.

4.1.5 Data Analysis

Raw Data

The living juvenile and dead glochidia raw data was entered into a spreadsheet for each fish, which were also assigned an ID based on the tank number and respective group. The grouping was confirmed by cross-checking the fish tag with a master list, containing the IDs for all present *S. cephalus* in the laboratory. Several values were calculated and highlighted from the raw data, including ‘4 days’, which was the total living or dead individuals from the first 4 days of the experimental recordings (November 10th – 13th, no values were recorded on November 9th); ‘Filtered Juvenile’, ‘Filtered Glochidia’, and ‘Total’, the number of living, dead, and total sampled individuals, respectively, for the duration of the experiment; and finally, ‘Transrate’, the transformation rate, or the percentage of filtered juveniles, or viable living mussels, found by the simple equation below (Equation 1).

$$\boldsymbol{Transrate} = \frac{\boldsymbol{Filtered\,Juvenile}}{\boldsymbol{Total}}$$

Equation 1. Transformation Rate

The transformation rate gives the hosting capability success rate for *S. cephalus* for each individual sample and moreover, for each group. It is this value that determines whether prior infections from *A. woodiana* have a significant effect on the capability of *S. cephalus* to host the glochidia of *A. anatina.*

Fish Parameters

At the conclusion of the experiment, the fish sizes were measured. First, the fish were weighed using a digital scale (0.01 gram precision) and the value was recorded in grams to the nearest tenth. Additionally, two values for length were also determined: standard length and total length, both in millimeters. Standard length describes the length measured from the tip of the fish snout to the end of the last vertebrae, in other words, the length excluding the caudal fin, or tail fin. The total length is a straight line measurement from the tip of the snout to the furthest tip of the caudal fin.

To determine the relative health of the fish based on their sizes, Fulton’s condition factor, *K*, was calculated for each individual fish. Equation 2, Fulton’s Condition Factor, is shown below.

$$K = 100\frac{W}{L^3}$$

Equation 2. Fulton's Condition Factor

In this equation, W refers to the weight in grams, while L is the total length in centimeters. The value is multiplied by 100 in order to obtain a value that is close to 1.

Statistical Analysis

Statistical analyses were conducted using the statistical program, *R*©. In order to select the appropriate statistical tests, the distribution of data needed to be classified as parametric or nonparametric. First, the data from each group was graphically plotted using a histogram and a quantile-quantile (q-q) plot. Parametric data dictates a histogram following a bell-shaped curve, while the q-q plot should show all data points falling in a straight line, meaning that the majority of the data would fall within the middle quantiles and not the outer ones. As a final measure, the Shapiro-Wilk test was conducted, with a significance level of $\alpha=0.05$, for each group with the null hypothesis being that the sample came from a normally distributed population (parametric data). In the animal science community, the Shapiro-Wilk test is regarded as having the highest power to test for normality (Ruxton et al. 2015).

To test the homogeneity of variances, Levene's test was chosen based on the distribution of the data. In this test, the null hypothesis states that the data comes from equal variances, or in other words, assumes homogeneity of variances. The chosen significance level was $\alpha=0.05$, so values less than this significance level would allow for rejection of the null hypothesis.

The Kruskal-Wallis test was selected to test whether there was a difference in means between any of the three groups. The null hypothesis is that there is no difference between the means of any of the groups at a confidence level of $\alpha=0.05$. Hence, a test statistic values lower than this threshold would allow for rejection of the null hypothesis and therefore indicate some difference among the three groups. Finally, a post-hoc Pairwise-Wilcoxon test was used to compare the differences in mean (or shift in distribution) between each pair of groups A, B, and C. Similar to Kruskal-Wallis, the null hypothesis states that there is no difference between any of the pairs at a confidence level of $a=0.05$, with values below allowing for rejection of the null hypothesis and indicating significant difference between those particular groups.

Chapter 4.2 Results

The experiment was conducted from November 6th 2015 (data collection) until December 1, 2015 (final count). All equipment and facilities were up to standard and maintained competent operation throughout the duration of the experiment. As noted, there were no mortalities of *S. cephalus* and none of the organisms involved were significantly harmed. Results indicate differences in hosting capabilities between naïve and primed host fish, rejecting the null hypothesis. Naïve fish were nearly twice as successful in hosting *A.* anatina as previously infected fish. Thusly, it was concluded that the presence of the invasive freshwater mussel *A. woodiana* impacts the ability for *A. anatina* to parasitize host fish and thus reproduce.

Significance Tests

All distribution tests indicated that the data was nonparametric. The tests were conducted on each group individually, with all of the histograms divergent from the bell-shaped curve indicative of parametric data, while all data points deviated from the best-fit line in the q-q plots. The normality test graphical representations of the histogram and q-q plot are shown in Figure 8 for groups A, B, and C. Using the Shapiro-Wilk test, the p-values were found to be 0.02221, 0.002687, and 0.002281 for groups A, B, and C, respectively. Since all of the p-values were below the alpha threshold of 0.05, the null-hypothesis that the data came from a normally distributed data set was rejected and the data was concluded to be nonparametric. Since there are adequate tests for nonparametric data, there was no need to transform, or normalize, the data. In addition, due to the low power of normality tests for sample sizes under 50 (Ruxton et al. 2015), it is believed that the transformation of the data would not have altered any of the results.

Levene's test produced a p-value of 0.3673. Therefore, the null hypothesis that there was homogeneity of variance was unable to be rejected. For the remaining tests, homogenous variance was assumed. The Kruskal-Wallis test is valid for determining differences in mean for nonparametric homogenous data (Ruxton and Beauchamp 2008). The results of the Kruskal-Wallis test ($p = 4.62e\text{-}05$) allowed for rejection of the null hypothesis, meaning that there exists significant difference between the means of at least two of the groups. Therefore, the post-hoc pairwise-Wilcoxon test was appropriate to determine where the difference was statistically present. Test values between each pair of the groups are shown in Table 1. Results led to a rejection of the null hypothesis of no difference in means, and instead indicate significant difference between both groups

A and C, and groups B and C, having values of 0.00018 and 0.00017, respectively. However, the pairwise comparison of groups A and B produced a test statistic value of 0.40741, stating that the null hypothesis cannot be rejected and there is no apparent difference between groups A and B. Furthermore, the box plot (Figure 9) gives a visual representation of the differences in means, or shifts in the distribution of the data. It is clear that groups A and B differ significantly from group C.

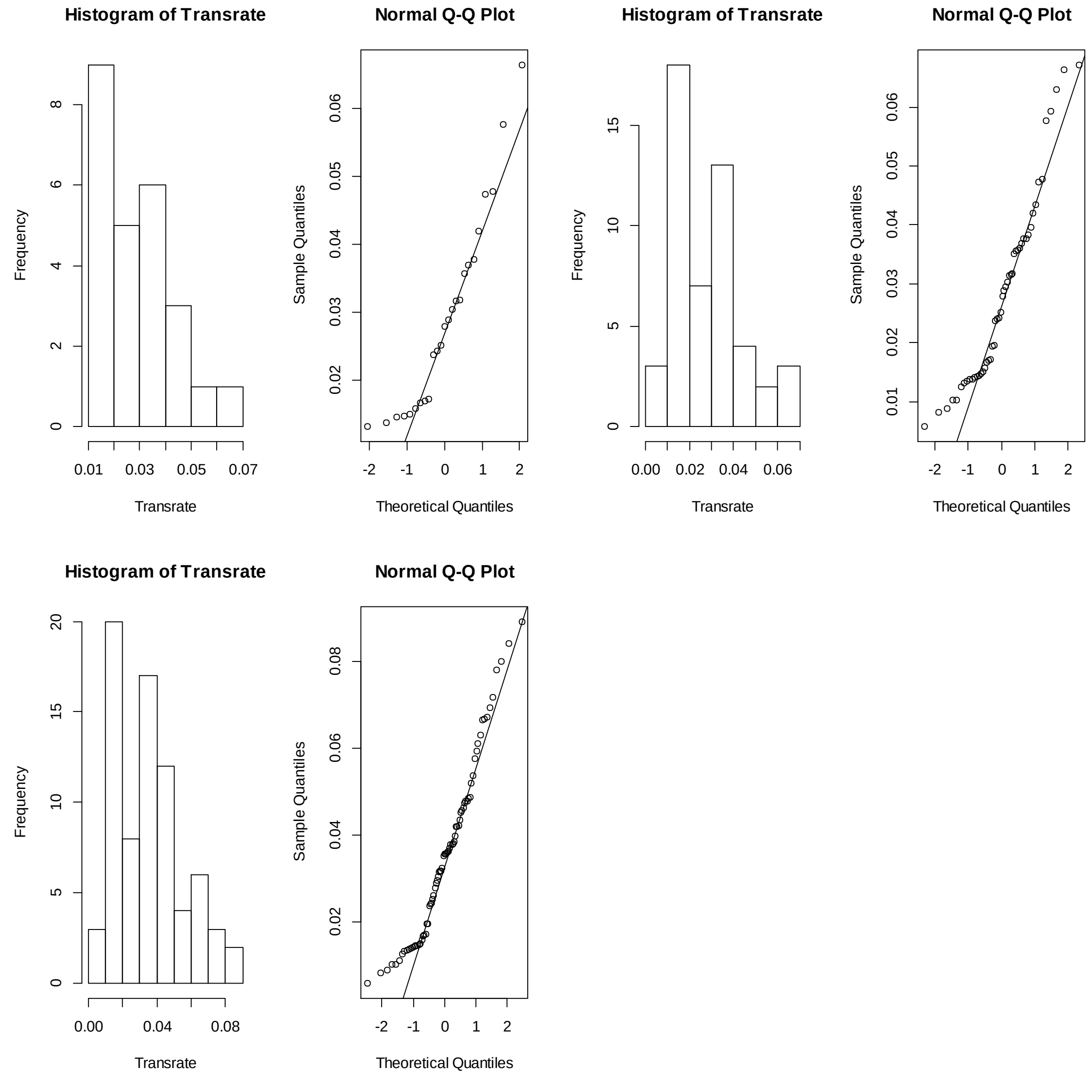

Figure 8. Graphical representations of normality for groups A (top left), B (top right), and C (bottom left)

Pairwise-Wilcox Test		
	A	**B**
B	0.40741	
C	0.00018	0.00017

Table 1. Pairwise-Wilcoxon test statistics showing differences of means between the groups of host fish

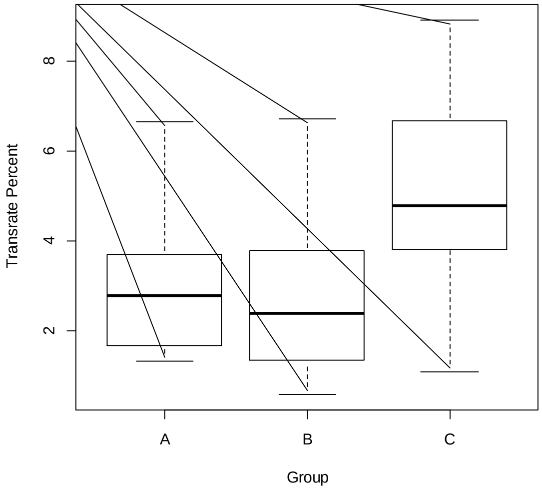

Figure 9. Boxplot representing the difference in means of groups A, B, and C

Total Numbers

During the first four days of the experiment, the number of glochidia that were sloughed ranged from 219 (C51) to 1219 (B31), showing a wide range among the 75 host fish. There were a total of 47445 sloughed glochidia during this period. The average detachment rate during the first four days was 632.6 glochidia for each host fish, or 158.15 glochidia per host fish each day. There were only seven individuals classified as living juveniles during this four-day period.

At the conclusion of the experiment, the number of developed juveniles ranged from 8 juveniles (B33 and B38) to 142 (C16), while the filtered glochidia varied from 628 (B38) to 2072 (C73). As expected the total number of filtered glochidia plus filtered juveniles occurred in the same fish as the total filtered glochidia, spanning from 636 (B38) to 2176 (C73). To put the experimental numbers in perspective, a total of 3933 living juvenile *A. anatina* were developed during the course of the experiment, while the number of undeveloped glochidia summed at 103722.

Transformation rate

The transformation rate was the lowest in fish B64, at a mere 0.58%. As a matter of fact, the five fish having the least success in terms of transformation rate were all from group B. Table 2 ranks the host fish with the lowest transformation success rate, listing the rank, the fish ID, number of filtered juveniles, total number of collected glochidia and juveniles, and the transformation rate as a percentage.

Rank	ID	Filtered Juvenile	Total	Trans %
71	B71	16	1558	1.03
72	B7	18	1760	1.02
73	B33	8	909	0.88
74	B68	10	1223	0.82
75	B64	9	1556	0.58

Table 2. Host fish with the lowest transformation rate, ranked 71-75

On the other hand, the six highest transformation rates were found in fish from group C, ranging from 8.92% in fish C27 to 6.93% in fish C15. These fish are represented in Table 3, with the same parameters at Table 2.

Rank	ID	Filtered Juvenile	Total	Trans %
1	C27	141	1581	8.92
2	C48	98	1165	8.41
3	C32	137	1714	7.99
4	C51	123	1576	7.80
5	C16	142	1978	7.18
6	C15	192	1328	6.93

Table 3. Host fish with the highest transformation rate, ranked 1-6

Factoring in all 25 fish from each group, host fish in group C as a whole had nearly double the transformation success rate as either group A or group B. Though group A was slightly more successful at developing live juveniles than group B, the difference was minor. Table 4 gives a summary of the transformation success rate, identifying the three fish groups, the total number of living juveniles, the total number of filtered juveniles and filtered glochidia, and the average transformation success rate for the group as a whole.

Group	Filtered Juveniles	Total	Trans %
A	1043	36106	2.89
B	899	31802	2.83
C	1991	39747	5.01

Table 4. Summary of the total number of filtered individuals and the average transformation rate

The experimental totals can be observed below in Figure 10, which clearly shows the total number of filtered glochidia, filtered juveniles, and overall total number of filtered individuals (juveniles plus glochidia) at the close of the experiment. The minimum count value has been adjusted to 30000 in order to clearly depict the differences between the groups. It can be seen that group C clearly had the most transformation success, with the highest count of living juveniles, glochidia, and consequently, total. Group B had the lowest counts in all three categories.

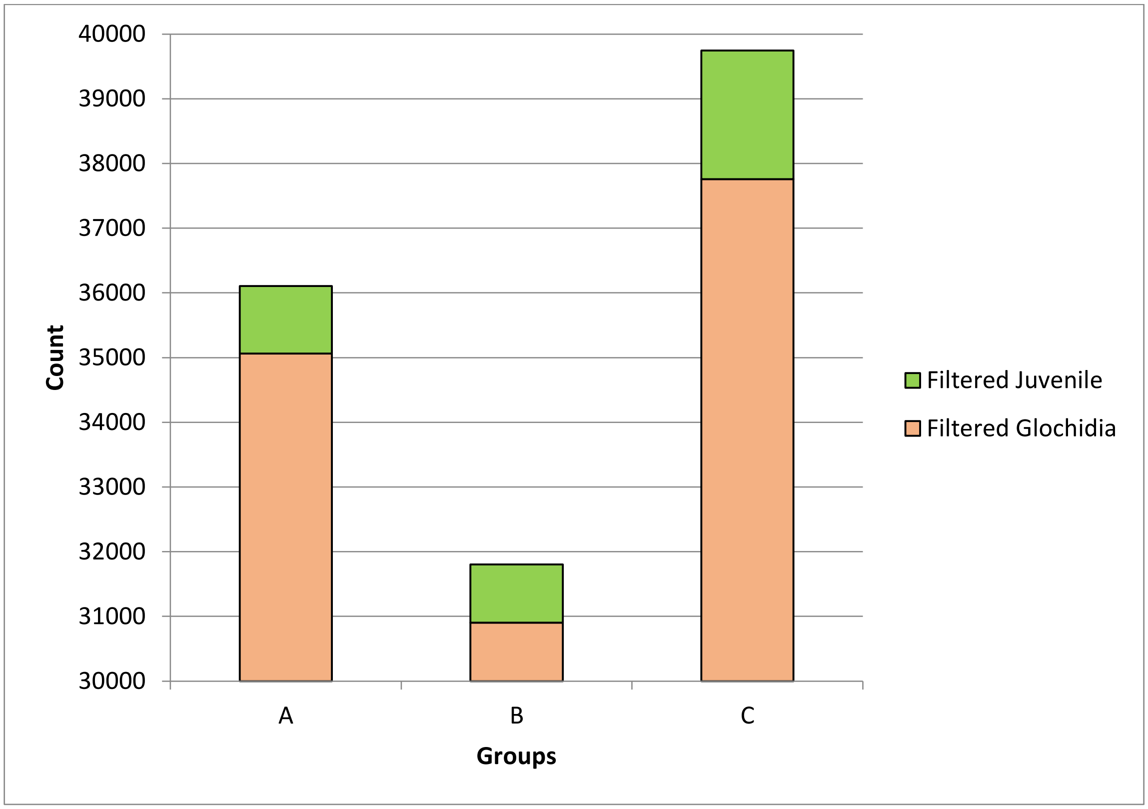

Figure 10. Representation of the filtered juvenile, filtered glochidia, and totals for each group

Fish Parameters

All fish used in the experiment were relatively similar in length. The standard lengths ranged from 101 to 144 mm, while the total lengths ranged from 117 to 169 mm. There were no discernable differences between groups of the fish, as the length for each of groups A, B, and C were recorded as (length ± standard deviation in millimeters) 150.2±8.6, 150.8±11.1, and 152.7±10.7. Weight ranged from 13.4 to 41.6 grams, again with negligible differences among groups – (in grams) group A: 26.7±4.9, group B: 27.3±5.9, and group C: 28.5±5.9.

Fulton's Condition Factor for standard weight varied from 0.67 to 0.89 for the entire set of fish, independent of group. When the data was summarized and the sizes were compared amongst the groups, there was less than a millimeter and less than a two millimeter difference for standard length and total length, respectively. Similarly, the recorded weight was less than two grams between the groups. Fulton's Condition Factor was determined to be 0.78 for both group A and group B, and 0.79 for group C. A summary of the fish parameters discussed above can be seen below in Table 5.

Group	Weight (g)	Standard Length (mm)	Total Length (mm)	Fulton's Condition Factor for Standard Weight
A	26.732	127.4	150.16	0.78
B	27.304	127.8	150.8	0.78
C	28.516	128.08	152.68	0.79

Table 5. Summary of fish parameters with weight, length (standard and total), and Fulton's Condition Factor

Chapter 4.3 Discussion of Experimental Data

Null Hypothesis

The null hypothesis stated that there would be no effects on the metamorphosis success rates of previously infected host fish compared to naïve host fish. However, the results of this experiment rejected the hypothesis. It can easily be observed from the count totals of both glochidia and juveniles, that the naïve group of *S. cephalus* (group C) was able to both host glochidia and develop juvenile freshwater mussels at a higher success rate than the fish previously primed with *A. woodiana*. This was further proved from statistical tests, which indicated there were significant differences between the means of groups A and B with group C.

Overall Transformation Rate

The overall transformation rate found in the experiment was lower than values found in similar experiments. In one trial, when comparing the nutrition and hosting viability of several fish species with *A. anatina*, the transformation rate on *S. cephalus* was recorded to be 7.3% (Douda 2015). However, the sample size used in the aforementioned experiment was only five fish individuals. The sample size could be too low for ascertaining an accurate transformation rate, therefore having a value non-indicative of larger sample sizes.

In an experiment using *R. amarus* as a host fish, the transformation rate of *A. anatina* was only 4%, compared with at 22% percent transformation rate with *A. woodiana* (Reichard et al. 2012). This may be attributed to the fact that bitterling are a parasite themselves and are known to use *A. anatina* as hosts, while having essentially no success in using *A. woodiana* as hosts. However, for a control trial with *L. cephalus,* the transformation rate of *A. anatina* was approximately 15% (Reichard et al. 2012). Though this was lower than the transformation rate of *A. woodiana* in that particular experiment, it was still significantly higher than the metamorphosis success rate findings of the diploma thesis.

Transformation Rate Variability of Groups

As indicated in the results, the experiment yielded differences in the transformation success rates between groups A, B, and C. The success rate of glochidial attachment and juvenile development on naïve fish was significantly higher than that on previously infected host fish.

The majority of the glochidia were sloughed off during the first four days of the experiment (45% of the total for group A, 56% for group B, and 34% for group C), which agree with other experiments. For primed fish, a large portion of glochidia are sloughed during the first day, whereas for control, or naïve, fish, the sloughing may be more gradual (Dodd et al. 2005). These values were negatively correlated to the transformation success rate for each group. For instance, group C had the lowest percentage of sloughed glochidia and the highest transformation rate. This was supported with findings from an experiment testing the compatibility of *U. crassus* and several species of European freshwater fish species, whereas the sloughing of unsuccessful glochidia that occurred during the first and fourth days of the experiment influenced the transformation success rate (Douda et al. 2012).

In another experiment involving the attachment of glochidia from *Lampsilis reeveiana* (Lea 1852) (broken rays mussel) on *Micropterus salmoides* (Lacepede 1802) (large-mouth bass), the transformation success rate decreased with each subsequent infection, from 67.9% to 38.1% to 28.0% on the third and final infection (Dodd et al. 2006). This corresponded to previous experiments, in which primed fish were only able host 56% of control fish, using the same species of host fish and anondontine and lampsiline freshwater mussel species (Dodd et al. 2005). These findings augment the experimental results, with declining transformation success rates with previous infections.

However, as time accrued between infections, the higher the transformation success rate became with groups of previously infected fish (Dodd et al. 2006). This seems to confirm that adaptive immunity due to previous priming dissipates over time. This positive correlation indicates that theoretically *A. anatina* would be more successful in infecting host fish that had been previously infected with *A. woodiana* as the time from said infection increased.

Fish Size

The low average total length and weight of the *S. cephalus* used in the experiment correspond to the fact that 1+ year old fish were used. For example, in two to three year-old *S. cephalus* sampled from the Sava River (Danube tributary) in Croatia, lengths varied from 157 - 235 millimeters and weights ranged from 33.1 - 129.4 grams (Dragun et al. 2009). Even the minimum values recorded in the Sava River exceeded the average length and weight for fish in each experimental group. Similarly, *Leuciscus cephalus* (synonym for *Squalius cephalus* (Linnaeus 1758)), collected from the confluence of the Svitava and Svtarka rivers in Brno, Czech Republic in 2004 ranged in mean total length from 239 – 303 millimeters, and mean weight of 120.78 – 347.68 grams (Lamková et al. 2007). Comparing with other species, for experiments done in freshwater ecosystems in northeastern France with *U. crassus* and compatible host species, the highest infestation and thus, highest number of encysted glochidia occurred in fish with average lengths between 550 and 600 millimeters (Lamand et al. 2016). Šlapanský et al. found that infection success by both *A. woodiana* and *U. tumidus* had risen with increasing fish size for young native fish species (2016).

The smaller sized fish were used to accommodate laboratory facilities and ensure that the fish were not previously infected with glochidia. The size of the fish used in the experiment can perhaps divulge the reason why the transformation success rate was low in group C. Generally, larger naïve fish are capable of hosting a larger number of glochidia (Rogers and Dimock 2003). However, this does not explain the lower transformation rate in primed groups A and B, as the smaller size should not be a significant factor in the increased adaptive immunity of the *S. cephalus* used in the experiment. In other words, previous infections should not dramatically alter the hosting success rate of smaller-sized fish. Moreover, since all the *S. cephalus* were relatively the same size, independent of group, the overall smaller sizes should not account for the discrepancies in transformation rate between the groups of host fish.

Fish Condition

Fulton's Condition Factor is a widely accepted manner of quantifying the condition or well-being of fish throughout fisheries and biological sciences, using a ratio of length to weight of the fish (Nash et al. 2006). The Fulton's Condition Factor values calculated during the experiment measure lower than the values found in the previously discussed experiments with *S. cephalus* in Croatia and the Czech Republic. Fulton's Condition Factor found in *S. cephalus* in the Sava River in Croatia measured at 0.94, 1.04, and 0.95 at three sampling sites, respectively (Dragun et al. 2009). Fulton's Condition Factor values calculated from *S. cephalus* at the Svitava and Svtarka rivers in Brno, Czech Republic were even higher, with values from 1.47-2.76 (Lamková et al. 2007). It is important to note that location, seasonality, and other factors can affect this value, though generally speaking, the higher condition factor, *K*, the greater assumed health of the fish (Nash et al. 2006).

Chapter 5: Conclusions

The objective of the conclusion is to serve as a summary and review of the diploma thesis. The two thesis aims are fully addressed, critiqued, and unified. Finally, interpretations and evaluations of current conservation efforts, in addition to informed suggestions and recommendations for conservation efforts going forward are discussed.

Chapter 5.1 Consequences of *Anodonta woodiana* in the Czech Republic

The unique anatomy and behavior of freshwater mussels make them keystone species and vital members of their ecosystems. Unfortunately, as of 2015, more than half of freshwater mussel species worldwide are listed on the *IUCN Red List of Threatened Species* (Lopes-Lima et al. 2016). The presence of alien freshwater mussel species and increased immunological resistance from host fish are severely threatening the reproduction of native freshwater mussels throughout their range.

Impacts of Anodonta woodiana

A. woodiana has taken over as the dominant freshwater mussel species in many of its newfound ecosystems by outcompeting native freshwater mussel species for resources and host fish. For example, *A. anatina* was found present at 80% of sampling sites that included *A. woodiana* in the Czech Republic (Beran 2008). The direct impact on *A. anatina* is profound. For instance, *A. woodiana* has directly decreased the ratio *A. anatina* from 17.8% to 8.6% (14.6% to 7.9% when measuring biomass) in Lake Balaton in Central Hungary (Benkő-Kiss et al. 2013). Furthermore, in a recent experiment, during the summer months 535 native and exotic host fish species were sampled from the Dyje River in the Czech Republic, containing *A. anatina*, *A. woodiana*, and *U. tumidus*; none of the 535 infected fish contained glochidia from *A. anatina*, while *A. woodiana* were the most successful infecting native fish species (Šlapanský et al. 2016). However, this may be attributed to the timing of the experiment, as *A. woodiana* are characterized by summer glochidial release. But, even without the presence of invasive species, *A. anatina* appears to be in peril, as populations in the Rhine river near Freiburg do not live longer than four years (Bauer 2001b).

Worrisome, the brooding season and release of glochidia from *A. woodiana* occurs in advance of native mussel species, with *A. woodiana* releasing glochidia in the summer

(Sarkany-Kiss et al. 2000, Afanasjev et al. 2001) and *A. anatina* releasing glochidia in the autumn or spring (from long-term brooding) (Hinzmann et al. 2013). *A. anatina* were observed in the aquatic laboratory of the Department of Zoology and Fisheries at CULS releasing glochida in the late autumn and periodically in small patches during the winter, before releasing the remaining glochidia in the spring (Karel Douda 2016 pers. comm.).

The ability of the invasive species to infect host fish prior to native mussels creates an adaptive immune response in potential host fish that later limits infections from native species (Rogers and Dimock 2003). This was exemplified by the current thesis research, with *A. anatina* metamorphosing at half the success rate on fish immune due to infections from *A. woodiana*, versus naïve fish. *A. anatina* residing the Czech Republic form a single species with all *A. anatina* individuals throughout Central and Western Europe, as proved from diagnostic loci, genetic distances, and gene flow analysis (Nagel and Badino 2001). Therefore, it can be assumed that from a biological standpoint, the results found in the diploma thesis would be similar for other *A. anatina* populations in the western and central European regions.

The characteristics and ecology of *A. woodiana* is problematic for native freshwater mussels and ecosystems throughout Europe. From experience sampling, the volume of *A. woodiana* biomass compares strongly against all other species in the Czech Republic. It is agreed upon by experts that the species has the ingenuity to adapt to ecosystems and the propensity to solidify itself as the dominant species amongst freshwater mussels. In addition to Europe, the species has also begun to invade ecosystems throughout Asia and Central America, while most ecologists would concur that it is only a matter of time before the species penetrates the ecosystems of North America.

Chapter 5.2 Experiment Review

Summary of Results

The primary experimental aim was to determine the effects of prior parasitic infections of *A. woodiana* on host fish on secondary infections by native freshwater mussels, namely *A. anatina*. The null hypothesis, that there was no effect on the metamorphosis success rates between previously infected fish and naïve fish, was disproved. Naïve host fish were nearly twice as successful at developing juvenile mussels as their primed counterparts. Specifically, naïve host fish were more capable of developing *A. anatina* by a ratio of 1.73:1 and 1.77:1 for groups primed with *A. woodiana* once (group A) and three times (group B), respectively. However, there does not seem to be highly distinguishable difference of transformation success rate based on the number of times host fish have been infected, with primed fish from groups A and B having similar transformation success rates. However, due to the acquired immunity from previous infections, the experimental findings strongly suggest that the presence of *A. woodiana* would negatively impact the development and status of freshwater mussels.

Experimental Limitations

The number of glochidia attached and transformed compared somewhat poorly to data found in previous experiments. This may be attributed to the relatively small size of the *S. cephalus* used in the experiment, or due to the less than ideal fish condition, calculated from Fulton's Condition Factor. As a previously discussed generalization, larger fish are able to host a larger number of glochidia (Rogers and Dimock 2003), though this only affects the attachment and development on naïve host fish. Moreover, *A. anatina,* as a species, has a history of lower transformation rates when compared to other freshwater mussels (Douda 2015).

The sample size of each host fish group was only 25 individuals, which is relatively small, and this could have attributed to some inconsistencies among the results. For statistical tests, generally a sample size of 50 individuals is recommended for strong results (Ruxton et al. 2015). Perhaps a larger sample size for each group would have allowed for more cogent results.

Of course, as with any experiment, there also exists the element of human error. A significant portion of the filters contained a large amount of debris, which could have strained the ability of the skilled research to make completely accurate counts. However, due to the high quantities of glochidia, a few errors would not likely impact the total numbers or proportions. A final reason for the lower glochidia counts and transformation rates could be due to the stress of the captive fish, though more research would be needed to solidify this as a probable reason.

Chapter 5.3 Going Forward

Current Conservation Efforts

Due to the overall threats against freshwater mussels and declining populations, first and foremost it is important to understand the problem and *then* begin to reverse trends. It is vital to increase awareness of the importance of these ecosystem engineers and the detriments that face ecosystems devoid of freshwater mussels. Fortunately, there have been a wider number of researchers and scientists focused on conserving these keystone species in recent years.

The development of conservation genetics and the understanding of host-general and host-specific species have allowed scientists to focus more on the development of juvenile mussels in an artificial setting. Moreover, the development of 'in vitro' fertilization, which is the fertilization of the egg and sperm independent of a host fish, has allowed scientists to manually propagate freshwater mussels. Preliminary results are showing that large numbers of juvenile mussels have been produced with transformation success rates of more than 90% (Lima et al. 2012). If further developed, this method could mitigate the effects from an invasive mussel such as *A. woodiana* on native mussel populations and accelerate conservation efforts for freshwater mussel species that are endangered. However, it is important to state that the effects of in vitro propagation are directed towards the short-term. Alternative solutions are needed for the long-term conservation of freshwater mussels.

Additional success in laboratory conditions has already occurred from the use of an immunosuppressant (Kirk and Layzer 1997), having allowed glochidia of freshwater mussels to attach to otherwise incompatible host fish species. Furthermore, the use of freshwater mussels in aquaculture facilities can lead to an increased understanding of the species and continued research efforts. Their inclusion in such facilities can ensure the progressive research and likelihood of solutions for conservation of freshwater mussels. Also, it is obligatory to halt the spread of glochidia traveling as parasites on transported fish from their home range. This can be done with stricter regulations and more diligent checks at border crossings and ports of entry. Finally, not only for the conservation of freshwater mussels, but also for other aquatic species, it is important to continually work on habitat restoration, channel naturalization, and defragmentation of habitats.

The development of conservation strategy outlines and regulations has focused efforts and increased communications between conservation entities. Frequent meetings, conferences, and the rise of organizations with departments and branches dedicated to freshwater mussel conservation has made the future brighter for unionids across Europe and North America. It is important for organizations to come into fruition in other continents as well, where the awareness and motivation for freshwater mussel conservation may be lacking.

Suggestions and Recommendations

The rise of *A. woodiana* in Europe has hopefully raised awareness as a silver lining to the negative effects of its invasion. From research efforts and results found in this diploma thesis, as well as other similar studies, it should be understood that this invasive species can significantly lower populations of native freshwater mussels in its adopted range. Due to its long life span and ability to spread rapidly and withstand severe environmental conditions, attempts at eradication of the species would be futile.

Perhaps controlled release of gravid females into cooler European waters could spur and heighten the development of native freshwater mussel species, as *Anodonta woodiana* tends to desire warmer water bodies (Kraszewski and Zdanowski 2007). This proposition would have to occur at the lower limits of colder temperatures, where resources would still be widely available. Unfortunately, due to its resistance and strength, A. *woodiana* has been found in colder regions in recent studies.

Another possibility may be to steer native *Anodonta* freshwater mussel species toward highly trophic bodies of water, as they have shown an aptitude for surviving in such conditions (Patzner and Muller 2001). Conversely, the invasive *A. woodiana* was found in bodies of water, classified as oligotrophic or mesotrophic (lower trophic levels) in Italy (Cappelletti et al. 2009).

In experiments comparing infections of glochidia between *A. woodiana* and *A. anatina*, *A. woodiana* were much more successful infecting *L. cephalus*, *R. amarus*, and species from East Asia, while *A. anatina* actually had a slightly higher success rate developing glochidia on *Barbus barbus* (Linnaeus 1758) (common barbell) (Reichard et al. 2012). Therefore, there may be other native host fish species that are not significantly affected by the presence of *A. woodiana*.

Finally, it is clear that there is currently a problem facing freshwater mussels in the Czech Republic and worldwide. The continuing awareness, education, and research into these delicate organisms are paramount to conservation efforts. In addition, the impacts of invasive species can be used as a template for freshwater mussel conservation plans. Furthermore, the understanding of the unique partnership of fish and freshwater mussels and the effects of fish immunity on glochidia attachment demands attention from biologists, aquatic researchers, and other scientists throughout the world.

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