Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Zoology and Fisheries

ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

Impact of municipal waste water pollution on endangered aquatic invertebrates - model group of freshwater bivalve molluscs and sertraline

Diploma Thesis

Author: Bc. Shuran Zhao

N-AMBO2 Waste Technology

Technology, Processing and Utilization of Waste

Supervisor: Ing. Karel Douda, Ph.D.

DECLARATION

I hereby declare that the present Diploma Thesis was composed by myself and that the work contained herein is my own. I also confirm all the sources I used are cited and listed in Bibliography according to the rules of faculty FAFNR, university CULS.

Prague, ...   Signature

ACKNOWLEDGEMENTS

I wish to express my deep appreciation to Ing. Karel Douda, Ph.D. for his valuable and constructive suggestions and supervision. Without him it would not be possible to complete this work. My special thanks are also extended to all people which have helped and contributed to the experimental part of this work and the affiliated article: Pavel Horký, Roman Grabic, Kateřina Grabicová, Tomáš Randák, Ondřej Slavík and Barbora Vodáková. Special thanks are given to Department of Zoology and Fisheries for enabling me to use the laboratory for implementing this experiment.

A lot of thanks also belongs to my parents for their encouragement and patience during my whole student life.

Finally, I would like to thank Bc. Adam Tetaur for his useful suggestions, encouragement and empathy and Ing. Dagmar Zádrapová for her support during the study.

Vliv znečištění z komunálních odpadních vod na ohrožené vodní bezobratlé - modelová skupina sladkovodní mlži a sertraline

Souhrn

Rostoucí množství léčivých přípravků a jejich soustředění ve vodním prostředí vzbuzuje mnoho obav. Nedostatečně vyčištěné odpadní vody se zbytky léčivých látek vypouštěné do přírodních vodních ploch a toků mohou přímo ohrozit organismy žijící níže po proudu. V této práci byl proveden experiment ve faktoriálním designu s organismy vystavenými a nevystavenými kontaminantu, založený na pozorování a kvantifikaci behaviorálních změn (míry přichycení, umístění glochidií po těle hostitele a úspěšnosti zapouzdření) u glochidií a ryb za účelem prozkoumání vlivů sertralinu na počáteční vývojové stádium sladkovodního mlže *Unio tumidus* (Philipsson, 1788) (Unionida) - glochidia. Jelikož mlži jsou v larválním stádiu obligátními parazity ryb, zařadili jsme do našeho výzkumu též rybu *Squalius cephalus* (Linnaeus, 1758) (Cypriniformes), pro sledování vlivu látky na interakce mezi hostitelem a parazitem. Sertraline je nejčastěji předepisovaným antidepresivem na světě, patří k selektivním inhibitorům zpětného vychytávání serotoninu (SSRI), které se používají k léčbě stavů, jako jsou deprese nebo úzkostné poruchy. V této práci byl použit design asymetrické expozice pro rybí hostitele a parazitická glochidia při koncentracích: *CONTROL* (0 µg L^{-1}) a *EXPOSED* (0.2 µg L^{-1}) pro ryby a *CONTROL* (0 µg L^{-1}), *LOW-EXPOSED* (0.2 µg L^{-1}) a *HIGH-EXPOSED* (4 µg L^{-1}) pro glochidia. Výsledky našeho experimentu ukázaly, že sertraline měl vliv jak na velmi náchylné počáteční vývojové stadium sladkovodních mlžů tak na jejich rybí hostitele. Chování glochidií a ryb bylo ovlivněno sertralinem především při počáteční fázi uchycení, což vyústilo ve větším množství glochidií přichycených na rybách a to hlavně na žábrách ryby, oproti zbytku těla. Výsledek této práce ukazuje signifikantní vliv látky na vztah mezi hostitelem a parazitem a zároveň byl schopný rozlišit dílčí vlivy na oba organismy v experimentu. Je potřeba také zmínit, že změněné chování u obou organismů bylo vypozorováno až při koncentraci sertralinu vyšší než environmentálně relevantní. Metoda použitá pro tento výzkum je schopná identifikovat ovlivněné parametry a odlišit efekt expozice hostitele od parazita, také jejich interakce a dodat kompletnější přehled o interakci mezi rybou a glochidiem. Výsledky této studie jsou důkazem toho, že test toxicity pomocí glochidií je citlivá a slibná metoda pro testování toxicity nových chemikálií, hlavně léčiv ve vodním prostředí. Glochidia a jejich hostitelé jsou vhodné organismy pro ekotoxikologické biotesty do budoucích studií jako modelové skupiny pro identifikaci vlivu farmaceutik na více druhové systémy v přírodních vodních zdrojích

Klíčová slova: sertraline; SSRI; *Unio tumidus*; velevrub nadmutý; mlži; glochidium; *Squalius cephalus*; environmentálně relevantní koncentrace; míra přichycení; žábry; ploutve;

Impact of municipal waste water pollution on endangered aquatic invertebrates - model group of freshwater bivalve molluscs and sertraline

Summary

Many concerns have been raised around pharmaceuticals due to their increasing number and concentration in the aquatic environment. Inadequately purified waste water effluent in nature reservoirs with medicine substances can directly threaten organisms living in downstream. In this work, an approach with full factorial design of non/exposed organisms based on the quantification of behavior response (the intensity of parasite attachment, spatial position of glochidia on host body, and encapsulation success) of glochidia and fish was used to investigate the effect of water-borne human pharmaceutical sertraline on early stage of freshwater mussel *Unio tumidus* Philipsson, 1788 (Unionida) - glochidium. We have included fish *Squalius cephalus* Linnaeus, 1758 (Cypriniformes) into our research for monitoring of host-parasite interaction as glochidium is an obligatory parasite on fish during larval stage. Sertraline is the most prescribed antidepressant worldwide, it belongs to Selective serotonin reuptake inhibitors (SSRIs), which are used to treat conditions such as depression or anxiety disorders. An asymmetrical exposure design was used for fish hosts and glochidia parasites at three concentrations sertraline: *CONTROL* (0 µg L^{-1}) and *EXPOSED* (0.2 µg L^{-1}) for fish and *CONTROL* (0 µg L^{-1}), *LOW-EXPOSED* (0.2 µg L^{-1}) and *HIGH-EXPOSED* (4 µg L^{-1}) for glochidia. The results pointed that sertraline was able to affect the highly sensitive life stage of freshwater mussels and their fish host as well as their interaction. Glochidia and fish were impacted by sertraline mainly in terms of the intensity of parasite attachment to fish and resulted in more encysting on gills in comparison to the remaining body parts. The result shows the influence of chemical exposure on the whole host-parasite relationship and it was also possible to identify the effects on individual partners. On the other hand, the proved significance in both fish and glochidia cases was detected with the level of contamination moderately exceeding nature relevant concentration. The approach used in this experiment is able to identify the affected parameters and distinguish the effect of host-exposure from parasite exposure or the influence of interaction and provide a more complete result about the host-parasite interaction. Also the results indicate that the early life-stage toxicity testing is a promising and sensitive approach for testing the toxicity of pharmaceuticals or other chemicals. Glochidia and their hosts can be recommended for future studies as bioassay organisms as well as they can be used as model groups for the identification of pharmaceutical multi-species impacts in the natural water bodies.

Keywords: sertraline; SSRI; *Unio tumidus*; freshwater mussel; glochidium; *Squalius cephalus*; environmentally relevant concentrations; attachment rate; gills; fins; infestation;

CONTENTS

1 INTRODUCTION

Freshwater mussels play an integral role in aquatic system as they provide essential ecosystem services that benefit many other organisms and some species even fulfill several criteria for indicator, keystone, flagship and umbrella species at one time (Geist 2010). Regardless of this fact, they are among the most threatened animal species, for instance almost one-third (29%) of the world's know mussel freshwater mussel species occur in North America (Stein et al. 2000) and 41% are listed as threated (Baillie et al. 2004). One of the biggest threats the mussels have been facing recently is water pollution. An increasing number of researches have pointed out the adverse effects of pharmaceuticals and personal care products on bivalves in their nature environment (Ribeiro et al. 2012; Petrie et al. 2014; Richardson & Ternes 2018). Concentration of pharmaceuticals in natural water bodies has been increasing last decades. The reason links to two most frequent causes: more often and common use of various medicine, e.g. anticonceptions, antidepressants, and insufficient technology of waste water treatment with pharmaceuticals. Inadequately purified water returning to nature reservoirs with medicine substances can threaten directly organisms living in downstream (Fent et al. 2006).

The key to the development of effective conservation strategies for this group is to dissolve their complicated life cycle including the parasitic larval stage when the glochidia become obligate parasites on fish host (Ćmiel et al. 2018) followed by metamorphosis. Glochidia are considered as an important part of mussel life and their metamorphosis rate is directly influencing the dynamics of mussel communities. Furthermore, they are highly sensitive organisms and their surviving is closely depended on their fish hosts (Graf 1997). Therefore, there is an emerging need for a complete analysis method to test the impact of pharmaceuticals on mussel larvae including interaction with fish host.

The model species of freshwater mussel used in this thesis is *Unio tumidus* Philipsson, 1788 (Bivalvia, Palaeoheterodonta, Unionida, Unionidea). This species is considered as vulnerable according to the Red List of Threatened Species of the Czech Republic (Beran et al. 2017). Their glochidia and their fish hosts *Squalius cephalus* Linnaeus, 1758 (Actinopterygii, Cypriniformes, Cyprinidae) were asymmetrically exposed to an active substance in antidepressant called sertraline. It belongs to SSRIs and can treats conditions as depression, anxiety etc. (AHFS 2013). By using these two model organisms this thesis presents a new complete multi-trophic analysis approach using sertraline as a stressor which are glochidia and fish confronted with. Furthermore the effect of sertraline on glochidia was analyzed to point out the potential risk posed for mussel larvae.

2 SCIENTIFIC HYPOTHESIS AND OBJECTIVES OF WORK

The main goal of this thesis was designed to assess the effect of short-term exposure of sertraline (which was found present in the Czech streams (Grabicova et al. 2017)) on the larval stage (glochidium) of freshwater mussel *U. tumidus*. With regard to the specific character that glochidia are encapsulating on fish host to complete their metamorphosis into juvenile mussels, the host-parasite interaction was considered and implicated into study design as well. We included a freshwater fish *S. cephalus* as a host to be exposed to sertraline and examined the interactions under the sertraline influence.

The hypothesis was whether sertraline have impact on mussel larvae infestation abilities and host defense assessed by several different indicators of mussel-fish interaction success.

3 LITERATURE OVERVIEW

Fresh water makes up approximately 0.8% of the Earth's surface, yet this tiny fraction of global water supports at least 100 000 species - almost 6% of all described species (Dudgeon et al. 2006). Biodiversity status in aquatic system is consequently driven by water quality. Pollution is one of the most prevalent causes of biodiversity loss and water quality degradation, from waste water and agriculture runoff, all of which links to human activities (Vaughn 2010; Moore & Bringolf 2018) .

3.1 Municipal waste water pollution and treatment

Waste water treatment plants (WWTPs) collect waste water from form households, industry, hospitals, as well as rainwater, depending on the technology and capacity of WWTP (Figure 1). They purify waste water from contamination using various physical, chemical and biological methods. Each method has different efficiency with some advantages and disadvantages. But none of them can reach 100 % removal efficiency of pollution. Also, with the emerging pollutants such as pharmaceuticals, illegal drugs or hormones, the removal efficiency is decreasing. Most of the municipal WWTPs carry out preliminary, primary and secondary water treatments which are very variable in removal efficiency ranging from 20 up to 100 % of removal rate (Verlicchi et al. 2012b). The output coming out from the treatment plant is called effluent. It is water purified from the pollutants and returned to the water cycle in nature with minimal impact on the environment. As municipal waste water effluents discharge complex mixtures of compounds including heavy metals, nitrates, ammonia, salts, PPCPs (analgesics, antibiotics, antidepressants, antihistamines and steroid hormones) and many other compounds (Verlicchi et al. 2012a; Grabicova et al. 2017), there are increasing concerns about the adverse effect on non-target aquatic organisms inhabiting the effluent dominated streams (De Lange et al. 2006; Gilroy et al. 2017).

The majority of WWTPs in the Czech Republic use a combination mechanic-biological treatment with activated sludge process. For the most of small up to middle WWTPs the activated sludge systems are often implemented as circulatory, with a managed aeration (Wanner 2017). Many of them have a regeneration zone to facilitate the recovery of storage capacity of bacteria and reuse the sludge. The redundant sludge can be used after dehydrating for generating biogas for energy production which is used for example in Central WWTP in

Prague (Jenicek et al. 2013). Between 1990 and 2007, the amount of pollution discharged from point sources decreased by 94.7% for biochemical oxygen demand, by 88% for chemical oxygen demand, by 90% for undissolved substances and by 14.6% for dissolved inorganic salts (Kráglová 2008).

The residual pollutants found in downstream, diluted by effluent from the WWTPs have been proved to be coming from the emerging pollutants which there is still not an appropriate technology to deal with. There are already available technologies to degrade and eliminate the emerging pollutants in the waste water in few countries over the world. For example, in Switzerland, a new water law requires the most important WWTPs in the country (for protection of Swiss lakes) to be equipped with the appropriate technology for at least 80 % removal of 12 selected substances from the list of emerging pollutants. Similar program has been launched in Germany too. Most of the time it is donated by EU funds, otherwise the sewerage rate would have to increase above the population acceptable level. It is expected that with a gradual legislative pressure to remove these substances from the waste water and improving technologies, the problem with emerging pollutants will be solved soon (Wanner 2017).

3.1.1 Pharmaceuticals and personal care products (PPCPs)

Last decades, many concerns around PPCPs have been raised because of their rising presence in nature and increasing number of researches showing biological effects on organisms at environmentally relevant concentrations (Luo et al. 2014; Ford & Fong 2016), but little is known about their effects on the wildlife that inhabit these waters (Weinberger & Klaper 2014).

PPCP include prescription and non-prescription human drugs and veterinary drugs, as well as their subsequent metabolites and conjugates, including antibiotics, hormones, anticonvulsants, antidepressants, lipid regulators, antihypertensives, and nonsteroidal anti-inflammatory drugs (Figure 2) (Cizmas et al. 2015). Nowadays there is over thousand active compounds in use, contained in even more medicines not only from hospitals. Besides PPCPs, disinfectants and various chemicals intended for diagnosis are in use too (Kummerer 2001).

PPCPs had been expected to be fully degradable in nature until first evidence proved their presence in the aquatic environment. The PPCPs are not completely metabolized by humans and enter the waste water treatment plants (WWTPs), which are not designed for treating such pharmaceuticals. The removal of micropollutants depends on physicochemical properties of micropollutants and treatment conditions and the removal effect varies between 12.5-100% (Luo et al. 2014).

In many cases PPCPs are able to pass through the waste water treatment process and end up in nature as unchanged parent compound or as metabolites at low levels (low to mid ng l^{-1} concentrations) (Brooks et al. 2005; Fent et al. 2006; Di Poi et al. 2014). Therefore WWTPs are identified to be the major sources discharging PPCPs to natural reservoirs and results in continual exposure of aquatic organisms (Minagh et al. 2009; Di Poi et al. 2014; Silva et al. 2014; aus der Beek et al. 2016).

Toxicity of these discharged compounds is the most worrying thing that needs attention of researchers to study their acute or chronical effect on wildlife. (Brausch et al. 2012) with his team made a comprehensive review of acute and chronical toxicities of PPCPs and their metabolites in aquatic ecosystems. Within the compounds investigated, dextropropoxyphene, sertraline, thioridazine, and diphenhydramine were highlighted as the most potential compounds for acute toxicity to the studied algal, invertebrate, and fish populations. Bacteria, fish, and amphibians were found to be less sensitive to the acute toxicity of analgesic drugs, while phytoplankton and invertebrates were found to be the most sensitive to the acute toxicity of these compounds (Brausch et al. 2012). Overall, the antiarrhythmic, antidepressant, antidiabetic, antiandrogenic, and synthetic estrogen compounds were not anticipated to exhibit acute risks at the environmentally relevant concentrations but with the respect to chronic toxicity, there were displayed high levels of chronic toxicity of pharmaceuticals to these aquatic organisms (Brausch et al. 2012). Except chronic toxicity, many studies were conducted to study the indirect effects of contaminants in aquatic ecosystems. The primary effect of contaminant inducing changes in behavior, competition and predation can alter species abundances or community composition later, which would not be associated with contaminants and can complicate the conservation plan making process (Fleeger et al. 2003).

Selective Serotonin Reuptake Inhibitors (SSRIs) are widely prescribed worldwide because of the active compounds treating wide range of disorders, as anxiety, panic disorder, obsessive-compulsive disorder, etc. (Fong & Ford 2014). The type and abundance of SSRIs found in different countries is strictly related to the local consumption rate (Calisto & Esteves 2009). According to the country survey of aus der Beek et al. (2016), the Czech Republic is one of the countries with 11-30 pharmaceuticals detected in surface/ground water and with 1-3 pharmaceuticals detected in tap/drinking water.

Another consideration attracting public attention is whether there is capacity of transfer of pharmaceuticals between trophic levels in aquatic ecosystems. This could lead to high concentrations of the contaminant in the bodies of animals with higher trophic positions (e.g., predators at the top of the food chain).

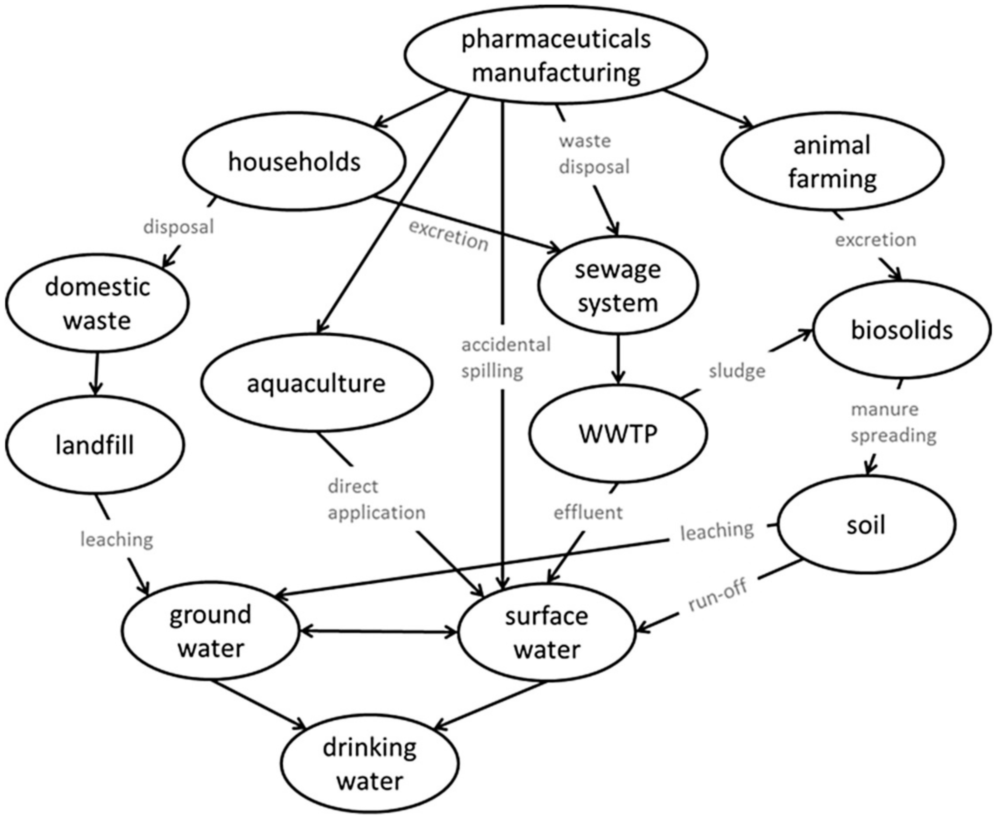

Figure 1 Source of pharmaceuticals which end up in the nature (Magureanu et al. 2015).

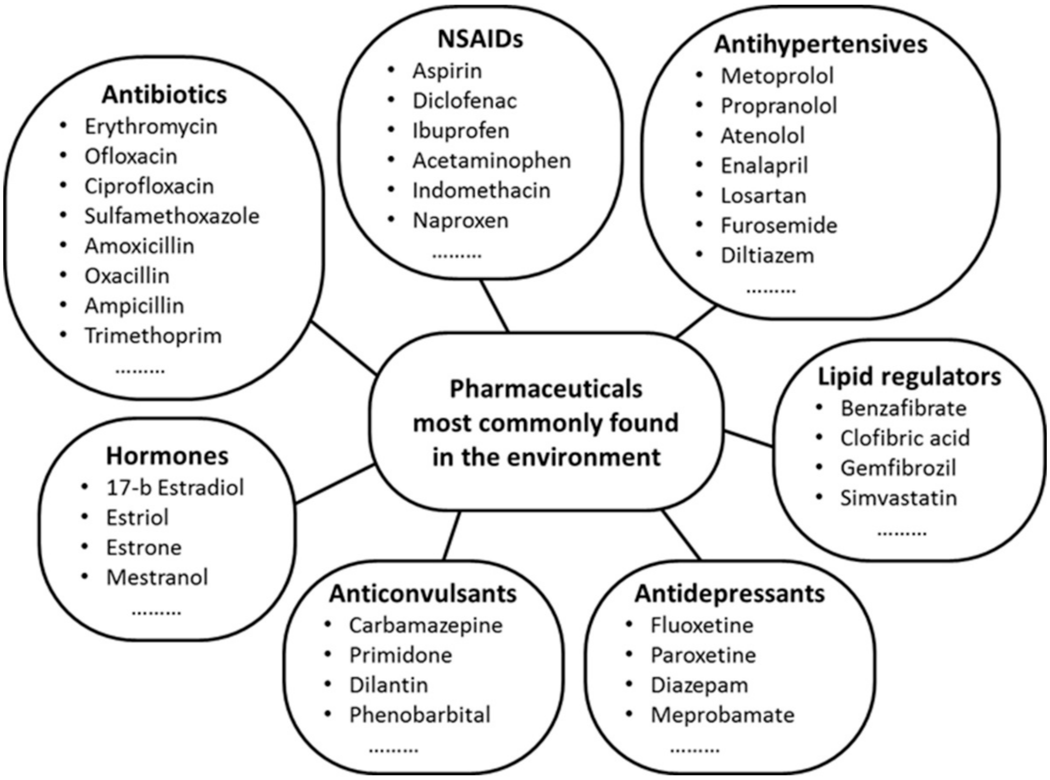

Figure 2 Pharmaceuticals frequently detected in the environment (Magureanu et al. 2015).

3.1.2 Sertraline

Sertraline belongs to the group of SSRI and is an active ingredient in Zoloft, which is one of the most commonly prescribed antidepressants in the world. It is used to treat conditions such as anxiety, panic disorder, and obsessive-compulsive disorder (Fong & Ford 2014). That is why sertraline is one of the most common substances entering the nature with effluent from sewage plant (Schultz et al. 2010).

Sertraline and other compounds among SSRIs present a growing concern for environmental biology and aquatic toxicology, especially sertraline was known for its potential risk of bioaccessibility and bioaccumulation (Brooks et al. 2005; Grabicova et al. 2015, 2017; de Solla et al. 2016; Dvořák et al. 2017) and was found to be the most sublethally toxic to many aquatic test organisms (Henry et al. 2004; Johnson et al. 2007; Minagh et al. 2009; Conners et al. 2009; Hazelton et al. 2014; Lamichhane et al. 2014). Gilroy et a l. (2017) has conducted a test on freshwater mussels over their life stage, including the larval parasites on fish. An acute toxicity was demonstrated with a 24-h LC50 of 0.06 mg L^{-1} of sertraline on glochidia. Although sertraline was not toxic to adult mussels at concentrations up to 0.3 mg L^{-1}, for the 28-d juvenile mussel sertraline was lethal (LC50 for sertraline was 0.04 mg L^{-1}) (Gilroy et al. 2017).

3.2 Freshwater organisms and their major threats

Even fresh water makes up only 0,01 % of water on our planet, this small fraction provides home for more than 100 000 species (Dudgeon et al. 2006). The freshwater ecosystems not only provide habitat for their inhabitants but also provide many important goods and services for human, besides storage of clean water, flood and erosion control. But with the increasing human population, the high value of freshwater ecosystems is often overlooked (Darwall et al. 2009).

Invertebrates create the majority of freshwater animals; molluscs are the most diverse but also most threatened group of animals. At the European level at least 43.7% (373) species of freshwater molluscs are considered threatened and at least 12.8% of them are Critically Endangered (Cuttelod et al. 2011). Freshwater biodiversity is experiencing much larger declines and the future extinction would be probably 5times bigger than of terrestrial fauna (Ricciardi & Rasmussen 1999). Water pollution, habitat modification linked with flow modification, alien invasion and overexploitation are the biggest threats impending the aquatic biodiversity (Figure 3).

Land use is a way how human use and modify the natural environment to their benefit. Altering the environment leads to many changes in the features of this environment and become often less beneficial to the inhabitants of this environment. Habitat loss due to the construction of dams, river revitalization, reduced surface storage, increased effective drainage area or other direct human interventions altered the dynamics of flows. Cumulatively, these changes in hydrology, geomorphology, nutrient cycling, and sediment dynamics have had profound implications for freshwater ecosystems (Blann et al. 2009) and has been shown to lead to many population declines, e.g. several fish species (Dudgeon et al. 2006). The decline of these fish species should have an impact on freshwater mussel species that depend on them (Kelner & Sietman 2000; Freeman et al. 2003; Haag 2012).

Overexploitation has created a threat especially for fish for many years. The subject of interest are mainly species with high commercial value. The overexploitation is directly responsible for the decline in fish abundance and biomass. Furthermore, the incidental capture of non-target fish species can also affect other less profitable species (Allan et al. 2006) increasing the chances to cause negative effects on fish-mussel interactions (Modesto et al. 2018).

Metal pollution has been creating a major problem for freshwater ecosystems over a long period due to the legacy of the past contamination and currently increasing metal emission. Along this, toxic exposure in the environment occurs as mixtures, it is needed to consider the combined effect with other compounds (de Souza Machado et al. 2016). Following this, Cravo et al. (2012) found complex patterns of spatial and temporal trends for covarying metals, hydrocarbons and tributyltin, with strong interaction of contaminants and water physico-chemistry (temperature, salinity, dissolved oxygen) on driving physiological responses of the bivalve molluscs.

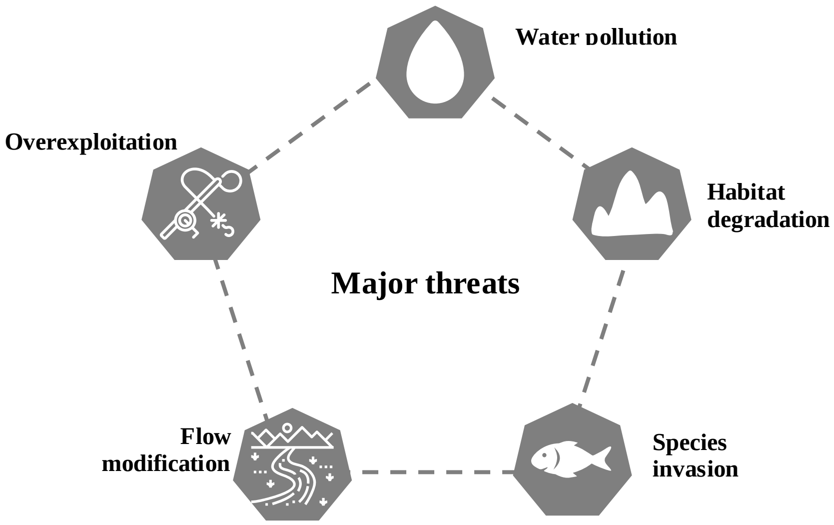

Figure 3 Five major threats which freshwater biodiversity is exposing to

3.2.1 Freshwater bivalves and their fish hosts

The subject of interest in our experiment is freshwater mussel *Unio tumidus* Philipsson, 1788 (Bivalvia, Palaeoheterodonta, Unionida, Unionidea). This family has the biggest species richness within the order Unionida with 620 species in 142 genera widely distributed across the freshwater ecosystems of Europe, Asia, North America and Africa (Bogan & Roe 2008). The Czech Republic hosts 8 species from freshwater bivalve mussels Unionida (Horsák et al. 2018). Red List of Threatened Species of the Czech Republic (Beran et al. 2017) recorded *U. tumidus* in the Czech Republic as a vulnerable species, which means this species can become endangered unless the circumstances that are threatening its survival improve. There has been a decline of population in the past, where the causes of the decline may not have disappeared or have not to be known or never be reversible. Nowadays this species inhabits locations of slowly flowing bigger streams with rich nutrients in Bohemia and Moravia including Třeboňsko, Southern Moravia and Polabí. Possible reason of habitat loss could be found in increasing pollution in streams as well as water flow modifications (Beran 2002). Their distribution covering Bohemian and Moravian streams is depicted in Figure 4 (Horsák et al. 2018).

The order Unionida, also known as pearly mussels or naiades, is a group of widely distributed aquatic bivalves residing freshwater environment like small ponds, lakes, and rivers and they have a unique life trait (Lopes-Lima et al. 2014; Douda 2015). All taxa included here are obligate aquatic organisms and spend their whole life cycle in freshwater (Bogan & Roe 2008). The overwhelming majority of glochidial hosts are fish, only very few species were known to successfully infect amphibians (Howard 1915; Barnhart et al. 1998; Watters & O'Dee 1998; Watters 2008). Glochidia are released by the female mussel into water. To survive, they must attach to the host fish (gills, fins or other tissue they come to contact with) followed by encystment. After encysting to a potential host, those glochidia that did not fall off from the host, are able to complete their metamorphosis. While encysting, which lasts days to months, the glochidia are dispersed by the fish to new sites. Adult mussels are able only of limited movement for short distances. Only the dispersal by fish hosts is an effective way to scatter. When the glochidia metamorphose, they drop from the host body to begin life as a juvenile mussel (Bogan 1993; Graf 1997). The whole process is complicated and driven by many factors which make this life stage highly sensitive and vulnerable. Female mussel release tens of thousands up to millions of glochidia during spawning event but very few of them successfully metamorphose into juvenile individuals and develop into free-living clams (McMahon 1991; Bauer 2006). Release timing is very important for glochidia survival success. Some species release glochidia very often over a short time period when fish abundance is increased (Trdan & Hoeh 2006; Zale & Neves 2008). In case of less suitable habitat with many disruptive conditions female mussel may use a sit-and-wait strategy when the adult wait over several months (in extreme case up to years) till they release their larvae.

The relationship between fish hosts and glochidia parasites appear to play a similarly important or larger role in mussels population dynamics and distribution than does food or habitats. Many studies have shown the valid evidence of mussels distribution and diversity that strongly depend on fish migration and species richness (Smith 1985; Theler & James 1987; Watters 1992; Lee et al. 1998). Except that, presence of suitable fish host, location of attachment on host body, fish defense mechanism and immunological reactions have been known to affect the time of encapsulation and have direct impact on metamorphosis success (Bauer & Wachtler 2001). Temperature may also affect the survival of glochidia on the host, while both glochidial development (Lefevre & C. Curtis 2005) and host immune response (Hruška 1992) are temperature-dependent.

Host-parasite interaction is mutual relationship which influences both partners of this interaction. Whereas glochidia are profiting, fish on the opposite are gaining more likely adverse effect from this relationship. Hosts loaded by glochidia parasites attached to gills caused increased ventilation by gills resulting in an increased energy cost of movements (Slavik et al. 2017), also a decrease movement, dispersal distance, body size were recorded in laboratory and wild. Further fish weakness caused by parasitism responses for higher predatory risk, reduced competitive ability and susceptibility to new parasites was demonstrated too (Crane et al. 2011; Horky et al. 2014; Filipsson et al. 2016; Gopko et al. 2018). Fish used in this experiment as the glochidial host is European chub (*S. cephalus*). This species was selected because it is a known host of *U. tumidus* (Lopes-Lima et al. 2017a) and is abundantly inhabiting central European waters. The reason why this fish shows high distribution and abundancy is thanks to their large ecological tolerance.

Unionida bivalves play integral role in aquatic system as essential ecosystem services that benefit many other organisms, e.g. water filter, nutrient cycling and being prey for other species (Vaughn 2010; Spooner et al. 2012). Once they were among the most abundant bivalve molluscs but now, unfortunately, these bivalves are among the most threatened freshwater taxa in the world (Carella et al. 2016; Lopes-Lima et al. 2017b; Modesto et al. 2018). Impact by human activities on aquatic environment is increasing and their long lifespan makes the exposure to the human impact even more significant (Dudgeon et al. 2006; Gagné et al. 2011). A total of 200 unionid species are on the IUCN Red List (Carella et al. 2016) and 202 of the nearly 300 unionid species known within The United States and Canada are listed by the Natural Heritage Network as presumed extinct, possibly extinct, critically endangered, endangered, or vulnerable (Master et al. 2000). About 30 North American taxa have become extinct in the last 100 years. They are facing one of the biggest decline but they receive much less publicity and attract disproportionately little research effort relative to vertebrates species. Continuous declines in mussel abundance may have irreversible effect in the whole aquatic biodiversity and may further adversely affect many other aquatic organisms whose life tightly relates to mussel community. In Europe the population status is sensitive and unstable. In few countries freshwater mussels are treated as critically endangered. To apply appropriate conservation program it is needed to have a comprehensive knowledge about themselves, their biology and ecology, habitats, niche and the interaction with fish (Haag & Williams 2014). Therefore freshwater mussels have become a subject of an increasing number of researches studying their behavior, effect of human activities on them (ecotoxicological organisms) or using them as the bioindicator to environmental stressors (Carella et al. 2016; Dvořák et al. 2017). Numerous laboratory studies have been conducted on freshwater mussels in order to understand the role of contaminants in waters in the decline of the populations (Valenti et al. 2005; Ingersoll et al. 2006). In particular, there have been studies that have assessed mussel sensitivity to a range of environmental contaminants including (Keller & Ruessler 1997), ammonia (Augspurger et al. 2003; Douda 2010; Soucek & Dickinson 2012; Moore & Bringolf 2018), cadmium (Wang et al. 2010; Markich 2017) and pharmaceuticals (Johnson et al. 2007; Bringolf et al. 2010; Hazelton et al. 2014; Stewart et al. 2014; Lamichhane et al. 2014; de Solla et al. 2016; Gilroy et al. 2017).

Czech Republic has already met the problem with pharmaceuticals. Based on country survey of aus der Beek et al. (2016), the Czech republic is one of the countries with 11-30 pharmaceuticals detected in surface/ground water and with 1-3 pharmaceuticals detected in tap/drinking water.

The effects of specific pharmaceuticals on freshwater mussel have been investigated, for example results of Bringolf et al. (2010) suggest, that fluoxetine accumulates in mussel tissue and has the potential to disrupt reproduction. Behavioral changes in adults are identified by Hazelton et al. (2013, 2014) with concentrations much closer to environmentally relevant concentration. This time they proved that fluoxetine altered movement in adults and therefore sediment nutrient cycling and oxygenation might be influenced consequently. Mussels are also known to be sensitive to some ionic compounds such as ammonia, potassium, copper, zinc and cadmium (Kováts et al. 2010; Moore & Bringolf 2018). Douda (2010) has documented that $N\text{-}NO^{-3}$ can have direct effect on spatial distribution of freshwater mussels, on the other hand juveniles showed certain tolerance towards $N\text{-}NO^{-3}$ than most previously tested freshwater macroinvertebrates. Mussels are long living organisms and capable of very limited movements, which force them exposing to all chemicals contained in their nature habitat. Some of the pharmaceuticals were identified to be very dangerous to mussels because of their stable and bioaccessible character in the environment (Dvořák et al. 2017; Grabicova et al. 2017; Rozman et al. 2017). There are already studies proving bioaccumulative ability of sertraline in mussels (Du et al. 2014; de Solla et al. 2016).

Influence on adult mussels has been well documented, but so far there is few of researches dealing with glochidia-larval stage of mussels. Glochidia are highly sensitive stage of mussel life cycle. As mentioned, glochidia are obligate ectoparasites and encyst by host tissue. Three to four weeks later following encystment, larvae undergo metamorphosis and become juvenile mussels. At this moment, they excysts from fish host (Aldridge & McIvor 2002). According to some hypothesis it came to such coevolution accidentally at the beginning. Now the advantage of such a strategy is dispersion from mature mussels, particularly in upstream direction. On the other hand it makes glochidia highly depended on fish hosts without who they are not able to complete the metamorphosis (Douda 2015). Besides that, fish size, immune system and host species are also essential key factors driving glochidial successful survival and dynamics of mussels communities. (Modesto et al. 2018). In some cases, mussels form an association with a very narrow range of suitable host fish sharing similar niches which results in a bigger assurance of favorable habitat for juvenile mussels. For example, experiment (Huber & Geist 2017) with *Anodonta cygnea* (Linnaeus 1758) from the same family Unionidae showed this species can be considered as generalist because of broad occurrence, different fish host species may have various effects on them concerning metamorphosis. But there is no detail information about preferences of *U. tumidus* in fish host species so far.

All these factors indicate that glochidia survival and mussel community dynamics are closely associated and limited by fish host availability and larval stage is the more vulnerable and important. Hence, the key leading to successful development of mussels' conservation should take into consideration their complicated life cycle, including the larval stage and the host-parasite interactions (Ćmiel et al. 2018).

On the other hand, there is an increasing number of researches regarding glochidia as appropriate testing organisms for ecotoxicity tests because of their high sensibility to chemicals (Lopes-Lima et al. 2014).

Future conservation actions should be focusing not only on one species, mussels or fish, but look at those species as a complex and apply program creating protection for both of them. Furthermore, many factors remain unknown for example fish compatibility to glochidia is not well explained. This is a useful information influencing glochidial survival success related to mussels population and later the mussels protection. Relatively new research has revealed that fish-glochidia relationships can vary both between geographically distinct lineages of species and within these lineages contrasting histories of sympatry (Reichard et al. 2015) which should be taken in concern as well.

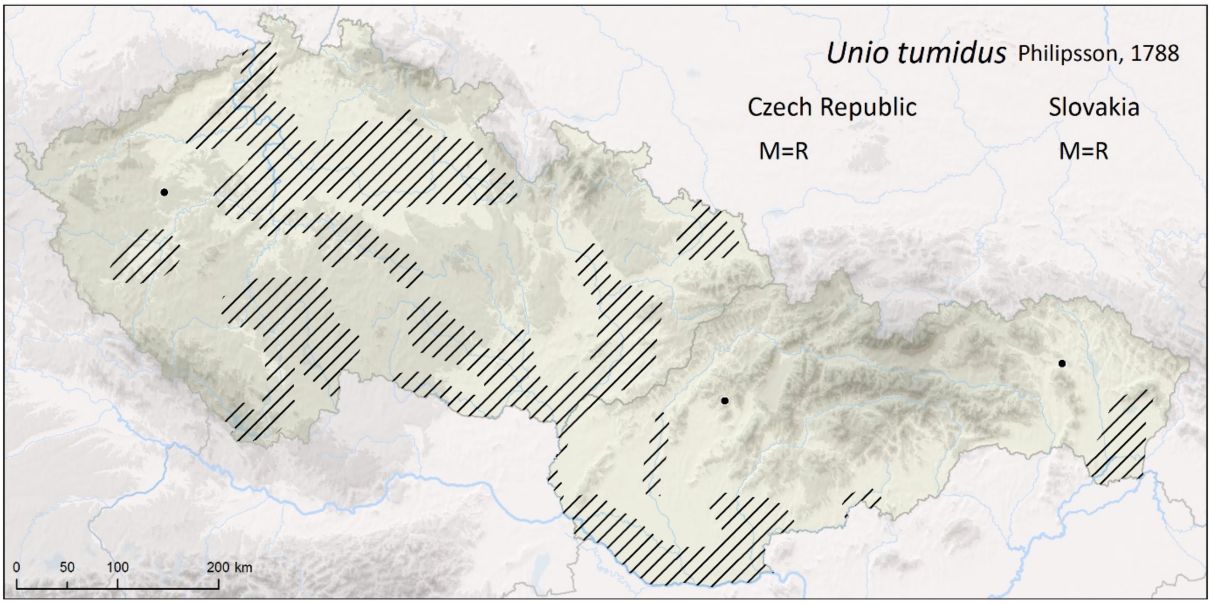

Figure 4 Distribution of U. tumidus in Czechia and Slovakia (Horsák et al. 2018).

4 MATERIALS AND METHODS

4.1 Chemicals and Water

Sertraline was received in concentration of 10 mg L^{-1} in ultrapure water and used for preparing the exposure solutions by dilution of the stock solution with Prague (Czech Republic) tap water into two concentrations: 0.2 µg L^{-1} (0.65 nmol L^{-1}) and 4 µg L^{-1} (13.06 nmol L^{-1}). For verifying the concentration in aquaria exposure solutions was applied method with liquid chromatography with tandem mass spectrometry (LC-MS/MS). Water samples were frozen at - 20°C until the analysis. The details concerning measuring method are not subject of this thesis and will be described in more detail in the related manuscript with wide collective of authors (Zhao et al. in prep).

4.2 Experimental animals

4.2.1 Host fish

We chose the European chub (*S. cephalus)* as the host fish for interacting with glochidia in our infection experiment. This species was selected because it is a known host of *U. tumidus* (Lopes-Lima et al. 2017a) and is abundantly inhabiting central European waters. *U. tumidus* used in our experiment were hatchery reared juveniles obtained from a local fish supplier (Vodňany, Czech Republic). Therefore no previous contact with glochidia was guaranteed. Sixty individuals of a similar size (1+ year old) in total were used for this experiment. The fish weights and standard lengths (means ± SD) at the end of the experiments were 6.05 ± 7.9 g and 75.7 ± 19.4 mm.

Two weeks prior to the start of the experiment, the fish were evenly divided into four separate holding tanks (200 L and 15 randomly selected individuals). Two of the identical tanks were intended for the exposure groups and the remaining two for the control groups. Furthermore, the tanks were placed in different laboratories to avoid the possibility of cross-contamination of the control group. Fish were fed once a day with commercial fish pellets for cyprinids (Biomar Group, Denmark). The pellets were equally distributed over the tanks to provide free access to food for all individuals and the fish were maintained on a photoperiod of 12 h of light and 12 h of darkness, which was the same regime as they were held in the hatchery. Two-thirds of the water volume was renewed with aged tap water every day to habituate the fish to the experimental regime. The water temperature was automatically controlled with air conditioning throughout the entire experiment and was maintained at an 20-22°C. All fish used for the experiments were randomly selected from all four tanks.

4.2.2 Glochidia

Glochidia of freshwater bivalve *U. tumidus* were chosen to be used as the test parasites in the experiment. Gravid mussels were collected from the Morava River (Czech Republic: 48°41'17"N, 16°59'25"E) and transferred to stock tanks (individual aerated aquaria with a volume of 20 L) at the Czech University of Life Sciences in Prague until the release of glochidia. The presence of sertraline in this river was not proved. Glochidia were carried out for exposure immediately after acquisition from the parent mussels by flushing the marsupial with water using a syringe (Douda et al. 2013) from three female mussels.

The viability of the glochidia isolated from each female was evaluated by their response to the addition of a concentrated solution of NaCl . The proportion of healthy glochidia snapping shut in response to the addition of saline solution was > 85 %.

Glochidia from three female mussels were pooled immediately into a water tank (5 L) prior to experimental inoculation. The glochidia were rinsed and separated from the clumps by whirling and were then allowed to settle and the water was decanted instantly before exposure to sertraline (Hanstén et al. 1996).

4.3 Design of exposure

Experiment of exposure of glochidia to sertraline was performed following standard methods of glochidia toxicity testing (Farris et al. 2006). Three levels of concentration were used: *CONTROL* (0 µg L^{-1}), *LOW-EXPOSED* (0.2 µg L^{-1}, equals to 0.65 nmol L^{-1}) and *HIGH-EXPOSED* (4 µg L^{-1}, equals to 13.06 nmol L^{-1}). Fish exposure was modeled with two levels of concentration (*CONTROL* and *EXPOSED* with 0.2 µg L^{-1} of sertraline). The real concentration of sertraline in exposure and control solutions were checked before and by the end of exposure period (Table 1).

There were 20 replicated exposure chambers prepared for each concentration. Each dish contained 200 mL of sertraline solution or aged tap water in case of control groups. Glochidia were used immediately after harvesting and assigned randomly and evenly possible to 60 dishes of sertraline solutions with approx. 2000 glochidia/dish. Dishes were sealed with plastic foil and rubber band for 24-hour exposure. The length of exposure was arranged on 24 hours due to the degradability of this chemical and glochidia's viability in term to simulate the most relevant environmental scenarios. In comparison with other mussel's stages of life, glochidia have relatively short viability after being released from female mussel into water. To maximize the efficiency of attachment, they remain viable only for few days to find a host and infest him. Hence the time when they are exposed to the chemicals in water is short. For this reason, 24-hour exposure of glochidia to sertraline was used ecologically relevant scenario (Douda et al. 2019).

In contrast, fish remain viable for a longer period and exposure of fish was customized for 6 weeks (42 days). Thirty sertraline-exposed fish and thirty control fish were held separately to be prevented from cross-contamination of control fish. Exposed fish were otherwise kept under the same conditions (e.g. water, temperature etc.) as control fish but with the pharmaceutical addition. Feeding and water changing (two thirds of water) was performed once a day. For keeping the concentration of sertraline in fish body, sertraline was added every other day to keep the concentration in water at ~ 1 µg L^{-1}. The real concentration of sertraline in exposure and control solutions were checked before and after water exchange for fish by liquid chromatography tandem mass spectrometry (Table 1).

4.3.1 Glochidia viability testing

Before starting a toxicity test, we verified glochidia viability (survival endpoint) by addition of chemical stimulus NaCl described by Wang et al. (2007a). This endpoint was performed to investigate prospective effect of the pharmaceutical on glochidia. At the end of 24-h exposure period, approximately 50 individuals from each group were put into dish filled with dechlorinated tap water. Each dish with glochidia was recorded by a camera installed on a microscope (10x magnification). Before starting the video recording, number of closed glochidia was written down. Camera started recording 1 min. before NaCl addition and kept recording 1 minute after first dose of NaCl added. Then we added second drop of NaCl solution (same place and intensity) and recorded another 15 seconds. Opened and closed glochidia were counted. Valve closure induced by NaCl is a survival endpoint ecologically relevant to attachment on fish (Wang et al. 2007a). Viability was tested by formula:

Viability (%) =

$$\frac{100 \times \begin{pmatrix} \text{total number of glochidia} - \text{number of closed glochidia before adding NaCl} \\ - \text{number of open glochidia after adding NaCl} \end{pmatrix}}{total\ number\ of\ glochidia\ in\ each\ dish}$$

4.3.2 Infestation experiment

The experiment was based on a controlled infestation of fish host *S. cephalus* by glochidia of *U. tumidus* to simulate environmentally relevant infection process and to evaluate parameters characterizing the initial stage of attachment and encapsulation which drives the success of survival under laboratory conditions.

The response variables of infestation success were studied in a full factorial design (3x2) resulting in six treatment combinations of control and exposed host-parasite interaction. All control and exposed groups were treated under identical conditions during the whole infestation experiment. Three parameters in total were monitored to analyze the infestation capability of sertraline-exposed glochidia and fish, as well as the effect of interaction with hosts. Glochidia infection success was tested with combination of non/exposed-glochidia and non/exposed-fish (3 levels of glochidia exposure: *CONTROL*, *LOW-EXPOSED* and *HIGH-EXPOSED*, 2 levels of fish exposure:*CONTROL* and *EXPOSED*) Each combination of glochidia and fish exposure treatments had 10 replications (60 in total). Shortly before infestation, 60 individuals of fish (30 exposed and 30 controls) were removed from the aquaria and placed into three-liter polycarbonate tanks (1 fish per tank) with 1-2 L of tap water. Glochidia were replaced from exposure dishes to infestation tanks with fish which started the experiment. Air stone with periodic smooth pipetting was used for water aeration and keeping the glochidia in the suspension. After attachment period of 15 minutes, fish infested by glochidia were transferred to new tanks and were monitored for next 24 hours. Infestation tanks with unsuccessful glochidia were filtered (139 µm) to count the number of unattached glochidia in the bath. After 24 hours of monitoring, fish were sacrificed, dissected and the number of attached glochidia was detected separately for different body parts.

Monitoring tanks with unsuccessful glochidia were filtered by the same way to determine the number of glochidia detached from the individual fish. Brains were dissected out of fish and sent to laboratory for analyzing the real concentration of pharmaceutical as sertraline is able of bioaccumulation in fish tissue (e.g. liver, brain and kidney) according to experiment of Grabicova et al. (2015, 2017).

Sum of glochidia in this experiment was calculated by counting the total number of glochidia recovered from the tank and fish during the whole monitoring period. Attachment rate was describing the initial attachment success and it was defined by number of glochidia initially attached to fish during the first 15 min. of infestation experiment divided by the total number of glochidia released into the experiment. In contrast, infection success was defined as proportion of number of attached glochidia by the end of experiment and number of all glochidia initially attached. The spatial distribution of glochidia over fish body (gill ratio) was calculated by glochidia attached to gills in comparison to the whole body.

4.3.3 Statistical analysis:

Data distribution was tested by Shapiro-Wilk test. The sertraline concentrations of water and fish brains and glochidia viability were compared by a Welch two sample t-test. The infection parameters (e.g. attachment rate, infection success and gill ratio) were compared by generalized linear models (GLM, 2 and more independent variables, with binomial distribution and quasi-binomial error structure) followed by Tukey's HSD test. Non-parametric data of attachment rate were tested by Kruskal Wallis test followed by Dunn test for multiple comparisons (Ogle et al. 2018). The dependent variables as attachment rate, infection success and gill ratio were treated as binary. The independent variables were fish exposure and glochidia exposure and treated as qualitative which is supplemented by quantitative variables of concentration of sertraline contained in fish brains showing influence on attached glochidia. Individual subsets of infection parameters were tested by Student's t-test. Values are displayed as mean ± SD if not specified otherwise. All analysis were carried out in R software (R Core Team 2016).

5 RESULTS

5.1 Exposure levels

Measured concentrations of sertraline in exposure solutions and control treatments (before and at the end of exposure for glochidia and before and after the water exchange for fish) are displayed in Table 1 and were consistent over the experiment for all groups (Welch two-sample t-test and paired-sample Wilcoxon test: $p > 0.05$). Control groups had lower concentration of sertraline than limit of quantification identify the control samples of water were sertraline-free.

For fish exposed to 0.2 μg L^{-1} of sertraline was found sertraline accumulated in their brains with average concentration at 2.21 μg g^{-1} ± 3.9 μg g^{-1} (Two-sample Wilcoxon test: $p = 0.029$) (Table 3 in Enclosures).

Table 1 Measured concentration of sertraline in the control and exposure solutions of both of glochidia and fish before the exposure started and at the end of glochidia exposure period (0-24 h) and for fish before and after water exchange (w. e.) during the host exposure period (30 d). Concentration determined by liquid chromatography tandem mass spectrometry (n = number of samples analyzed). The concentration for sertraline was 0.65 nmol L^{-1} (0.2 μg L^{-1}) and 13.06 nmol L^{-1} (4 μg L^{-1})

SERTRALINE	***Time***	***Group***		
Glochidia		*CONTROL* (μg L^{-1})	*EXPOSED* (μg L^{-1})	
			LOW-EXPOSED	*HIGH-EXPOSED*
	Before exposure	<0.01	0.24 ± 0.03	3.95 ± 0.25
	After exposure	<0.01	0.1575 ± 0.0375	3.7 ± 0.8
Fish		*CONTROL* (μg L^{-1})	*EXPOSED* (μg L^{-1})	
	Before w. e.	<0.011 ± 0.001	0.20 ± 0.06	
	After w. e.	<0.011 ± 0.001	0.26 ± 0.09	

5.2 Glochidia viability and infestation success

5.2.1 Viability of glochidia

The viability of glochidia *U. tumidus* simulated by NaCl was approximately the same over three levels of concentration. The percentage of reacting glochidia was 90.93 % in *CONTROL* group, 91.46 % in *LOW-EXPOSED* group and 90.93 % in *HIGH-EXPOSED* group. Statistical significance among groups was not proved (GLM: all $p > 0.05$).

5.2.2 Attachment rate

In contrast to the glochidia viability, initial attachment success was found different among groups. *HIGH-EXPOSED* glochidia had the highest percentage (11.96 %) of attachment rate during the 15minute infestation than other two groups (both had 8.96 %). Statistically *HIGH-EXPOSED* glochidia revealed different attachment rate (GLM: df= 2,57; $p = 0.0196$) than other groups as well (Figure 6 A). In addition, sum of sertraline and its metabolites (norsertraline) in fish brains as the independent variable has confirmed the difference in attachment rate (GLM: df= 2,57; $p = 0.0246$). This reflected a different attachment success among non-exposed and heavily exposed glochidia. But no significant difference was proved within control glochidia and glochidia with low content of sertraline.

The opposite site of this relationship appeared to be affected too. There was an ascending trend in between fish groups showing more glochidia on exposed fish (10.9 % ± 2.3%) than on control (8.9% ± 2.2 %) (Figure 6 A). Attachment rate has showed a slight statistical difference among fish groups (GLM: df= 1,56; $p = 0.0614$). By collected data from fish brains we tested whether there was a difference between concentration of sertraline (as a quantitative independent variable instead of categorical) in fish brain and glochidia exposure. The sum of sertraline and norsertraline in fish brain confirmed the significance with GLM test (GLM: df= 1,56; $p = 0.0454$). The verified value indicated an increase of attachment success of glochidia with increasing sertraline in fish brains. Then we checked the differences at the individual level and found a fish subset revealing a significant difference (Welch Two Sample t-test: df = 12.98, p-value = 0.0065) between control and exposed fish within non-exposed glochidia group at individual level (Figure 5 A). At the level of glochidia exposed to high level of sertraline, fish showed a slight difference in attachment rate (Figure 5C) but the statistically no difference was observed ($p > 0.05$). See Table 2 to check all p values with tested endpoints.

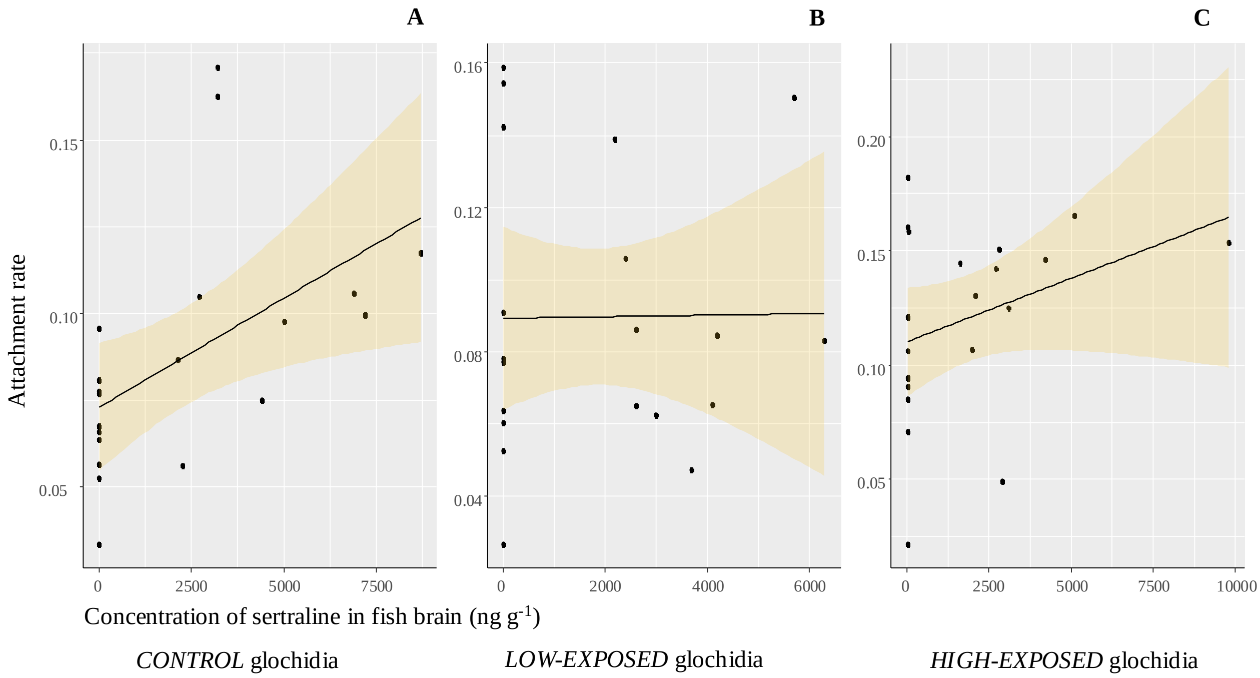

Figure 5 Attachment rate described by concentration of sertraline detected in fish brain at individual level (each figure depicts different glochidia groups). Dependent variable is attachment rate and independent is concentration of sertraline in fish brain. Figure 5A is comparing attachment rate with sertraline in fish brain within control glochidia. Figure 5B depicts *LOW-EXPOSED* glochidia with 0.2 µg L^{-1} and figure 5C shows *HIGH-EXPOSED* glochidia of 4 µg L^{-1} sertraline. There was a relationship between attachment rate and sertraline in fish brain of fish infected to control glochidia. A slight connection can be observed at the level of glochidia exposure 4 µg L^{-1}.

5.2.3 Infection success

In contrast to attachment rate, the infection success indicating encapsulation success did not show the significance within fish and glochidia groups (all groups have $p > 0.05$) (Table 2 and Figure 6 B), even percentage of parasite exposure has showed a slight change between *HIGH-EXPOSED* (4 µg L^{-1}) (64.22 %) and control (0 µg L^{-1}) individuals (68.67 %). Neither sertraline in fish brains has shown any dissimilarity among groups ($p > 0.05$). We also tested differences at the individual level between fish or glochidia subset and none of results dismissed the null hypothesis.

5.2.4 Gill ratio

In the case of gill ratio, a significant effect of glochidia exposure could be observed between *CONTROL* and *HIGH-EXPOSED* glochidia (GLM: df= 2,57; p = 0.00307). At individual level the exposed fish were attached to gills by more control glochidia (101.9 %) than fish without exposure (87.1 %). The effect of parasite exposure (*HIGH-EXPOSED*) demonstrated even a bigger difference in control fish (102.5 %) compared to the exposed fish (160.2 %). More glochidia on gills than on the remaining part of body to both control and exposed fish were depicted in Figure 6 C. Sertraline in fish brain confirmed to have effect on gill ratio at *HIGH-EXPOSED* glochidia while we tested with sum of sertraline and norsertraline (GLM: df= 2,57; p = 0.00333).

Table 2 Sublethal endpoints of acute toxicity test with host-parasite interaction. Glochidia and fish were exposed to different levels of sertraline. Generalized linear model with quasi-binomial error structure was used. Significant p-value are highlighted in red color and weakly significant are highlighted in yellow color. Attachment rate and gill ratio were significant with glochidia of 4 $\mu g\ L^{-1}$ of sertraline and fish exposure.

	Explanatory variables	***Attachment rate***	***Infection success***	***Gill ratio***
		P-value	P-value	P-value
Categorial independent variable (expressed by fish groups)	Exposed glochidia	0.01959 *	0.1286	0.003075 **
	Exposed fish	0.06142 .	0.2377	0.563441
	Glochidia:Fish	0.07588 .	0.2079	0.561072
Continuous independent variable (expressed by concentration of sertraline in fish brain)	Exposed glochidia	0.02464 *	0.1402	0.003333 **
	Sum sertraline in fish brain	0.04541 *	0.1255	0.848630
	Glochidia:Fish brain	0.34148	0.2666	0.885081

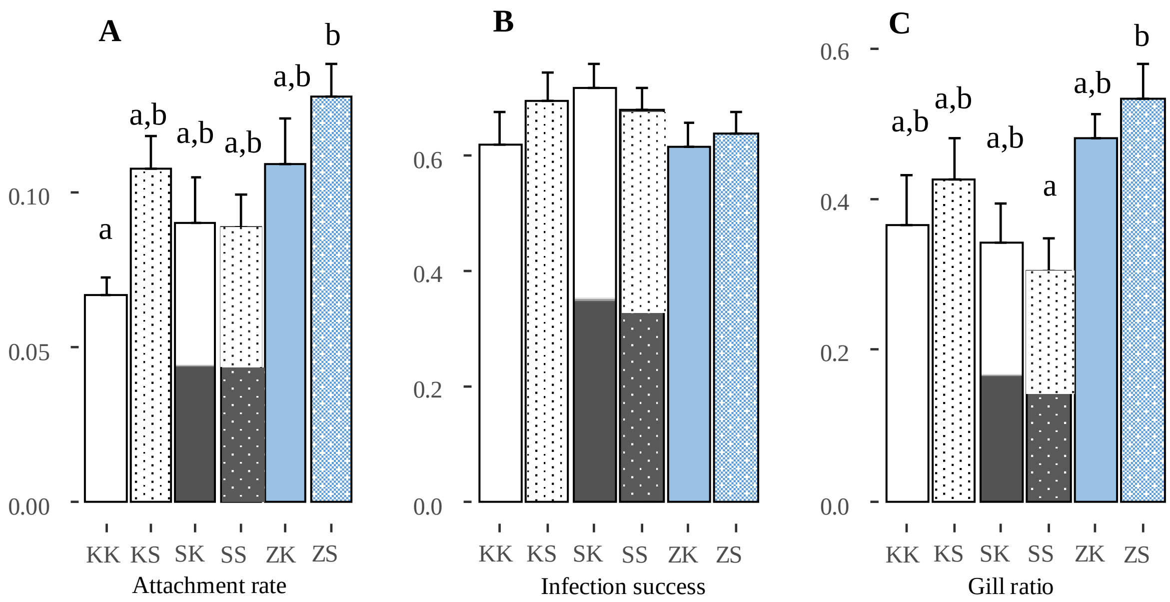

Figure 6: Sublethal host-parasite interaction parameters (Attachment rate - proportion of glochidia attached to fish; Infection success - proportion of successfully encapsulated glochidia; Gill ratio - proportion of glochidia attached to gills) were used as toxicity test endpoints. First letter in group indicates fish hosts exposure statement (K = *CONTROL*; S = *EXPOSED*). The second letter shows the exposure statement of glochidia (K = *CONTROL*; S = *LOW-EXPOSED*; Z = *HIGH-EXPOSED*). First two white columns are indicating control glochidia, the middle two are glochidia with 0.2 µg L^{-1} of sertraline and the last blue are glochidia of 4 µg L^{-1}.Significant results of multiple comparisons performed by Dunn test for multiple comparisons for attachment rate (non-parametric data) and Tukey's post hoc test (p<0.05) are indicated by different letters. See the results section and Table 2 for the significance of individual factors.

6 DISCUSSIONS

Our experiment was designated to study the ecological impact of sertraline on the mussel glochidia host-parasite interaction. Different scenarios of asymmetrical exposure of multitrophic systems were applied to compare with control treatment and defined influenced behavior of host or parasite. Designed experiment precisely determined which participant's exposure was associated with the change in endpoints. Results of this study highlight the importance of the presence of host-parasite relationship in ecotoxicological studies, without which the experiment can be incomplete and results can be misrepresented (Gustafson et al. 2016).

This study showed sertraline exposure affects initial attachment success, moreover in detail the experiment has proved that *HIGH-EXPOSED* glochidia had effect in this case. According to our collected data, sertraline induced a higher attachment in glochidia, but this was valid only for the high concentration. At lower level (0.2 µg L^{-1}) of sertraline the exposure was not supposed to alter the glochidia attachment behavior. On the other hand, the glochidial encapsulation success in combination with fish immunity, did not show any affected changes. Even at the highest designed concentration (4 µg L^{-1}) sertraline did not show the impairable effect to glochidia viability after 24-h of encapsulation. A presumable explanation for this might be that residual sertraline on glochidia could interfere fish immune response and then host-parasite interaction, which was similar to an experiment with fluoxetine made by (Hazelton et al. 2013). Also exposure length might play fundamental role in success of alteration by sertraline. Markich (2017) indicated that the sensitivity of each mussel species to metals increased 2.5-fold with increasing exposure time from 24 to 72 h.

Small but statistically significant differences were observed in spatial position of glochidia on host body. The high parasite exposure reflected difference from control glochidia which proved that sertraline facilitated parasite encystment to gills at the expense of fish. This body part is a strategical way for the success to glochidia, according to Itoh et al. (2003) gills create bigger chance for metamorphosis into juvenile. But for fish the gill encapsulation results in increased potential respiratory stress and higher risk to subsequent infection with other parasites e.g. trematodes which is a fish gill parasite (Filipsson et al. 2017; Gopko et al. 2018).

Viability test did not demonstrate statistical differences over all host and parasite exposure treatments in compare with other endpoints. The possible reason for this effect includes sertraline exposure being lower than lethal concentration (LC50) on glochidia. This fact is underlaid with experiment conducted by Gilro y et al. (2017a) with 24-h LC50 concentration at 60 µg L^{-1} of sertraline and our highest exposure was 4 μg/L, which is about 15-fold lower than the proved LC50. Another study based on toxicity test of zebrafish *Danio rerio* embryo showed low mortality rate even at 100 µg L^{-1} of sertraline. 100-fold higher concentration (10 000 µg L^{-1}) led to the 100% mortality of all examined organisms (Ribeiro et al. 2015). Test assessed on *Daphnia magna* showed toxicity of sertraline at 1.15 mg/L (1 150 µg L^{-1}) as LC50. But although concentrations used in this study were not lethal, it did not mean that sertraline left no effects on them. Evidence of consequence of sertraline was found at other endpoints mentioned above. According to many sources, sertraline was one of the most toxic selective serotonin reuptake inhibitors to aquatic invertebrate (Henry et al. 2004; Johnson et al. 2007; Minguez et al. 2014; Gilroy et al. 2017). However, for certain chemical, toxicity can vary in life stages and species (Cherry et al. 2002; Augspurger et al. 2003). Furthermore, sertraline toxicity is inconsistent as pH value of its matrix is changing. Valenti Jr. et al. (2009) have found sertraline toxicity as pH dependent in their study which detected the variability in LC50 of sertraline for examined organism *Pimephales promelas,* sertraline toxicity increased 9-fold as 647, 205, and 72 µg L^{-1} at pH 6.5, 7.5, and 8.5.

Fish from this host-parasite interaction was affected by sertraline in attachment phase too. Even there was proof neither by fish exposure nor by glochidia exposure on the second endpoint (infection success) describing capability of glochidia to stay attached on fish and the encapsulation success, we can suggest an effect of sertraline on fish as well as on glochidia. Exposed fish were more prone to glochidial attachment then control fish and more glochidia were found attached to gills then to the remaining part of fish body.

Altered fish behavior connected with sertraline in brain is underlined by other already published studies demonstrating that sertraline and it’s metabolites are classified as potentially bioaccumulative and bioaccessible compound in tissues of fish living in effluent-dominated streams (Grabicova et al. 2015; Xie et al. 2015; de Solla et al. 2016; Dvořák et al. 2017). Brooks et al. (2005) observed higher concentrations of antidepressants and their metabolites in the brain and liver of exposed fish than in muscle. Further (Grabicova et al. 2017) in their study demonstrated that sertraline was the only one chemical among other 10 tested detected in the fish brain. This support the output of this experiment that suggested an increasing trend of sertraline in fish brain which were not exposed by sertraline but these fish were infested by glochidia exposed to sertraline. We suggest there might be a potential drift of sertraline within host-parasite relationship and sertraline might be bioaccumulative in fish brain.

An increasing number of studies are finding biological effects of at lower concentrations but not at higher concentrations (De Lange et al. 2006; Conners et al. 2009; Painter et al. 2009; Guler & Ford 2010; Bossus et al. 2014; Fong & Ford 2014). For example, Conners et al. (2009) have proved biological changes on metamorphose of *Xenopus* tadpoles while exposing to low and moderate concentration of sertraline and at moderate concentration to fluoxetine whereas tadpoles to high concentration behaved most similarly to controls. These non-monotonic responses are characteristic of occurrence of hormesis in dose responses, while low concentration activate adaptive stressors (in a beneficial way) and high concentration can harm (Fong & Ford 2014). Which gives us a warning that pharmaceuticals at environmentally relevant concentration might already impair the habitants of aquatic system. Therefore we suggest taking into account this factor while designing and evaluating the toxicity tests. Data gained by Grabicova et al. (2017) showed concentration of sertraline detected in the water sample from a Czech stream Zivny, where in November 2012 was reached the highest concentration: 10 + 4 ng/L, which is in agreement with experiment of Golovko et al. (2014). They concluded seasonal effect on concentration variability for determined pharmaceuticals (e.g. sertraline) that concentrations in waste water were higher during winter. The max. concentration in effluent was found at 6 ng/L (January 2012) in a WWTP in České Budějovice, Czech Republic. All data mentioned above representing a very low level of sertraline contamination in Czech streams in compare with designed concentration used in this experiment, thanks to the higher removal efficiency (RE) of specified method (Golovko et al. 2014), otherwise the removal efficiency is fluctuating depending on the used approaches (Manoli et al. 2019). Furthermore organisms inhabiting effluent-dominated streams are exposed to mixture of pharmaceuticals of various classes. Existing toxicity test with invertebrates has already proved that mixture of pharmaceuticals can produce additive effect (Henry & Black 2007). Risk of interaction of pharmaceutical mixture should be considered too.

It can be supposed that as the highly sensitive stage of the whole life cycle of mussels, glochidia are responsive to the stressor sertraline from the aquatic environment. This suggestion was underlying by many other reports, which indicated that glochidia and juvenile mussels are more sensitive to some chemicals in comparison with usually tested aquatic organisms, e.g. Cladocera, amphipod and fish, to name few (Henry et al. 2004; Bringolf et al. 2007; Wang et al. 2007b, 2007a; Gilroy et al. 2017).

These results highlight the potential of mussel glochidia bioassay as a promising and sensitive approach for testing the toxicity of new chemicals, esp. pharmaceuticals in aquatic environment than adult mussels thanks to their sensitive character (Gilroy et al. 2017). The approach used in this experiment can be applied in other host-parasite interaction to distinguish the effect of host-exposure from parasite or the influence of interaction and provide a more complete result about the host-parasite interaction. This experiment points out the importance of asymmetrical effects of exposure on the interaction output which determined a different level of effective concentration.

7 CONCLUSIONS

To conclude, our experiment was determined to examine the effect of sertraline on glochidia of freshwater mussels and the interaction with their fish hosts. The results demonstrated that sertraline was able to affect the highly sensitive life stage of freshwater mussels. Glochidia were impacted by sertraline in the attachment success and spatial position of attachment. Overall, the result of this thesis indicate that the early life-stage toxicity testing is a promising and sensitive approach for testing the toxicity of pharmaceuticals or other chemicals. Glochidia and their hosts can be recommended for future studies as bioassay organisms as well as they can be used as model groups for the identification of pharmaceutical multi-species impacts in the natural water bodies.

8 BIBLIOGRAPHY

AHFS. 2013. AHFS DI Monographs. Drugscom. Available from http://www.drugs.com/monograph (accessed 31.03.2019).

Aldridge DC, McIvor AL. 2002. Gill evacuation and release of glochidia by unio pictorum and unio tumidus (bivalvia : unionidae) under thermal and hypoxic stress. Journal of Molluscan Studies **69**:55–59.

Allan J, Abell R, Hogan Z, Revenga C, Taylor B, Welcomme RL, Winemiller K. 2006. Overfishing of Inland Waters. BioScience **55**:1041.

Augspurger T, Keller AE, Black MC, Cope WG, Dwyer FJ. 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry **22**:2569–2575.

aus der Beek T, Weber FA, Bergmann A, Hickmann S, Ebert I, Hein A, Küster A. 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. Environmental Toxicology and Chemistry **35**:823–835.

Baillie JEM, Hilton-taylor C, Stuart SN. 2004. 2004 IUCN Red List of Threatened Species. A Global Species Assessment.

Barnhart C, Riusech F, Baird M. 1998. Host of salamander mussel, Simpsonaias ambigua, and snuffbox, Epioblasma triquetra, from the Meramee River system, Mo. Triannu Unionid Rep:16–34.

Bauer G. 2006. Reproductive Strategy of the Freshwater Pearl Mussel Margaritifera margaritifera. The Journal of Animal Ecology **56**:691.

Bauer G, Wachtler K. 2001. Ecology and Evolution of the Freshwater Mussels Unionoida. Ecology and Evolution of the Freshwater Mussels Unionoida. Springer Berlin Heidelberg, New York.

Beran L. 2002. Aquatic molluscs of the Czech Republic–distribution and its changes, habitats, dispersal, threat and protection, Red List. Sborník přírodovědného klubu v Uherském Hradišti, Supplementum **10**:258.

Beran L, Juřičková L, Horsák M. 2017. Mollusca. - In: Hejda R., Farkač J. & Chobot K. [eds], Red list of threatened species of the Czech Republic Invertebrates. Příroda. Prague.

Blann KL, Anderson JL, Sands GR, Vondracek B. 2009. Effects of Agricultural Drainage on Aquatic Ecosystems: A Review. Critical Reviews in Environmental Science and Technology **39**:909–1001.

Bogan AE. 1993. Freshwater bivalve extinctions (mollusca: Unionoida): A search for causes. Integrative and Comparative Biology **33**:599–609.

Bogan AE, Roe KJ. 2008. Freshwater bivalve (Unioniformes) diversity, systematics, and evolution: status and future directions. Journal of the North American Benthological Society **27**:349–369.

Bossus MC, Guler YZ, Short SJ, Morrison ER, Ford AT. 2014. Behavioural and transcriptional changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and sertraline. Aquatic Toxicology **151**:46–56.

Brausch JM, Connors KA, Brooks BW, Rand GM. 2012. Human Pharmaceuticals in the Aquatic Environment: A Review of Recent Toxicological Studies and Considerations for Toxicity Testing. Reviews of environmental contamination and toxicology **218**:1–99.

Bringolf RB, Cope WG, Barnhart MC, Mosher S, Lazaro PR, Shea D. 2007. Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos, and permethrin) to glochidia and juveniles of Lampsilis siliquoidea. Environmental Toxicology and Chemistry **26**:2101–2107.

Bringolf RB, Heltsley RM, Newton TJ, Eads CB, Fraley SJ, Shea D, Cope WG. 2010. Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. Environmental Toxicology and Chemistry **29**:1311–1318.

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, Lewis RJ. 2005. Determination of select antidepressants in fish from an effluent-dominated stream. Environmental Toxicology and Chemistry **24**:464–469.

Calisto V, Esteves VI. 2009. Psychiatric pharmaceuticals in the environment. Chemosphere **77**:1257–1274.

Carella F, Villari G, Maio N, De Vico GD. 2016. Disease and disorders of freshwater unionid mussels: A brief overview of recent studies. Frontiers in Physiology **7**: 489.

Cherry DS, Van Hassel JH, Farris JL, Soucek DJ, Neves RJ. 2002. Site-specific derivation of the acute copper criteria for the Clinch River, Virginia. Human and Ecological Risk Assessment **8**:591–601.

Cizmas L, Sharma VK, Gray CM, McDonald TJ. 2015. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environmental chemistry letters **13**:381–394.

Ćmiel AM, Zajac K, Lipińska AM, Zajac T. 2018. Glochidial infestation of fish by the endangered thick-shelled river mussel Unio crassus. Aquatic Conservation: Marine and Freshwater Ecosystems:535–544.

Conners DE, Rogers ED, Armbrust KL, Kwon JW, Black MC. 2009. Growth and development of tadpoles (Xenopus laevis) exposed to selective serotonin reuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. Environmental Toxicology and Chemistry **28**:2671–2676.

Crane AL, Fritts AK, Mathis A, Lisek JC, Barnhart MC. 2011. Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, Etheostoma caeruleum? Animal Behaviour **82**:817–823.

Cravo A et al. 2012. A multibiomarker approach in the clam Ruditapes decussatus to assess the impact of pollution in the Ria Formosa lagoon, South Coast of Portugal. Marine Environmental Research **75**:23–34.

Cuttelod A, Seddon M, Neubert E. 2011. European Red List of Non-marine Molluscs. Luxembourg. Publications Office of the European Union.

Darwall W, Smith K, Allen D, Seddon M, Reid GM, Clausnitzer V, Kalkman VJ. 2009. Freshwater biodiversity: a hidden resource under threat. In IUCN. Wildlife in a changing world: An Analysis of the 2008 IUCN Red List of Threatened Species. Gland, Switzerland.

De Lange HJ, Noordoven W, Murk AJ, Lürling M, Peeters ETHM. 2006. Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. Aquatic Toxicology **78**:209–216.

de Solla SR, Gilroy AM, Klinck JS, King LE, McInnis R, Struger J, Backus SM, Gillis PL. 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel Lasmigona costata in a river receiving wastewater effluent. Chemosphere **146**:486–496.

de Souza Machado AA, Spencer K, Kloas W, Toffolon M, Zarfl C. 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity.

Science of The Total Environment **541**:268–281.

Di Poi C, Evariste L, Serpentini A, Halm-Lemeille MP, Lebel JM, Costil K. 2014. Toxicity of five antidepressant drugs on embryo–larval development and metamorphosis success in the Pacific oyster, Crassostrea gigas. Environmental Science and Pollution Research **21**:13302–13314.

Douda K. 2010. Effects of nitrate nitrogen pollution on central european unionid bivalves revealed by distributional data and acute toxicity testing. Aquatic Conservation: Marine and Freshwater Ecosystems **20**:189–197.

Douda K. 2015. Velcí mlži a jejich hostitelské vazby. Živa **5**:222–224. Nakladatelství Academia. Prague

Douda K, Lopes-Lima M, Hinzmann M, Machado J, Varandas S, Teixeira A, Sousa R. 2013. Biotic homogenization as a threat to native affiliate species: Fish introductions dilute freshwater mussel's host resources. Diversity and Distributions **19**:933–942.

Douda K, Zhao S, Vodáková B, Horký P, Grabicová K, Božková K, Grabic R, Slavík O, Randák T. 2019 (in press). Host-parasite interaction as a toxicity test endpoint using asymmetrical exposures. Aquatic Toxicology.

Du B et al. 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream. Philosophical Transactions of the Royal Society of London **369**.

Dudgeon D et al. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society **81**:163–182.

Dvořák T, Száková J, Vondráčková S, Košnář Z, Holečková Z, Najmanová J, Tlustoš P. 2017. Content of Inorganic and Organic Pollutants and Their Mobility in Bottom Sediment from the Orlík Water Reservoir (Vltava River, Czech Republic). Soil and Sediment Contamination **26**:584–604.

Farris JL, Van Hassel JH. 2006. Freshwater bivalve ecotoxicology. SETAC/CRC Press, Boca Raton, FL, USA.

Fent K, Weston AA, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology **76**:122–159.

Filipsson K, Brijs J, Näslund J, Wengström N, Adamsson M, Závorka L, Österling EM, Höjesjö J. 2017. Encystment of parasitic freshwater pearl mussel (Margaritifera margaritifera) larvae coincides with increased metabolic rate and haematocrit in juvenile brown trout (Salmo trutta). Parasitology Research **116**:1353–1360.

Filipsson K, Petersson T, Piccolo JJ, Österling EM, Wengström N, Höjesjö J, Näslund J. 2016. Heavy loads of parasitic freshwater pearl mussel (Margaritifera margaritifera L.) larvae impair foraging, activity and dominance performance in juvenile brown trout (Salmo trutta L.). Ecology of Freshwater Fish **27**:70–77.

Fleeger JW, Carman KR, Nisbet RM. 2003. Indirect effects of contaminants in aquatic ecosystems. Science of The Total Environment **317**:207–233.

Fong PP, Ford AT. 2014. The biological effects of antidepressants on the molluscs and crustaceans: A review. Aquatic Toxicology **151**:4–13.

Ford AT, Fong PP. 2016. The effects of antidepressants appear to be rapid and at environmentally relevant concentrations. Environmental Toxicology and Chemistry **35**:794–798.

Freeman MC, Pringle CM, Greathouse E a, Freeman BJ. 2003. Ecosystem-level consequences of migratory faunal depletion caused by dams. American Fisheries Society Symposium.

Gagné F, André C, Cejka P, Hausler R, Fournier M. 2011. Evidence of neuroendocrine disruption in freshwater mussels exposed to municipal wastewaters. Science of the Total Environment **409**:3711–3718.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (Margaritifera margaritifera L.): A synthesis of conservation genetics and ecology.

Gilroy EAM et al. 2017. The effects of pharmaceuticals on a unionid mussel (Lampsilis siliquoidea): An examination of acute and chronic endpoints of toxicity across life stages. Environmental Toxicology and Chemistry **36**:1572–1583.

Golovko O, Kumar V, Fedorova G, Randak T, Grabic R. 2014. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere **111**:418–426.

Gopko M, Chowdhury MMR, Taskinen J. 2018. Interactions between two parasites of brown trout (*Salmo trutta*): Consequences of preinfection. Ecology and Evolution **8**:9986–9997.

Grabicova K, Grabic R, Blaha M, Kumar V, Cerveny D, Fedorova G, Randak T. 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Research **72**:145–153.

Grabicova K, Grabic R, Fedorova G, Fick J, Cerveny D, Kolarova J, Turek J, Zlabek V, Randak T. 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Research **124**:654–662.

Graf DL. 1997. Sympatric speciation of freshwater mussels (Bivalvia: Unionoidea): a model. American Malacological Bulletin **14**:35–40.

Guler Y, Ford AT. 2010. Anti-depressants make amphipods see the light. Aquatic Toxicology **99**:397–404.

Gustafson KD, Belden JB, Bolek MG. 2016. Atrazine reduces the transmission of an amphibian trematode by altering snail and ostracod host-parasite interactions. Parasitology Research **115**:1583–1594.

Haag WR. 2012. North American Freshwater Mussels: Natural History, Ecology, and Conservation. Page North American Freshwater Mussels. Cambridge University Press.

Haag WR, Williams JD. 2014. Biodiversity on the brink: An assessment of conservation strategies for North American freshwater mussels. Hydrobiologia **735**:45–60.

Hanstén C, Heino M, Pynnönen K. 1996. Viability of glochidia of Anodonta anatina (Unionidae) exposed to selected metals and chelating agents. Aquatic Toxicology **34**:1–12.

Hazelton PD, Cope WG, Mosher S, Pandolfo TJ, Belden JB, Barnhart MC, Bringolf RB. 2013. Fluoxetine alters adult freshwater mussel behavior and larval metamorphosis. Science of the Total Environment **445–446**:94–100.

Hazelton PD, Du B, Haddad SP, Fritts AK, Chambliss CK, Brooks BW, Bringolf RB. 2014. Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels. Aquatic Toxicology **151**:27–35.

Henry T, Black M. 2007. Mixture and single substance acute toxicity of selective serotonin reuptake inhibitors in Ceriodaphnia dubia. Environmental toxicology and chemistry **26**:1751–1755.

Henry TB, Kwon J-W, Armbrust KL, Black MC. 2004. Acute and chronic toxicity of five selective serotonin reuptake inhibitors in Ceriodaphnia dubia. Environmental toxicology and chemistry **23**:2229–2233.

Horky P, Douda K, Maciak M, Zavorka L, Slavik O. 2014. Parasite-induced alterations of host behaviour in a riverine fish: The effects of glochidia on host dispersal. Freshwater Biology **59**:1452–1461.

Horsák M et al. 2018. Check-list and distribution maps of the molluscs of the Czech and Slovak Republics.

Howard AD. 1915. Some exceptional cases of breeding among the Unionidae. Nautilus **29**:4–11.

Hruška J. 1992. The freshwater pearl mussel in South Bohemia: Evaluation of the effect of temperature on reproduction, growth and age structure of the population. Arch Hydrobiology **126**:181–191.

Huber V, Geist J. 2017. Glochidial development of the freshwater swan mussel (Anodonta cygnea, Linnaeus 1758) on native and invasive fish species. Biological Conservation **209**:230–238.

Ingersoll CG, Kernaghan NJ, Gross TS, Bishop CD, Wang N, Roberts A. 2006. Laboratory toxicity testing with freshwater mussels. Pages 95–134 in J. L. Farris and J. H. van Hassel, editors. Freshwater Bivalve Ecotoxicology.

Itoh T, Oda N, Maruyama T. 2003. Availability of host fish for glochidia of the freshwater unionid mussel Pronodularia japanensis. Japanese Journal of Ecology **53**:187–196. Plankton Laboratory, Department of Ocean Sciences, Tokyo University of Fisheries, Tokyo 108-8477, Japan.

Jenicek P, Kutil J, Benes O, Todt V, Zabranska J, Dohanyos M. 2013. Energy self-sufficient sewage wastewater treatment plants: is optimized anaerobic sludge digestion the key? Water Science and Technology **68**:1739–1744.

Johnson DJ, Sanderson H, Brain RA, Wilson CJ, Solomon KR. 2007. Toxicity and hazard of selective serotonin reuptake inhibitor antidepressants fluoxetine, fluvoxamine, and sertraline to algae. Ecotoxicology and Environmental Safety **67**:128–139.

Keller AE, Ruessler DS. 1997. The toxicity of malathion to unionid mussels: Relationship to expected environmental concentrations. Environmental Toxicology and Chemistry **16**:1028–1033.

Kelner DE, Sietman BE. 2000. Relic populations of the ebony shell, fusconaia ebena (bivalvia: Unionidae), in the upper mississippi river drainage. Journal of Freshwater Ecology.

Kováts N, Abdel-Hameid N-A, Kovács K, Paulovits G. 2010. Sensitivity of three unionid glochidia to elevated levels of copper, zinc and lead. Knowledge and Management of Aquatic Ecosystems. 04.

Kráglová L. 2008. The environment of the Czech Republic: Water. Prague.

Kummerer K. 2001. Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - a review.

Lamichhane K, Garcia SN, Huggett DB, DeAngelis DL, La Point TW. 2014. Exposures to a selective serotonin reuptake inhibitor (SSRI), sertraline hydrochloride, over multiple generations: Changes in life history traits in Ceriodaphnia dubia. Ecotoxicology and Environmental Safety **101**:124–130.

Lee H-L, DeAngelis D, Koh H-L. 1998. Modeling spatial distribution of the Unionid mussels and the core-satellite hypothesis. Water Science and Technology **38**:73–79.

Lefevre G, C. Curtis W. 2005. Reproduction and parasitism in the Unionidæ. Journal of Experimental Zoology **9**:79–116.

Lopes-Lima M et al. 2017a. Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biological Reviews **92**:572–607.

Lopes-Lima M et al. 2017b. Phylogeny of the most species-rich freshwater bivalve family (Bivalvia: Unionida: Unionidae): Defining modern subfamilies and tribes. Molecular Phylogenetics and Evolution **106**:174–191.

Lopes-Lima M, Teixeira A, Froufe E, Lopes A, Varandas S, Sousa R. 2014. Biology and conservation of freshwater bivalves: Past, present and future perspectives. Hydrobiologia **735**:1–13.

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S, Wang XC. 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment.

Magureanu M, Mandache NB, Parvulescu VI. 2015. Degradation of pharmaceutical compounds in water by non-thermal plasma treatment. Water Research **81**:124–136.

Manoli K, Morrison LM, Sumarah MW, Nakhla G, Ray AK, Sharma VK. 2019. Pharmaceuticals and pesticides in secondary effluent wastewater: Identification and enhanced removal by acid-activated ferrate(VI). Water Research **148**:272–280.

Markich SJ. 2017. Sensitivity of the glochidia (larvae) of freshwater mussels (Bivalvia: Unionida: Hyriidae) to cadmium, cobalt, copper, lead, nickel and zinc: Differences between metals, species and exposure time. Science of the Total Environment **601–602**:1427–1436.

Master LL, Stein BA, Kutner LS, Hammerson GA. 2000. Vanishing Assets: Conservation Status of U.S. Species. Pages 93–118 Precious Heritage: The Status of Biodiversity in the United States. Oxford University Press, New York.

McMahon R. 1991. Mollusca: Bivalvia. Pages 315–399 in J. Thorp and A. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, New York.

Minagh E, Hernan R, O'Rourke K, Lyng FM, Davoren M. 2009. Aquatic ecotoxicity of the selective serotonin reuptake inhibitor sertraline hydrochloride in a battery of freshwater test species. Ecotoxicology and Environmental Safety **72**:434–440.

Minguez L, Farcy E, Ballandonne C, Lepailleur A, Serpentini A, Lebel JM, Bureau R, Halm-Lemeille MP. 2014. Acute toxicity of 8 antidepressants: What are their modes of action? Chemosphere **108**:314–319.

Modesto V, Ilarri M, Souza AT, Lopes-Lima M, Douda K, Clavero M, Sousa R. 2018. Fish and mussels: Importance of fish for freshwater mussel conservation. Fish and Fisheries **19**:244–259.

Moore AP, Bringolf RB. 2018. Effects of nitrate on freshwater mussel glochidia attachment and metamorphosis success to the juvenile stage. Environmental Pollution **242**:807–813.

Painter MM, Buerkley MA, Julius ML, Vajda AM, Norris DO, Barber LB, Furlong ET, Schultz MM, Schoenfuss HL. 2009. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas). Environmental Toxicology and Chemistry **28**:2677–2684.

Petrie B, Barden R, Kasprzyk-Hordern B. 2014. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Research **72**:3–27.

R Core Team. 2016. R: A Language and Environment for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria. The R Foundation for Statistical Computing, Vienna.

Reichard M, Douda K, Przybyłski M, Popa OP, Karbanová E, Matasová K, Rylková K, Polačik M, Blažek R, Smith C. 2015. Population-specific responses to an invasive species. Proceedings of the Royal Society B: Biological Sciences **282**:20151063. The Royal Society.

Ribeiro AR, Castro PML, Tiritan ME. 2012. Chiral pharmaceuticals in the environment.

Ribeiro S, Torres T, Martins R, Santos MM. 2015. Toxicity screening of diclofenac, propranolol, sertraline and simvastatin using danio rerio and paracentrotus lividus embryo bioassays. Ecotoxicology and Environmental Safety **114**:67–74.

Ricciardi A, Rasmussen JB. 1999. Extinction rates of North American freshwater fauna. Conservation Biology **13**:1220–1222.

Richardson SD, Ternes TA. 2018. Water Analysis: Emerging Contaminants and Current Issues.

Rozman D, Hrkal Z, Váňa M, Vymazal J, Boukalová Z. 2017. Occurrence of pharmaceuticals in wastewater and their interaction with shallow aquifers: A case study of Horní Beřkovice, Czech Republic. Water (Switzerland) **9**.

Schultz MM, Furlong ET, Kolpin DW, Werner SL, Schoenfuss HL, Barber LB, Blazer VS, Norris DO, Vajda AM. 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment and selective uptake in fish neural tissue. Environmental Science and Technology **44**:1918–1925.

Silva LJG, Pereira AMPT, Meisel LM, Lino CM, Pena A. 2014. A one-year follow-up analysis of antidepressants in Portuguese wastewaters: Occurrence and fate, seasonal influence, and risk assessment. Science of the Total Environment **490**:279–287.

Slavik O, Horky P, Douda K, Velisek J, Kolarova J, Lepic P. 2017. Parasite-induced increases in the energy costs of movement of host freshwater fish. Physiology and Behavior **171**:127–134.

Smith DG. 1985. Recent Range Expansion of the Freshwater Mussel Anodonta implicata and Its Relationship to Clupeid Fish Restoration in the Connecticut River System. Freshwater Invertebrate Biology **4**:105–108.

Soucek DJ, Dickinson A. 2012. Acute toxicity of nitrate and nitrite to sensitive freshwater insects, mollusks, and a crustacean. Archives of Environmental Contamination and Toxicology **62**:233–242.

Spooner DE, Vaughn CC, Galbraith HS. 2012. Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. Oecologia **168**:533–548.

Stein BA, Adams JS, Master LL, Morse LE, Hammerson G. 2000. A Remarkable Array: Species Diversity in the United States. Pages 55–92 Precious Heritage: The Status of Biodiversity in the United States. Oxford University Press, New York.

Stewart AM, Grossman L, Nguyen M, Maximino C, Rosemberg DB, Echevarria DJ, Kalueff A V. 2014. Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns. Aquatic Toxicology **156**:269–273.

Theler J, James L. 1987. Prehistoric Freshwater Mussel Assemblages of the Mississippi River in Southwestern Wisconsin. The Nautilus **101**:143–150.

Trdan RJ, Hoeh WR. 2006. Eurytopic Host Use by Two Congeneric Species of Freshwater Mussel (Pelecypoda: Unionidae: Anodonta). American Midland Naturalist **108**:381.

Valenti Jr. TW, Perez-Hurtado P, Chambliss CK, Brooks BW, Valenti Jr TW, Perez-Hurtado P, Chambliss CK, Brooks BW. 2009. Aquatic toxicity of sertraline to Pimephales promelas at environmentally relevant surface water pH. (Special Issue: Pharmaceuticals and personal care products in the environment). Environmental Toxicology and Chemistry **28**:2685–2694.

Valenti TW, Cherry DS, Neves RJ, Schmerfeld J. 2005. Acute and chronic toxicity of mercury to early life stages of the rainbow mussel, Villosa iris (Bivalvia: Unionidae). Environmental toxicology and chemistry **24**:1242–6.

Vaughn CC. 2010. Biodiversity Losses and Ecosystem Function in Freshwaters: Emerging Conclusions and Research Directions. BioScience **60**:25–35.

Verlicchi P, Al Aukidy M, Galletti A, Petrovic M, Barceló D. 2012a. Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment.

Verlicchi P, Al Aukidy M, Zambello E. 2012b. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review.

Wang N et al. 2007a. Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (unionidae). Environmental Toxicology and Chemistry **26**:2036–2047.

Wang N et al. 2007b. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). Environmental Toxicology and Chemistry **26**:2048–2056.

Wang N, Ingersoll CG, Ivey CD, Hardesty DK, May TW, Augspurger T, Roberts AD, Van Genderen E, Barnhart MC. 2010. Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. Environmental Toxicology and Chemistry **29**:2053–2063.

Wanner J. 2017. Čištění odpadních vod v ČR: vývoj a současná situace. Available from http://vodnihospodarstvi.cz/cisteni-odpadnich-vod-cr/ (accessed 31.03.2019).

Watters GT. 1992. Unionids, Fishes, and the Species-Area Curve. Journal of Biogeography **19**:481.

Watters GT. 2008. Glochidial metamorphosis of the freshwater mussel Lampsilis cardium (Bivalvia: Unionidae) on larval tiger salamanders, Ambystoma tigrinum ssp. (Amphibia: Ambystomidae). Canadian Journal of Zoology **75**:505–508. NRC Research Press Ottawa, Canada.

Watters GT, O’Dee SH. 1998. Metamorphosis of Freshwater Mussel Glochidia (Bivalvia : Unionidae) on Amphibians and Exotic Fishes. The American Midland Naturalist **139**:49–57. The University of Notre Dame.

Weinberger J, Klaper R. 2014. Environmental concentrations of the selective serotonin reuptake inhibitor fluoxetine impact specific behaviors involved in reproduction, feeding and predator avoidance in the fish Pimephales promelas (fathead minnow). Aquatic Toxicology **151**:77–83.

Xie Z, Lu G, Li S, Nie Y, Ma B, Liu J. 2015. Behavioral and biochemical responses in freshwater fish Carassius auratus exposed to sertraline. Chemosphere **135**:146–155.

Zale A V., Neves RJ. 2008. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology **60**:2535–2542. NRC Research Press Ottawa, Canada.

Zhao S, Douda K, Horky P, Grabic R, Grabicova K, Slavik O, Randak T. 2019 (in preparation). The sublethal effects of sertraline on freshwater mussel parasitic larvae and their interaction with fish hosts. Unpublished manuscript.

9 LIST OF ABBREVIATIONS

- PPCP: pharmaceuticals and personal care product
- WWTP: waste water treatment plant
- *U. tumidus*: *Unio tumidus* (freshwater mussel)
- *S. cephalus*: *Squalius cephalus* (fish)
- SSRI: selective serotonin reuptake inhibitors