CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

FACULTY OF ENVIRONMENTAL SCIENCES

DEPARTMENT OF ECOLOGY

Fakulta životního prostředí

The environmental biology and conservation of the flagship groups of stream macroinvertebrate species in the Czech Republic

Vliv faktorů prostředí na vybrané skupiny ohrožených bentických bezobratlých ve vodních tocích ČR

Karel Douda

PhD Thesis

disertační práce

Supervisor: Doc. Mgr. Jan Růžička, Ph.D.   Praha, 2011

I thereby declare that I wrote the Ph.D. thesis myself using the results of my own work or collaborative work with my colleagues and with help of other publication resources which are properly cited.

Prohlašuji, že jsem vypracoval disertační práci samostatně, s využitím výsledků mé vlastní práce, výsledků spolupráce s mými kolegy a dalších publikovaných zdrojů, které jsou náležitě citovány.

Karel Douda

In Prague,   30.10. 2011   …………………………….

Content

Acknowledgements

Introduction

The current period of global mass extinctions has elicited increasing efforts to protect Earth's biological diversity (Chapin et al. 2000). Novel data, methodologies and concepts are needed in the field of biodiversity conservation to reduce the detrimental effects of human activities on the biota (e.g. Margules & Pressey 2000, Geist 2011).

This work addresses stream benthic macroinvertebrate species, a critically important constituent of one of the most threatened environments worldwide. Streams and rivers are under increasing pressure from human activities that alter their qualitative parameters, functions (Carpenter *et al.* 1992) and the structure of their associated communities (Dudgeon *et al.* 2006). Because rivers provide a variety of goods and services for human societies, they are often subject to extensive exploitation and are especially vulnerable. Running waters in a natural state are characterised primarily by high spatial and temporal diversity, longitudinal connectivity, a one-directional flow of energy and materials and predominantly allochtonous inputs (Allan 2001). As a result, natural stream habitats represent a continuum of habitat conditions, as described by the River Continuum Concept (Vannote et al. 1980). The primary threats to the biodiversity of running waters include water pollution, habitat degradation, flow modifications, diseases, overexploitation and invasions by exotic species (Dudgeon *et al.* 2006, Malmquist & Rundle 2002, Richter et al. 1997).

Current research on the environmental biology and conservation of stream macroinvertebrate species focuses mainly on relationships with the physico-chemical parameters of the habitat (water quality, flow regime, substrate) (e.g., Augspurger et al. 2003, Brainwood et al. 2006), species interactions (e.g., Kozubíková et al. 2008, Schwalb et al. 2011) and conservation genetics (e.g., Geist 2010). It is also acknowledged that sustainable conservation strategies for freshwater biodiversity can be most effective if they integrate multiple levels of biological organisation (Geist 2011). Single-species approaches may be ineffective in preparing solutions for conservation authorities addressing biodiversity protection at a particular site. Furthermore, the management regimes of several target species can conflict. However, an ecosystem approach may also sometimes be problematic, primarily because of problems with the definition of its goals and cost-effectiveness. As a result, a surrogate species approach (flagship, indicator or umbrella species) may often be useful for reaching conservation goals (Simberloff 1998).

This thesis addresses Central European species of two groups of benthic macroinvertebrates, freshwater mussels and crayfish. Both of these groups represent functionally highly important constituents of freshwater habitats

5

(Nyström *et al.* 1996; Vaughn & Hakenkamp 2001). Because of their general sensitivity, they are often used as indicator species for the overall quality of the aquatic environment (Fureder *et al.* 2003; Webb *et al.* 2008). Moreover, their tight interspecific relationships and frequently high cultural value make them suitable 'flagship species' for the protection of freshwaters (Caro 2010).

Populations of unionid bivalves severely declined worldwide during the 20th century (Richter et al., 1997; Lydeard et al., 2004; Bogan, 2008; Christian and Harris, 2008). In Central Europe, many species disappeared from large parts of their former areas of occurrence and population abundances in the remaining localities decreased significantly (Bauer and Wachtler, 2001). Identification of key stressors at particular localities is difficult with regard to the many changes that Central European freshwaters have undergone within the past century and a variety of factors potentially limiting species distributions (Van Hassel and Farris, 2007). The main harmful factors threatening freshwater mussels are reported to be water pollution (Goudreauet al., 1993; Augspurger et al., 2003), habitat degradation (Bogan, 1993; Watters, 1996; Brainwood et al., 2006), host unavailability (e.g. Strayer 2008) and the invasion of alien bivalves (e.g. Haag et al. 1993). Many unresolved questions concerning freshwater mussel species conservation in Central European region have emerged in the past few decades.

Several recent studies observed a relationship between the impaired population status of threatened freshwater mussels and elevated $N\text{-}NO_3^-$ concentrations in running waters (Bauer, 1988; Hochwald, 2001; Douda, 2006, Köhler, 2006; Zettler and Jueg, 2007). Nevertheless, the methods based on analysis of spatial co-occurrence of stressors and responses cannot confirm causality (Clements et al., 2002). Many studies demonstrate the need to integrate descriptive and experimental approaches in order to develop causal arguments for ecological assessment (Clements et al., 2002). The fact that causal mechanisms responsible for the observed relationship are unknown, and no experimental data or comprehensive studies involving more species are available causes uncertainty in targeting conservation strategies (Köhler, 2006). It remains unknown whether the elevated $N\text{-}NO_3^-$ level at freshwater mussel localities should be interpreted as a mere indicator of impaired site conditions or whether it has a direct negative effect on mussel populations.

Another challenging issue for European conservation biologists concerned with the protection of freshwater mussels is the availability of fish hosts for mussels' glochidia. The coextinction of species is hypothesized to represent one of the most frequent forms of biodiversity loss (Dunn et al., 2009). Identifying a species' host-affiliate relationships and the impairment of these relationships facilitates the prioritisation of

conservation actions and is important in reducing the effort required to manage threatened species (Koh et al., 2004; Marvier & Smith, 1997; Moir et al., 2010). An increasing body of literature addresses the effects of host fish availability on freshwater mussel dispersion (Schwalb, Poos & Ackerman, 2010) and population status (McNichols, Mackie & Ackerman, 2010; Schwalb et al., 2011; Spooner et al., 2011). Nevertheless, the effect of potential host limitation on the conservation status of endangered unionid bivalves remains poorly documented, and this lack of information also causes uncertainty in the prioritisation of conservation actions (Schwalb et al., 2011).

In contrast to the threats to affiliate species that result from a narrow host spectrum, broad host generalism may play a critical role in facilitating species invasion. Several freshwater mussel species that have free living dispersion larvae represent some of the most problematic invasive species worldwide (Higgins and Vander Zanden 2010). For example, recent invasions of *Dreissena polymorpha*, *Corbicula fluminea* and *Limnoperna fortunei* have altered entire aquatic environments in invaded regions by their filtration and burrowing activities (Higgins and Vander Zanden 2010; Karatayev et al. 2007b; Sousa et al. 2009; Strayer 2009), and they have severely affected native communities (mainly the native mussel populations) by altering habitats and competing for resources (Karatayev et al. 1997; Sousa et al. 2008b; Ward and Ricciardi 2007). In contrast to these invasive species with free-living larvae, invasive behaviour is extremely rare between unionid bivalves, presumably because of their tight host-affiliate relationship with local fish species. However, the general acceptance of this truism might be the reason that the threats resulting from unionid bivalve invasions were long overlooked. In this thesis, I investigated the invasion potential of *Anodonta (Sinanodonta) woodiana*, a species of East Asian unionid mussel established worldwide despite having an obligatory parasitic stage.

An invasion by A. woodiana has several potential negative consequences for the native unionid mussel species. First, A. woodiana may directly compete with native mussels for food. Competition for food is considered to be the main factor threatening the native populations of North American unionids in areas that have been invaded by D. polymorpha (Haag et al. 1993). Second, *A. woodiana* is expected to compete with native unionids for hosts: (1) by direct competition between glochidia for space on hosts; (2) via the activation of innate host tissue responses; or (3) by triggering an adaptive immune response by hosts, making them immunised against repeated infections after contact with glochidia (Rogers and Dimock 2003). Potential long-term consequences of *A. woodiana* invasions for native mussels also exist via evolutionary changes in the host acceptance of glochidia. Unionids and their fish hosts co-evolved over a long time period, which resulted in a variety of species-specific

adaptations in their host parasite relationships (Barnhart et al. 2008). Indeed, all unionid species native to Central Europe have a certain level of host specialisation, reflecting their spatial and habitat requirements (Bauer 2001). It is possible that native unionids and their hosts are in co-evolutionary equilibrium, where potential costs of glochidia rejection outweigh potential costs of acceptance (Takasu et al. 1993). If this equilibrium is shifted by *A. woodiana* glochidia, hosts may evolve a response to the increased glochidia encounter rate.

One of the chapters in this thesis addresses freshwater crayfish, organisms phylogenetically and ecologically distinct from bivalves. However, because crayfish and bivalves share the same habitats, crayfish also face one of the principal detrimental processes in freshwaters – human-induced changes in water quality. Hence, although a different taxon is considered in this chapter, the environment, stress factors and study methods are similar. The viability of crayfish populations, like that of the bivalves, is affected by a combination of abiotic and biotic factors. Moreover, in recent times, biotic interactions have been considered to be much more important for structuring Central European crayfish populations than abiotic factors. Crayfish plague, caused by the parasitic oomycete *Aphanomyces astaci*, is spread by invasive crayfish species throughout European freshwaters (Kozubíková et al. 2008, 2009). Currently, crayfish plague is the focus of intense research efforts. However, the other sources of environmental pressure on crayfish populations appear to remain relatively understudied.

In summary, this thesis presents four studies examining different aspects of the detection of impacts on threatened benthic macroinvertebrate species. I address both threats resulting from abiotic environmental conditions (water pollution, Chapters 1, 2) and threats resulting from biotic interactions (host availability, Chapters 3, 4). Chapter 1 addresses the issue of causality in stress detection. This study combines both experimental and observational approaches to identify the effects of nitrate nitrogen pollution on freshwater mussels. Chapter 2 demonstrates the importance of the selection of the proper length of an environmental gradient for the study of species responses stress detection. The chapter investigates the distribution of indigenous and alien crayfish along stream water quality gradients in the Czech Republic. Chapter 3 shows the importance of the proper verification of resource availability for declining species. The aim of the study is to assess the potential threats to *U. crassus* in Central Europe resulting from a lack of host resources. Finally, Chapter 4 deals with the potential threats resulting from the introduction of a strong competitor. It examines the invasion potential of *Anodonta (Sinanodonta) woodiana* in Europe based on its host use, host availability and temperature tolerance.

General purpose and main aims

This study addresses different aspects of the identification of threats to endangered species of stream benthic invertebrates. Its primary foci are the limitation of freshwater mussels and crayfish by water quality impairment and the issues associated with the host limitation of freshwater mussels.

Specific questions:

(1)

Should the elevated nitrate levels observed at impaired freshwater mussel localities in Central Europe be interpreted as an indicator of impaired site conditions? Alternatively, do these elevated nitrate levels have a direct negative effect on mussel populations?

(2)

Is there any relationship between the present distribution of crayfish and water quality conditions in the Czech Republic?

(3)

How can the host limitation of unionid bivalves be effectively detected? What is the role of host limitation in the present pan-European decline of *U. crassus*?

(4)

The aim of the investigations presented in Chapter 4 was to examine the invasive capacity of *A. woodiana*, a potentially strong competitor with the native unionid bivalves. What is the role of host specificity in the persistence and spread of freshwater mussels? Can the success of the invasive Asian freshwater mussel *Anodonta woodiana* in Europe be explained by its wide host generalism?

Literature

Allan J.D. 2001. Stream Ecology: Structure and Function of Running Waters. Kluwer Academic Publishers: Dordrecht, The Netherlands.

Augspurger, T., Keller, A.E., Black, M.C., Cope, W.G., and Dwyer, F.J., 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry 22(11):2569-2575.

Bauer, G., 1988. Threats to the freshwater pearl mussel Margaritifera margaritifera L in Central Europe. Biological Conservation 45:239–253.

Bauer, G., Wachtler, K., 2001. Environmental relationships of naiads: threats, impact on the ecosystem, indicator function. In Ecology and Evolution of the Freshwater Mussels Unionoida, Bauer G, Wachtler K (eds). Springer: Heidelberg, Germany; 311–315.

Bogan, A.E., 1993. Fresh-water bivalve extinctions (Mollusca, Unionoida) – a search for causes. American Zoologist 33(6):599-609.

Bogan, A.E., 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia 595:139-147.

Brainwood, M., Burgin, S., and Byrne, M., 2006. Is the decline of freshwater mussel populations in a regulated coastal river in south-eastern Australia linked with human modification of habitat? Aquatic Conservation-Marine and Freshwater Ecosystems 16(5):501-516.

Caro, T. 2010. Conservation by Proxy: Indicator, Umbrella, Keystone, Flagship, and Other Surrogate Species. Island Press. University of California, Davis, USA

Carpenter, S.R., Fisher, S.G., Grimm, N.B. & Kitchell, J.F., 1992. Global Change and Freshwater Ecosystems. Annual Review of Ecology and Systematics 23:119-139.

Clements W.H., Carlisle D.M., Courtney L.A., Harrahy E.A. 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. Environmental Toxicology and Chemistry 21: 1138-1146.

Chapin, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C., & Diaz, S., 2000. Consequences of changing biodiversity. Nature 405, 234-242.

Christian, A.D. and Harris, J.L. 2008. An introduction to directions in freshwater mollusk conservation: molecules to ecosystems. Journal of the North American Benthological Society 27:345-348.

Douda, K. 2006. Mlži čeledi Unionidae v lotických biotopech povodí řeky Lužnice. Diploma thesis. Czech University of Life Sciences. Prague. in Czech.

Dudgeon, D., A.H. Arthington, M.O. Gessner, Z.I. Kawabata, D.J. Knowler, C. que, R.J. Naiman, A.H. Prieur-Richard, D. Soto, M.L.J. Stiassny, & C.A. Sullivan, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182.

Dunn, R.R., Harris, N.C., Colwell, R.K., Koh, L.P. & Sodhi, N.S. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists? P. Roy. Soc. B–Biol. Sci. 276, 3037–3045.

Fureder, L., B. Oberkofler, R. Hanel, J. Leiter, & B. Thaler. 2003. The freshwater crayfish *Austropotamobius pallipes* in South Tyrol: Heritage species and bioindicator. Bulletin Francais de la Peche et de la Pisciculture 81-95.

Geist, J., 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of Conservation Genetics and Ecology. Hydrobiologia 644, 69-88.

Geist, J., 2011. Integrative freshwater ecology and biodiversity conservation. Ecological Indicators 11, 1507-1516.

Goudreau, S.E., Neves, R.J., and Sheehan, R.J., 1993. Effects of waste-water treatment-plant effluents on fresh-water mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia 252:211-230.

Haag W.R., Berg D.J., Garton D.W., Farris J.L. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (Dreissena polymorpha) in western Lake Erie. Can J Fish Aquat Sci 50:13–19.

Higgins S.N., Vander Zanden M.J. 2010. What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecological Monographs 80:179–196.

Hochwald, S., 2001. Plasticity of life-history traits in Unio crassus. Pages 127-141 in G. Bauer, & K. Wachtler editors. Ecology and evolution of the freshwater mussels Unionoida. Springer-Verlag Berlin Heidelberg.

Karatayev A.Y., Padilla D.K., Minchin D., Boltovskoy D., Burlakova L.E. (2007) Changes in global economies and trade: the potential spread of exotic freshwater bivalves. Biological Invasions 9:161–180.

Karatayev A.Y., Burlakova L.E., Padilla D.K. 1997. The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in eastern Europe. Journal of Shellfish Research 16:187–203.

Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C. & Smith, V.S. 2004. Species coextinctions and the biodiversity crisis. Science 305, 1632–1634.

Köhler, R., 2006. Observations on impaired vitality of Unio crassus (Bivalvia: Najadae) populations in conjunction with elevated nitrate concentration in running waters. Acta Hydrochimica et Hydrobiologica 34:346-348.

Kozubíková, E., A. Petrusek, Z. Ďuriš, M. P. Martín, J. Diéguez-Uribeondo, & B. Oidtmann, 2008. The old menace is back: recent crayfish plague outbreaks in the Czech Republic. Aquaculture 274:208-217.

Lydeard, C., Cowie, R.H., Ponder, W.F., Bogan, A.E., Bouchet, P., Clark, S.A., Cummings, K.S., Frest, T.J., Gargominy, O., Herbert, D.G., Hershler, R., Perez, K.E., Roth, B., Seddon, M., Strong, E.E., and Thompson, F.G., 2004. The global decline of nonmarine mollusks. Bioscience 54:321-330.

Malmqvist, B., Rundle, S., 2002. Threats to the running water ecosystems of the world. Environmental Conservation 29, 134-153.

Margules, C.R., Pressey, R.L., 2000. Systematic conservation planning. Nature 405, 243-253.

Marvier, M.A. & Smith, D.L. 1997. Conservation implications of host use for rare parasitic plants. Conservation Biology 11, 839–848.

McNichols, K.A., G.L. Mackie, & J.D. Ackerman, 2010. Host fish quality may explain the status of endangered Epioblasma torulosa rangiana and Lampsilis fasciola (Bivalvia: Unionidae) in Canada. Journal of the North American Benthological Society 30:60-70.

Moir, M.L., Vesk, P.A., Brennan, K.E.C., Keith, D.A., Hughes, L. & McCarthy, M.A. 2010. Current constraints and future directions in estimating coextinction. Conservation Biology 24, 682–690.

Nyström, P.E., C.H.R.I. Brönmark &, W.I.L.H. Granéli, 1996. Patterns in benthic food webs: a role for omnivorous crayfish? Freshwater Biology 36:631-646.

Richter, B.D., Braun, D.P., Mendelson, M.A., and Master, L.L., 1997. Threats to imperiled freshwater fauna. Conservation Biology 11:1081-1093.

Rogers C.L., Dimock R.V. 2003. Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia : Unionidae) after multiple infections. Journal of Parasitology 89:51–56

Barnhart M.C., Haag W.R., Roston W.N. 2008. Adaptations to host infection and larval parasitism in Unionoida. J N Am Benthol Soc 27:370–394

Schwalb, A., Poos, M. & Ackerman, J. 2010. Movement of logperch – the obligate host fish for endangered snuffbox mussels: implications for mussel dispersal. Aquatic Sciences 73, 223–231.

Schwalb, A.N., K.A.R.L. Cottenie, M.S. Poos, & J.D. Ackerman, 2011. Dispersal limitation of unionid mussels and implications for their conservation. Freshwater Biology. DOI: 10.1111/j.1365-2427.2011.02587.x

Simberloff, D., 1998. Flagships, umbrellas, and keystones: Is single-species management passe in the landscape era? Biological Conservation 83, 247-257.

Sousa R., Nogueira A.J.A., Gaspar M., Antunes C., Guilhermino L. 2008. Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): possible implications for ecosystem functioning. Estuar Coast Shelf S 80:289–295

Spooner, D.E., M.A. Xenopoulos, C.H.R.I. Schneider, & D.A. Woolnough, 2011. Coextirpation of host-affiliate relationships in rivers: the role of climate change, water withdrawal, and host-specificity. Global Change Biology 17:1720-1732.

Strayer D.L. 2009. Twenty years of zebra mussels: lessons from the mollusk that made headlines. Front Ecol Environ 7:135–141

Taraschewski H 2006. Hosts and parasites as aliens. J Helminthol 80:99–128.

Strayer D.L., 2008. Freshwater mussel ecology: a multifactor approach to distribution and abundance. University of California Press, Berkley, California.

Takasu F., Kawasaki K., Nakamura H., Cohen J. & Shigesada N. 1993. Modeling the population dynamics of a cuckoo–host association and the evolution of host defenses. American Naturalist 142: 819–839.

Van Hassel J.H., Farris J.L. 2007. Freshwater bivalve ecotoxicology. In: Freshwater bivalve ecotoxicology, Farris JL, Van Hassel JH (eds). Society of Environmental Toxicology and Chemistry: Pensacola, FL; 1-17.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., & Cushing, C.E., 1980. River Continuum Concept. Can J Fish Aquat Sci 37, 130-137.

Ward J.M., Ricciardi A. 2007. Impacts of *Dreissena* invasions on benthic macroinvertebrate communities: a meta-analysis. Diversity and Distributions 13:155–165

Watters, G. T., 1996. Small dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. Biological Conservation 75:79-85.

Vaughn, C.C., & C.C. Hakenkamp, 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431-1446.

Webb, K., C. Craft, & E. Elswick, 2008. The evaluation of the freshwater western pearl mussel, *Margaritifera falcata* (Gould, 1850), as a bioindicator through the analysis of metal partitioning and bioaccumulation. Northwest Science 82:163-173.

Zettler, M.L., & U.Jueg, 2007. The situation of the freshwater mussel Unio crassus (Philipsson, 1788) in north-east Germany and its monitoring in terms of the Habitats Directive. Mollusca 25:165-174.

Chapter 1

Effects of nitrate nitrogen pollution on Central European unionid bivalves revealed by distributional data and acute toxicity testing

Karel Douda

Effects of nitrate nitrogen pollution on Central European unionid bivalves revealed by distributional data and acute toxicity testing

KAREL DOUDA [a,b,1]

[a] *T G M - Water Research Institute, Podbabska 30, 160 62 Prague 6, Czech Republic*
[b] *Czech University of Life Sciences Prague (CULS), Department of Ecology, Kamycka 1176, 165 21 Prague 6, Czech Republic*

ABSTRACT

1. Studies from Central Europe have shown a relationship between the impaired population status of threatened freshwater mussel species and elevated nitrate nitrogen ($N-NO_3^-$) concentrations in running waters.
2. Causal mechanisms, however, remain unknown, and no experimental data or comprehensive studies involving more species are available, which causes uncertainty in prioritising conservation actions.
3. This study uses both descriptive and experimental approaches to identify the effects of nitrates on freshwater mussels and demonstrates the need for integrating different research methods for development of conservation strategies for threatened species.
4. Spatial co-occurrence of five native freshwater mussel species (*Anodonta anatina*, *Pseudanodonta complanata*, *Unio pictorum*, *Unio tumidus*, *Unio crassus*) and $N-NO_3^-$ concentrations were examined in a 7th-order river catchment (Lužnice River, Czech Republic) with anthropogenically-induced increasing $N-NO_3^-$ levels and declining populations of these species during the 20th century.
5. Acute toxicity of $N-NO_3^-$ was then estimated for artificially reared juveniles of *A. anatina* and *U. crassus* using both lethal and sublethal test endpoints.
6. Results showed that the probability of occurrence of all species was significantly reduced in reaches with elevated $N-NO_3^-$ levels.
7. In contrast, the results of toxicity testing revealed that the juvenile stages of the two tested species were less sensitive to $N-NO_3^-$ than most previously tested freshwater macroinvertebrates. The detected 96-hour median lethal $N-NO_3^-$ concentrations were two orders of magnitude higher than the limits derived from distributional data.
8. Despite the probable absence of a direct negative effect of $N-NO_3^-$ on freshwater mussel populations, $N-NO_3^-$ has potential to be used as an effective indicator of biotope conditions. Identification of causal mechanisms responsible for the observed relation between unionids and $N-NO_3^-$ will require further research.

KEY WORDS: benthos; endangered species; freshwater mussels; invertebrates; nutrient enrichment; river; stream; water quality

---

[1] Correspondence to: K. Douda, T G M - Water Research Institute, Podbabska 30, 160 62 Prague 6, Czech Republic. E-mail: karel_douda@vuv.cz

INTRODUCTION

Freshwater mussel populations severely declined worldwide during the 20th century (Richter *et al.*, 1997; Lydeard *et al.*, 2004; Bogan, 2008; Christian and Harris, 2008). In Central Europe, many species disappeared from vast areas and population abundances in the remaining localities decreased significantly (Bauer and Wachtler, 2001). Identification of key stressors at particular localities is difficult due to the many changes that Central European streams have undergone within the past century and a variety of factors potentially limiting species distributions (Van Hassel and Farris, 2007b). The main harmful factors are reported to be water pollution (e.g., Goudreau *et al.*, 1993; Augspurger *et al.*, 2003) and habitat degradation (e.g., Bogan, 1993; Watters, 1996; Brainwood *et al.*, 2006). Several recent studies from Central Europe observed a relationship between impaired population status of threatened freshwater mussels and elevated N-NO$_3^-$ concentrations in running waters (Bauer, 1988; Hochwald, 2001; Köhler, 2006; Zettler and Jueg, 2007). As a consequence of nitrogen pollution, N-NO$_3^-$ concentrations in surface waters can actually exceed values of 25 mg L^{-1} (Camargo *et al.*, 2005). In contrast, localities with undisturbed populations of the thick-shelled river mussel (*Unio crassus*) have reported values of up to 2.0 – 2.3 mg L^{-1} (Hochwald, 2001; Köhler, 2006; Zettler and Jueg, 2007), and only up to 0.5 L^{-1} for the freshwater pearl mussel (*Margaritifera margaritifera*) (Bauer, 1988; Bílý and Simon, 2007). Both species are seriously threatened in Europe and are the subjects of many conservation efforts (Buddensiek, 1995; Cosgrove and Hastie, 2001; Perez-Quintero, 2007). Despite the fact that understanding the role of N-NO$_3^-$ may help to focus conservation measures, no experimental data or comprehensive studies involving more species are available (Köhler, 2006). The main toxic effect of nitrogen on aquatic animals is considered to be the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen (Camargo and Alonso, 2006). The uptake of NO$_3^-$ in aquatic animals seems to be more limited than uptake of NH$_4^+$ or NO$_2^-$, which contributes to the relatively low toxicity of NO$_3^-$ (Jensen, 1996; Cheng and Chen, 2002; Camargo and Alonso, 2006). As a result, comparatively few studies have been conducted to experimentally assess N-NO$_3^-$ toxicity in aquatic animals (Camargo *et al.*, 2005); however, some recent ecotoxicological studies showed some groups of freshwater animals to be highly sensitive to N-NO$_3^-$ (reviewed by Camargo *et al.*, 2005). The safe level recommended to protect these species from N-NO$_3^-$ pollution is 2.0 mg L^{-1}, a value that is often exceeded in impaired Central European unionid bivalve habitats. Because no relevant experimental data on nitrate toxicity to freshwater mussels are available, it remains unknown whether the elevated N-NO$_3^-$ level at freshwater mussel localities should be interpreted as a mere indicator of impaired site conditions or whether it has a direct negative effect on mussel populations.

Identification of factors affecting viability and persistence of threatened species is essential for development of conservation strategies (Pickett *et al.*, 1997). Many studies demonstrate the need to integrate descriptive and experimental approaches in order to develop causal arguments for ecological assessment (Clements *et al.*, 2002). The methods based on analysis of spatial co-occurrence of stressors and responses cannot confirm causality (Clements *et al.*, 2002). Moreover, the small numbers of remnant populations of threatened species often limit the use of these methods in the field of conservation biology (Van Dyke, 2008). A combination of this approach with experimental methods is therefore needed for identification of key limiting factors that allow subsequent focusing on the appropriate conservation measures (Van Hassel and Farris, 2007c). Over the past 20 years, unionids have been the subject of numerous ecotoxicological studies, many of which developed and validated methods for conducting toxicity tests (Van Hassel and Farris, 2007a). Glochidia, juvenile stages and adult mussels have been successfully used in ecotoxicologial studies (Cope *et al.*, 2008). In this study, I used early post-larval stages of juvenile mussels immediately after their release from host-fish, which are easy to produce and highly sensitive to water- and sediment-associated contaminants due to their feeding and burrowing activities (Bishop *et al.*, 2007; Cope *et al.*, 2008). Early juvenile stages are also thought to be the critical life-stage in populations of *M. margaritifera* and *U. crassus* in Central Europe, because aging populations and an absence of recruitment are often observed in impaired localities (Zettler and Jueg, 2007; Osterling *et al.*, 2008).

The aim of this study was to examine the relationship between Central European unionids and N-NO$_3^-$ using a combination of descriptive and experimental approaches. The first step was to analyse the spatial co-occurrence of five native freshwater mussels species (*Anodonta anatina*, *Pseudanodonta complanata*, *Unio pictorum*, *U. tumidus* and *U. crassus*) and N-NO$_3^-$ concentrations in a 7th-order catchment that has experienced anthropogenically-induced increasing N-NO$_3^-$ levels and declining populations of these species during the 20th century. Second, this study assessed the acute toxicity of N-NO$_3^-$ on artificially reared juvenile mussels of two species: *A. anatina,* the most common species with a widespread distribution in Central European running waters, and *U. crassus,* which suffered a severe decline in the 20th century.

METHODS

Study area

The Lužnice River (Elbe drainage basin, Central Europe) is a 7th-order stream, 199 km long with a 4,226 km^2 drainage area. The river predominantly flows through the area with underlying geology formed by metamorphic or igneous rocks and a fluvial sediment basin 347-990 m above sea level. The discharge at the river mouth is 24.3 m^{3}s^{-1}. The average river slope is 0.28% (with only 0.08 to 0.1% in the lower part of the stream). The river system flows through agricultural areas with mean population densities of approximately 100 inhabitants.km^{-2} and 37% forest cover. Water quality in the study area is substantially influenced by both non-point and point source anthropogenic pollution. Selected water quality characteristics recorded at studied sites (Fig. 1.1) are presented in Table 1.1. Suitable host fish species for the larval stage of studied freshwater mussels (Hochwald, 1997; Weber, 2005; Blazek and Gelnar, 2006) are common in the study area (Hartvich *et al.*, 1998). Freshwater mussel population densities were historically several-fold higher than at present (Beran, 2002).

Distributional study

Data on N-NO$_3^-$ concentrations were acquired from long-term water quality monitoring programmes of local water management authorities. The sampling sites represent spatially and physicochemically distinct reaches within the Lužnice River basin. A total of 36 sampling sites located on 18 streams (stream orders from 3 to 7) were used for analysis (Fig. 1.1). Between 21 and 121 monthly measurements per site were available for the period of 1995–2005 (totalling 2,981 measurements of N-NO$_3^-$ concentration). Median values calculated from all N-NO$_3^-$ data recorded at particular sites were used in analysis. Because of seasonal variation in N-NO$_3^-$ concentrations, medians suppress the extreme values generally recorded in cold season and during elevated run-off events (Van Herpe and Troch, 2000; Köhler, 2006), which do not correspond to the period of early juvenile mussel development (Pekkarinen, 1993; Hochwald, 2001). Stream reaches with water quality sampling sites were sampled for the presence of mussel species. The maximum distance between the mussel sampling site and the respective water quality site was constrained by the presence of stream confluences or point sources of pollution (e.g., sewage treatment plants) and was always less than 5 km of stream distance. Sampling was carried out in 2003-2006 by means of a visual inspection of the biotope while moving up the stream, either wading or using a boat. The shoreline was inspected visually to a depth of 60 cm with the use of a glass-bottomed viewing-bucket at potential unionid habitats (undisturbed habitats with stable bottom sediments). In addition, fine bottom sediments were excavated with a 10 L bucket or skimmer dredge and sieved on 3 mm mesh screen. The minimal sampling time per reach 45 minutes was prolonged in case of poor sampling conditions (high turbidity, elevated water level). The appropriateness of these methods for detecting rare species was confirmed by the fact that a 3-fold increase in sampling time at five sites did not reveal any additional species. The concentration of N-NO$_3^-$ in the

set of 36 sampling sites was analysed with respect to particular species occurrence using GLM (logistic regression) (Jongman *et al.*, 2005). This method creates a presence/absence response curve for each species, which describes the probability of the species being present (dependent variable) as a function of the explanatory environmental variable. Logit functions $g(x) = \beta_0 + \beta_1 x + \beta_2 x^2$ (β_0 - intercept; β_1, β_2 - parameters of logit response curves) were plotted against N-NO$_3^-$ concentration and the significance of model parameters was tested by comparing deviance reduction of models that included the estimated parameter with those that did not include the parameter (Chi-square, $p < 0.05$). Percentage deviance reduction of fitted models was also computed (Peeters and Gardeniers, 1998). All the analyses were done using the R 2.7.0 software package (Anonymous, 2008).

Toxicity testing
Methods for obtaining juvenile mussels were adjusted to the different spawning biology of *A. anatina* and *U. crassus* and different host fish species. Only non-lethal techniques were employed to obtain glochidia for subsequent transformation on host fishes and the number of parent mussels was minimised in order to avoid possible negative effects on studied populations. Gravid females of *A. anatina* were collected in January 2008 from Lužnice River near Bechyně, brought to the laboratory and acclimated to a temperature of 20ºC over five days. Glochidia were obtained by flushing the marsupium with water using a syringe (methods in Dodd *et al.*, 2005). A random subsample of 30 glochidia was tested for viability with sodium chloride according to Wang *et al.* (2007a). Two gravid females (length 93.0, 86.0 mm) that had glochidial viability over 90% were used for inoculation. The host fish (host suitability earlier reported by Blazek and Gelnar (2006)) were hatchery-reared European perch (*Perca fluviatilis*). Nine specimens (mean 142.8, SD 9.3 mm total body length) were inoculated by placing them in an aerated glochidial suspension of approximately 1300 infective glochidia per litre of dechlorinated tap water (DTW) for 30 minutes in a 10 L polyethylene (PE) box.
Gravid females of *U. crassus* were collected in June 2008 from the same locality. Females were separately held in the laboratory in 10 L of renewed aerated river water until the spontaneous uniphase release of egg-glochidia clumps, which is necessary for gaining ripe glochidia of this species (Engel, 1990). Glochidia of two gravid *U. crassus* females (length 74.7, 64.3 mm) that had glochidial viability over 90% were pooled and used for inoculation. Host fish (host suitability earlier reported by e.g., Engel, (1990) and Hochwald, (1997)) were Eurasian minnows (*Phoxinus phoxinus*) obtained by electrofishing from Židova Strouha Creek (Lužnice River basin). Thirteen specimens (mean 49.0, SD 12.2 mm total body length) were inoculated by the same method with approximately 4700 infective glochidia per litre.
After inoculation the fish were moved to 50 L PE tanks of DTW with 3 mm nets on the bottom and fed daily with *Chironomus* sp. larvae. Juvenile mussels were collected at 2 to 4 day intervals by siphoning the bottom of tanks and isolating them from debris using nylon screens (mesh size 139 and 507 μm; UHELON). The newly transformed juveniles were held in dishes with aerated DTW with no feeding up to the end of the excysting period. Toxicity tests were conducted with 1- to 10-day-old juvenile mussels.
Static 96-hour toxicity tests (with five increasing concentrations and control) were conducted in five replicates with five juvenile mussels per test chamber (50 ml Petri dish with 30 ml of test solution) for each species (in total 150 juveniles per species). Only the juvenile mussels exhibiting foot movements were used for testing. Test solutions were created from DTW (hardness 158 mg L^{-1} as CaCO$_3$) with addition of an appropriate amount of sodium nitrate (NaNO$_3$). At the beginning of each replicate test five juveniles were preserved in 70% ethanol for subsequent measurement of initial shell lengths (total 25 juveniles/species). The concentrations used (control, 25, 100, 500, 1000 and 2000 mg L^{-1}) were set by a previous range-finding study for *A. anatina*. N-NO$_3^-$ concentrations and other parameters (N-NH$_4^+$, N-NO$_2^-$, pH, dissolved oxygen, conductivity) were measured at the beginning and end of each test on composite samples of each

treatment using standard methods (ISO standard norm protocols). Survival was determined visually (within 30 seconds/specimen) after 48 hours and at the end (96 hours) of each test under a microscope (magnification 50-200x). Previous observations on *A. anatina* showed that juveniles exposed to increased concentrations of N-NO$_3^-$ minimised their foot movement activity first. Subsequently, they stopped exhibiting adductive valve movements. Finally, ciliary activity together with heart beat terminated, followed by rapid decomposition of organs (K. Douda unpublished). Hence, two sublethal endpoints (foot movement, valve movement), which characterise changes in juvenile behaviour, and one lethal endpoint (indicated by ciliary activity) were used in testing. Characteristics of the juveniles classified as live were as follows:
A. foot movement – juvenile exhibits active foot movement with foot extended out of valve
B. valve movement – juvenile exhibits valve movement or minor foot movement inside valve
C. ciliary activity – juvenile exhibits ciliary activity on the surface of mantle margin or foot
At the end of the tests, all surviving mussels were pooled within treatments and preserved in 70% ethanol for subsequent growth measurements. The maximum length of juveniles oriented in the ventral position was measured to the nearest 0.001 mm using a stereomicroscope (magnification 50x) with digital camera and image analysis software UTHSCSA Image Tool 2.0 (Anonymous, 1997). Normality and variance homogeneity were checked with the Shapiro-Wilkinson normality test and Bartlett test of homogeneity of variances, respectively. Significant reductions in growth relative to controls were tested for concentrations with no mortality (25, 100 and 500 mg L^{-1}). Pairwise comparisons of growth in treatments were done using the Dunnet test (with adjusted *p* values using the Bonferroni method). Median lethal concentrations (LC$_{50}$) were computed from recorded mortalities with the Spearman-Karber method or its Trimmed modification if necessary in software TSK 1.5 (Anonymous, 1990). Comparisons of mortality rates between control and other treatments were computed by Fisher's exact test. Relative sensitivity of species was tested by comparing mortalities using generalised linear models (GLM) with the binomial distribution (Venables and Ripley, 2002). Analyses were done using the R 2.7.0 software package (Anonymous, 2008).

RESULTS
Distributional study
In total, 2,761 living or recently dead (i.e., the nacreous layer surface was still lustrous and the periostracum was still coloured) mussels of studied species were found in the 36 sampled reaches. The most frequent species was *A. anatina* (detected at 31 reaches), and the rarest was *U. crassus* (detected at five reaches). The upper limit of N-NO$_3^-$ concentration (median value) found in sites of occurrence of *U. crassus* and *P. complanata* was 2.0 mg L^{-1}, and 4.3 mg L^{-1} in the *U. tumidus, U. pictorum* and *A. anatina* localities. Table 1.2 shows the estimated values for parameters β_0, β_1 and β_2 from the equation of logit function "g" and the percentage reduction in deviance of fitted models. The estimated parameter β_2 was significantly different from zero in the case of *U. crassus* and the response curve was therefore symmetrical and bell-shaped. For the other species the response curve was sigmoid with increasing probability of occurrence in lower values (Fig. 1.2).

Toxicity testing
Water quality parameters measured during acute test exposures (summarised in Table 1.3) indicated that test dilutions were similar with respect to water quality conditions. The concentration of N-NO$_3^-$ increased slightly during the tests due to evaporation (increase up to 10%). No mortality was recorded in control exposures throughout the testing period. Percent mortalities indicated by different endpoints at particular test dilutions are shown in Figure 1.3. Detected values of 96-hour LC$_{50}$ for N-NO$_3^-$ indicated by lethal endpoint were 922 mg L^{-1} (95% confidence limit (c.l.) 813-1046) and 1272 mg L^{-1} (95% c.l. 1150-1406) for *A. anatina* and *U. crassus*, respectively. Other calculated values of LC$_{50}$ are shown in Table 1.4. A test of

relative sensitivity of species brood stocks showed significantly higher survival of *U. crassus* for all used endpoints (binomial logistic regression; $p \leq 0.001$, df = 1, n = 300).

Shell lengths of juveniles at the beginning of the tests were 253 ±16 μm (mean ± SD) for *U. crassus* and 396 ± 16 μm (mean ± SD) for *A. anatina* (n = 25 for each species). Growth measurements taken at the end of the test are presented in Table 1.5. Both species exhibited significant growth in the control and two lowest test dilutions over the 96-hour test duration (all $p < 0.01$, Dunnet test). Mean percent increase in length in control was 7.6% (= 20.9 μm) for *U. crassus* and 4.3% (=18.0 μm) for *A. anatina*. Only in 500 mg N-NO$_3^-$ L^{-1} nominal concentration was growth significantly reduced relative to the control ($p < 0.05$ for *A. anatina*, $p < 0.001$ for *U. crassus*, both Dunnet test).

DISCUSSION

Analysis of the spatial distribution of unionids within the running waters of the Lužnice River basin showed a clear relationship with N-NO$_3^-$ concentration. These results are in agreement with other studies relating N-NO$_3^-$ to freshwater mussels populations in Central Europe. Bauer (1988) showed, using a comparison of age-specific mortality and water chemistry, that 46% of the variation of calculated mortality of *M. margaritifera* could be explained by the N-NO$_3^-$ concentration. In contrast to populations in unpolluted rivers with concentrations around 0.5 mg L^{-1}, survivorship curves were drastically altered at levels of 1.5-3 mg L^{-1}. Hochwald (1997) stated that in brooks with healthy, reproducing populations of *U. crassus,* the average N-NO$_3^-$ concentrations were under 2.2 mg L^{-1}, whereas in localities with no present reproduction N-NO$_3^-$ ranged between 3.6 – 5.0 mg L^{-1} (in growing season). Other similar results have also been published by, e.g., Köhler (2006) and Zettler and Jueg (2007). This study found the upper limit of N-NO$_3^-$ concentration (median value) in *U. crassus* localities to be 2.0 mg L^{-1}, which is consistent with the fact that the mussels still occasionally reproduce in these sites (Douda, 2007). However, as only five sites contained *U. crassus*, the interpretation of these results cannot be considered as indicative of absolute environmental tolerances, as many other factors are likely to be important in determining presence/absence at a given site.

The results of this study also indicate that the presence of the other studied unionid species is associated with lower N-NO$_3^-$ values. Shapes of response curves are indicative of monotonic increasing of probability of species occurrence with decreasing N-NO$_3^-$ levels, except in the case of *U. crassus*, where the response curve had a unimodal character. This corresponds to the fact that besides the sites with elevated N-NO$_3^-$ median levels, *U. crassus* was also absent in sites with especially low concentrations. A possible explanation for this observation is the presence of oxygen deficits, which were recorded in those sites with especially low nitrogen concentrations (Douda, 2007). It has been shown in *Unio pictorum* that mussels forego reproduction when under hypoxic stress (Aldridge & McIvor, 2003) and other negative effects linked to hypoxia are probable. A low dissolved oxygen concentration probably has a more pronounced effect on *U. crassus* as it inhabits strictly lotic habitats (Bauer, 2001), and the ability to regulate oxygen is related to the degree of hypoxia that a species normally experiences in its habitat type (Chen *et al.*, 2001).

Results of acute toxicity tests showed that direct effects on juvenile movement, growth and survival during a four-day period of N-NO$_3^-$ exposure were detectable at levels that were two orders of magnitude higher than in-stream concentrations in the studied area. The computed values of 96-hour LC$_{50}$ tests of N-NO$_3^-$ (922 and 1272 mg L^{-1} for *A. anatina* and *U. crassus*, respectively) place these species among the least sensitive groups of freshwater animals. Moreover, the response of juveniles might be attributed to acute shock exposure in the laboratory, compared with prolonged low-level exposure to N-NO$_3^-$ in the field. Published values of 96-hour LC$_{50}$ N-NO$_3^-$ for freshwater invertebrates range from 62.5 (Amphipoda) to 1042 mg L^{-1} (Gastropoda) (reviewed by Camargo *et al.*, 2005). This alignment is indicative of a remarkable tolerance of the early juvenile phases of the studied species to N-NO$_3^-$. In the context of environmentally realistic values, it seems improbable that N-NO$_3^-$ directly limits these species by harming their juvenile stage. Another indication that N-NO$_3^-$ toxicity to juvenile mussels is not responsible for the observed distribution of these species is provided by analyses of the relative sensitivity of species. Broodstock of *A. anatina* were identified as significantly more sensitive to N-NO$_3^-$ in spite of the fact that distributional data imply higher tolerance to N-NO$_3^-$. Moreover, this species is still commonly distributed in Central European running waters and, unlike *U. crassus,* has not declined widely. Several studies have shown that *A. anatina* is even able to utilise the increase in nutrients and increase its population density in eutrophicated localities (reviewed in Patzner and Muller, 2001). The results of the toxicity tests might have been influenced by the different vitality of the broodstock of each species, caused mainly by the different timing of reproduction and glochidia collection. However, null mortality in the control treatment implies good health status of tested juveniles. Most toxicity studies use survival as the test endpoint, although most researchers recognise the need to develop sublethal biomarkers for these animals (Gooding *et al.*, 2006). This is in accordance with the results of this study, which showed that by employing sublethal endpoints (growth, movement), it is possible to detect significant effects at concentrations around one-half of the lethal endpoints, which are indicated by the cessation of juvenile ciliary activity. Although these values considerably exceed concentrations measured in the field, chronic effects may exist. For example, Wang *et al.* (2007b) estimated that chronic (28-day) toxicity of total ammonia was 11-18-fold lower than the acute (4-day) values for three freshwater mussel species. Further research is therefore needed to evaluate possible chronic effects.

The difference between the limiting values inferred from distributional data and toxicity tests can be also explained by the presence of harmful factor(s) closely related to elevated N-NO$_3^-$ levels in running waters. Water chemistry processes in stream bottom sediments may alter interstitial water quality, which is of general importance for successful recruitment of juveniles developing in this environment (Buddensiek *et al.*, 1993; Geist and Auerswald, 2007). It is thus supposed that in streams with elevated N-NO$_3^-$ levels, the processes of denitrification in the bottom release nitrite nitrogen (N-NO$_2^-$), which may have a toxic effect on the juvenile mussels buried in the sediment (Patzner and Muller, 2001). Other nitrogen transformation pathways in bottom sediments may lead to transformation of nitrate nitrogen to ammonia (Burgin and Hamilton, 2007), which is extraordinarily harmful to juvenile unionids (Augspurger *et al.*, 2003). Because no relevant data on the importance of these processes in juvenile mussel microhabitats are available, further research is needed in this field.

Another explanation for the recorded relationship between the occurrence of species and N-NO$_3^-$ concentrations is suggested by land use characteristics. N-NO$_3^-$ agricultural runoff has dramatically increased mainly because of the use of inorganic nitrogen fertilisers and leaching of nitrate via subsurface water drainage systems in extensively managed areas (Jacobs and Gilliam, 1985; Jordan *et al.*, 1997). Increased N-NO$_3^-$ levels thus indicate extensive agricultural practices that are connected with other potentially negative effects on freshwater mussels, e.g., modified properties of sediment and particulate organic matter flows, altered temperature regimes of streams, inputs of fertilisers and pesticides (Allan, 2001). Focusing conservation measures for freshwater mussel populations on the mere elimination of N-NO$_3^-$ inputs to river networks, regardless of N-NO$_3^-$ sources, would therefore probably have little effect by itself. New approaches involving landscape ecology methods (reviewed by Newton *et al.*, 2008) and analyses of river bed nitrogen pathways are needed to identify the processes responsible for the observed decline of Central European freshwater mussel species and for subsequent prioritising of conservation measures.

This study showed that the negative association between nitrate and unionids cannot be a result of short-term toxicity and demonstrates the need for integrating different research methods for the development of conservation strategies for threatened species. Despite the probable absence of a direct negative effect of N-NO$_3^-$ on freshwater mussel populations, N-NO$_3^-$ has a potential to be used as an effective indicator of some types of

habitat degradation. Monitoring N-NO$_3^-$ levels in the localities of endangered unionid species may provide forewarning of negative trends in habitat conditions. When elevated N-NO$_3^-$ levels are recorded, conservation authorities should verify the unionid population status and try to identify the causes of elevated nitrate values. N-NO$_3^-$ might also be used as an indicator of the effectiveness of conservation measures adopted in catchments. However, the identification of causal mechanisms responsible for the observed relation of unionids to N-NO$_3^-$ still needs further research.

REFERENCES
Aldridge DC, McIvor AL. 2003. Gill evacuation and release of glochidia by Unio pictorum and Unio tumidus under thermal and hypoxic stress. *Journal of Molluscan Studies* **69**: 55-59.

Allan JD. 2001. *Stream Ecology: Structure and function of running waters*. Kluwer Academic Publishers: Dordrecht, The Netherlands.

Anonymous. 1990. *Trimmed Spearman–Karber (TSK) Program Version 1.5*. US Environmental Protection Agency: Cincinnati, OH.

Anonymous. 1997. *UTHSCSA Image Tool - IT Version 2.0*. University of Texas Health Science Center at San Antonio: San Antonio, TX.

Anonymous. 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing: Viena, Austria.

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.

Bauer G. 1988. Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in Central Europe. *Biological Conservation* **45**: 239-253.

Bauer G. 2001. Framework and driving forces for the evolution of naiad life histories. In: *Ecology and evolution of the freshwater mussels Unionoida*, Bauer G, Wachtler K. (eds). Springer-Verlag: Heidelberg, Germany; 233-255.

Bauer G, Wachtler K. 2001. Environmental relationships of naiads: Threats, impact on the ecosystem, indicator function. In: *Ecology and evolution of the freshwater mussels Unionoida*, Bauer G, Wachtler K. (eds). Springer-Verlag: Heidelberg, Germany; 311-315.

Beran L. 2002. Aquatic molluscs of the Czech Republic – distribution and its changes, habitats, dispersal, threat and protection, red list. *Journal of the society for Natural Science Uherské Hadiště* - Supplementum **10**: 1-258.

Bílý M, Simon O. 2007. Water quality issues in the protection of oligotrophic streams with the occurrence of pearl mussel (*Margaritifera margaritifera*) in the Czech Republic. *Acta Universitatis Carolinae, Environmentalica* **21**: 21-30.

Bishop CD, Hudson R, Farris JL. 2007. Propagation and culture of freshwater mussels. In: *Freshwater bivalve ecotoxicology*, Farris JL, Van Hassel JH (eds). Society of Environmental Toxicology and Chemistry: Pensacola, FL; 65-94.

Blazek R, Gelnar M. 2006. Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: Unionidae) on host fishes. *Folia Parasitologica* **53**: 98-106.

Bogan AE. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. *American Zoologist* **33**: 599-609.

Bogan AE. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. *Hydrobiologia* **595**: 139-147.

Brainwood M, Burgin S, Byrne M. 2006. Is the decline of freshwater mussel populations in a regulated coastal river in south-eastern Australia linked with human modification of habitat? *Aquatic Conservation: Marine and Freshwater Ecosystems* **16**: 501-516.

Buddensiek V. 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: A contribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation* **74**: 33-40.

Buddensiek V, Engel H, Fleischauerrossing S, Wachtler K. 1993. Studies on the chemistry of interstitial water taken from defined horizons in the fine sediments of bivalve habitats in several Northern German lowland waters. 2. Microhabitats of *Margaritifera margaritifera* L., *Unio crassus* (Philipsson) and *Unio tumidus* (Philipsson). *Archiv fur Hydrobiologie* **127**: 151-166.

Burgin AJ, Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment* **5**: 89-96.

Camargo JA, Alonso A, Salamanca A. 2005. Nitrate toxicity to aquatic animals: A review with new data for freshwater invertebrates. *Chemosphere* **58**: 1255-1267.

Camargo JA, Alonso Á. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment *International* **32**: 831-849.

Chen L-Y, Heath AG, Neves RJ. 2001. Comparison of oxygen consumption in freshwater mussels (Unionidae) from different habitats during declining dissolved oxygen concentration. *Hydrobiologia* **450**: 209-214.

Cheng S-Y, Chen J-C. 2002. Study on the oxyhemocyanin, deoxyhemocyanin, oxygen affinity and acid-base balance of *Marsupenaeus japonicus* following exposure to combined elevated nitrite and nitrate. *Aquatic Toxicology* **61**: 181-193.

Christian AD, Harris JL. 2008. An introduction to directions in freshwater mollusk conservation: Molecules to ecosystems. *Journal of the North American Benthological Society* **27**: 345-348.

Clements WH, Carlisle DM, Courtney LA, Harrahy EA. 2002. Integrating observational and experimental approaches to demonstrate causation in stream biomonitoring studies. *Environmental Toxicology and Chemistry* **21**: 1138-1146.

Cope WG, Bringolf RB, Buchwalter DB, Newton TJ, Ingersoll CG, Wang N, Augspurger T, Dwyer FJ, Barnhart MC, Neves RJ, Hammer E. 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. *Journal of the North American Benthological Society* **27**: 451-462.

Cosgrove PJ, Hastie LC. 2001. Conservation of threatened freshwater pearl mussel populations: River management, mussel translocation and conflict resolution. *Biological Conservation* **99**: 183-190.

Dodd BJ, Barnhart MC, Rogers-Lowery CL, Fobian TB, Dimock Jr RV. 2005. Cross-resistance of largemouth bass to glochidia of unionid mussels. *Journal of Parasitology* **91**: 1064-1072.

Douda K. 2007. The occurrence and growth of *Unio crassus* (Mollusca: Bivalvia: Unionidae) in Lužnice River basin in respect to water quality. *Acta Universitatis Carolinae, Environmentalica* **21**: 57-63.

Engel H. 1990. *Untersuchungen zur Autökologie von Unio crassus (Philipsson) in Norddeutschland*. University of Hannover: Hannover, Germany.

Geist J, Auerswald K. 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). *Freshwater Biology* **52**: 2299-2316.

Gooding MP, Newton TJ, Bartsch MR, Hornbuckle KC. 2006. Toxicity of synthetic musks to early life stages of the freshwater mussel *Lampsilis cardium*. *Archives of Environmental Contamination and Toxicology* **51**: 549-558.

Goudreau SE, Neves RJ, Sheehan RJ. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. *Hydrobiologia* **252**: 211-230.

Hartvich P, Lusk S, Halačka K, Havlová J, Švarc J. 1998. Diversity of the ichthyofauna and migration permeability of the River Lužnice. *Sborník Biodiverzita ichtyofauny ČR* **2**: 137-148.

Hochwald S. 1997. Populationsökologie der Bachmuschel (*Unio crassus*). *Bayreuther Forum Ökologie* **50**: 1-171.

Hochwald S. 2001. Plasticity of life-history traits in *Unio crassus*. In: *Ecology and evolution of the freshwater mussels Unionoida*, Bauer G, Wachtler K. (eds). Springer-Verlag: Heidelberg, Germany; 127-141.

Jacobs TC, Gilliam JW. 1985. Riparian losses of nitrate from agricultural drainage waters. *Journal of Environmental Quality* **14**: 472-478.

Jensen FB. 1996. Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). *Aquatic Toxicology* **34**: 95-104.

Jongman RHG, ter Braak CJF, van Tongeren OFR. 2005. *Data analysis in community and landscape ecology*. Cambridge University Press: Cambridge, United Kingdom.

Jordan TE, Correll DL, Weller DE. 1997. Relating nutrient discharges from watersheds to land use and streamflow variability. *Water Resources Research* **33**: 2579-2590.

Köhler R. 2006. Observations on impaired vitality of *Unio crassus* (Bivalvia: Najadae) populations in conjunction with elevated nitrate concentration in running waters. *Acta hydrochimica et hydrobiologica* **34**: 346-348.

Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG *et al*. 2004. The global decline of nonmarine mollusks. *BioScience* **54**: 321-330.

Newton TJ, Woolnough DA, Strayer DL. 2008. Using landscape ecology to understand and manage freshwater mussel populations. *Journal of the North American Benthological Society* **27**: 424-439.

Osterling EM, Greenberg LA, Arvidsson BL. 2008. Relationship of biotic and abiotic factors to recruitment patterns in *Margaritifera margaritifera*. *Biological Conservation* **141**: 1365-1370.

Patzner RA, Muller D. 2001. Effects of eutrophication on unionids. In: *Ecology and evolution of the freshwater mussels Unionoida*, Bauer G, Wachtler K. (eds). Springer-Verlag: Heidelberg, Germany; 327-335.

Peeters ETHM, Gardeniers JJP, 1998. Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwater Biology* **39**: 605-615.

Pekkarinen M. 1993. Reproduction and condition of unionid mussels in the Vantaa river, south Finland. *Archiv für Hydrobiologie* **127**: 357-375.

Perez-Quintero JC. 2007. Diversity, habitat use and conservation of freshwater molluscs in the lower Guadiana River basin (SW Iberian Peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems* **17**: 485-501.

Pickett S, Ostfeld RS, Shachak M, Likens GE. 1997. *Ecological basis of conservation: heterogeneity, ecosystems, and biodiversity*. Chapman and Hall: New York, NY.

Richter BD, Braun DP, Mendelson MA, Master LL. 1997. Threats to imperiled freshwater fauna. *Conservation Biology* **11**: 1081-1093.

Van Dyke F. 2008. *Conservation biology: foundations, concepts, applications*. Springer-Verlag: New York, NY.

Van Hassel JH, Farris JL. 2007a. A review of the use of unionid mussels as biological indicators of ecosystem health. In: *Freshwater bivalve ecotoxicology*, Farris JL, Van Hassel JH (eds). Society of Environmental Toxicology and Chemistry: Pensacola, FL; 19-49.

Van Hassel JH, Farris JL. 2007b. Freshwater bivalve ecotoxicology. In: *Freshwater bivalve ecotoxicology*, Farris JL, Van Hassel JH (eds). Society of Environmental Toxicology and Chemistry: Pensacola, FL; 1-17.

Van Hassel JH, Farris JL. 2007c. Linking bioacumulation and biological effects to chemicals in water and sediment: a conceptual framework for freshwater bivalve ecotoxicology. In: *Freshwater bivalve ecotoxicology*, Farris JL, Van Hassel JH (eds). Society of Environmental Toxicology and Chemistry: Pensacola, FL; 215-255.

Van Herpe Y, Troch PA. 2000. Spatial and temporal variations in surface water nitrate concentrations in a mixed land use catchment under humid temperate climatic conditions. *Hydrological Processes* **14**: 2439-2455.

Venables WN, Ripley BD. 2002. *Modern Applied Statistics with S*. Springer-Verlag: New York, NY.

Wang N, Augspurger T, Barnhart MC, Bidwell JR, Cope WG, Dwyer FJ, Geis S, Greer IE, Ingersoll CG, Kane CM *et al*. 2007a. Intra- and interlaboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* **26**: 2029-2035.

Wang N, Ingersoll CG, Greer IE, Hardesty DK, Ivey CD, Kunz JL, Brumbaugh WG, Dwyer FJ, Roberts AD, Augspurger T *et al*. 2007b. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry* **26**: 2048-2056.

Watters GT. 1996. Small Dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. *Biological Conservation* **75**: 79-85.

Weber E. 2005. Population size and structure of three mussel species (Bivalvia: Unionidae) in a northeastern German river with special regard to influences of environmental factors. *Hydrobiologia* **537**: 169-183.

Zettler ML, Jueg U. 2007. The situation of the freshwater mussel *Unio crassus* (Philipsson, 1788) in north-east Germany and its monitoring in terms of the Habitats Directive. *Mollusca* **25**: 165-174.

Table 1.1 Range of water quality conditions in the study area measured at the set of 36 studied sites between 1995-2005; (mean+-SD is presented for the site with the minimal and maximal calculated mean, n = 21 – 120)

parameter	units	range		
BOD_5	$(mg\ L^{-1})$	2.2 ± 1.3	-	7.8 ± 4.6
COD_{Cr}	$(mg\ L^{-1})$	15.5 ± 6.4	-	49.6 ± 18.9
conductivity	$(\mu S\ cm^{-1})$	14.6 ± 1.7	-	40.7 ± 5.1
total suspension	$(mg\ L^{-1})$	6.6 ± 3.7	-	59.0 ± 136.9
$N-NH_4^-$	$(mg\ L^{-1})$	0.06 ± 0.05	-	0.88 ± 1.02
$N-NO_3^-$	$(mg\ L^{-1})$	1.1 ± 0.7	-	5.8 ± 2.0
dissolved O_2	$(mg\ L^{-1})$	7.9 ± 2.9	-	11.3 ± 3.3
total P	$(mg\ L^{-1})$	0.07 ± 0.03	-	0.45 ± 0.23
pH		7.2 ± 0.2	-	8.0 ± 0.8

Table 1.2 Estimated values for the parameters of sigmoid (*U. crassus*) and Gausian (other species) logit response curves (β_0, β_1, β_2) describing probability of occurrence; R - percentage deviance reduction of fitted models; significance of parameters were tested by Chi-square test of deviance reduction, ns – not significant (p<0.05).

species	β_0	β_1	β_2	R
A. anatina	10.6560	-2.4619	ns	58.8
P. complanata	1.4127	-1.2729	ns	20.5
U. tumidus	2.0309	-1.1342	ns	21.6
U. pictorum	1.5396	-0.6032	ns	9.6
U. crassus	-240.9143	265.3243	-72.5560	61.1

Table 1.3 Measured water quality parameters in particular test treatments (mean ± SD, n=4).

Test $N-NO_3^-$ concentration	$N-NO_3^-$ $(mg\ L^{-1})$	$N-NH_4^+$ $(mg\ L^{-1})$	$N-NO_2^-$ $(mg\ L^{-1})$	pH	dissolved O_2 $(mg\ L^{-1})$	conductivity $(\mu S.cm^{-1})$
2000	2060 ± 95.2	< 0.12	< 0.028	8.11 ± 0.12	8.35 ± 0.27	14328 ± 387.5
1000	1005 ± 23.7	< 0.10	< 0.026	8.14 ± 0.17	8.38 ± 0.32	7670 ± 189.2
500	530 ± 22.3	< 0.12	< 0.021	8.14 ± 0.15	8.34 ± 0.18	4188 ± 175
100	102 ± 4.1	< 0.10	< 0.021	8.16 ± 0.15	8.35 ± 0.25	1168 ± 47.5
25	26.2 ± 1.2	< 0.13	< 0.020	8.13 ± 0.18	8.4 ± 0.24	588 ± 33.8
control	5.1 ± 0.1	< 0.13	< 0.020	8.06 ± 0.28	8.45 ± 0.18	430 ± 35.7

Table 1.4 Median lethal concentrations and 95% confidence limits (c.l.) of $N-NO_3^-$ for *U. crassus* and *A. anatina* computed by Spearman-Karber method (for test endpoint characteristics see Methods; n.s. - no partial kill observed, which is necessary to compute LC_{50}).

toxicant	test duration (hours)	test endpoint	*U. crassus* LC_{50} $(mg\ L^{-1})$	*U. crassus* 95% c.l. $(mg\ L^{-1})$	*A. anatina* LC_{50} $(mg\ L^{-1})$	*A. anatina* 95% c.l. $(mg\ L^{-1})$
$N-NO_3^-$	48	Foot movement	822	674-1003	550	420-720
		Valve movement	1307	1196-1428	n.s.	-
		Ciliary activity	n.s.	-	1383	1285-1488
	96	Foot movement	504	401-633	369	284-480
		Valve movement	1140	1003-1295	721	648-802
		Ciliary activity	1272	1150-1406	922	813-1046

Table 1.5 Length measurements of juvenile mussels *U. crassus* and *A. anatina* in particular treatments after 96-hour exposure to $N-NO_3^-$ (mean ± SD); an asterisk * indicates a significant reduction relative to control, p < 0.05.

Nominal concentration	*U. crassus* Length (µm)	*U. crassus* % increase	*A. anatina* Length (µm)	*A. anatina* % increase
control	274 ± 20	7.6	414 ± 27	4.3
25 $mg\ L^{-1}$	276 ± 16	8.3	423 ± 26	6.4
100 $mg\ L^{-1}$	278 ± 14	9.0	421 ± 20	5.9
500 $mg\ L^{-1}$	258 ± 11*	1.9	400 ± 19*	1.0
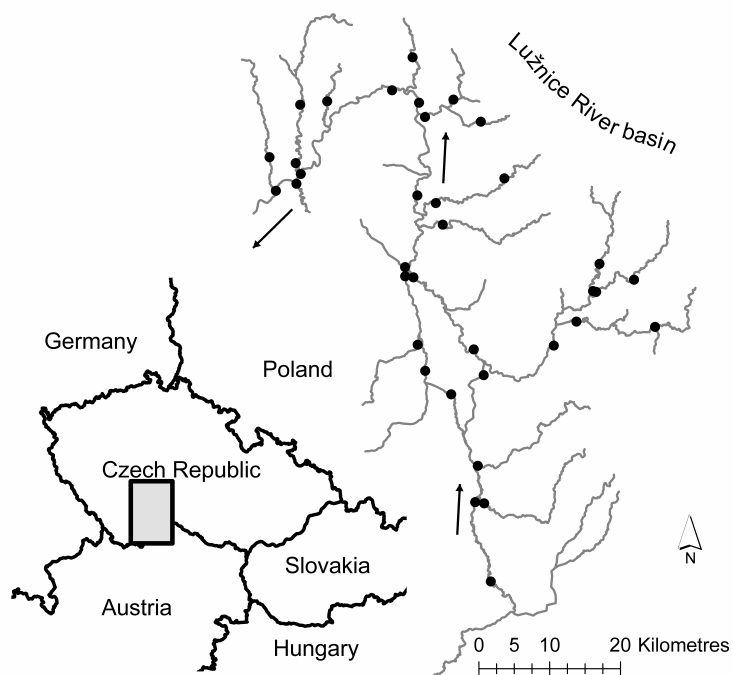
Figure 1.1 Location of the study area (Lužnice River basin) and 36 sampling sites (•) for water quality and unionid bivalves presence/absence.
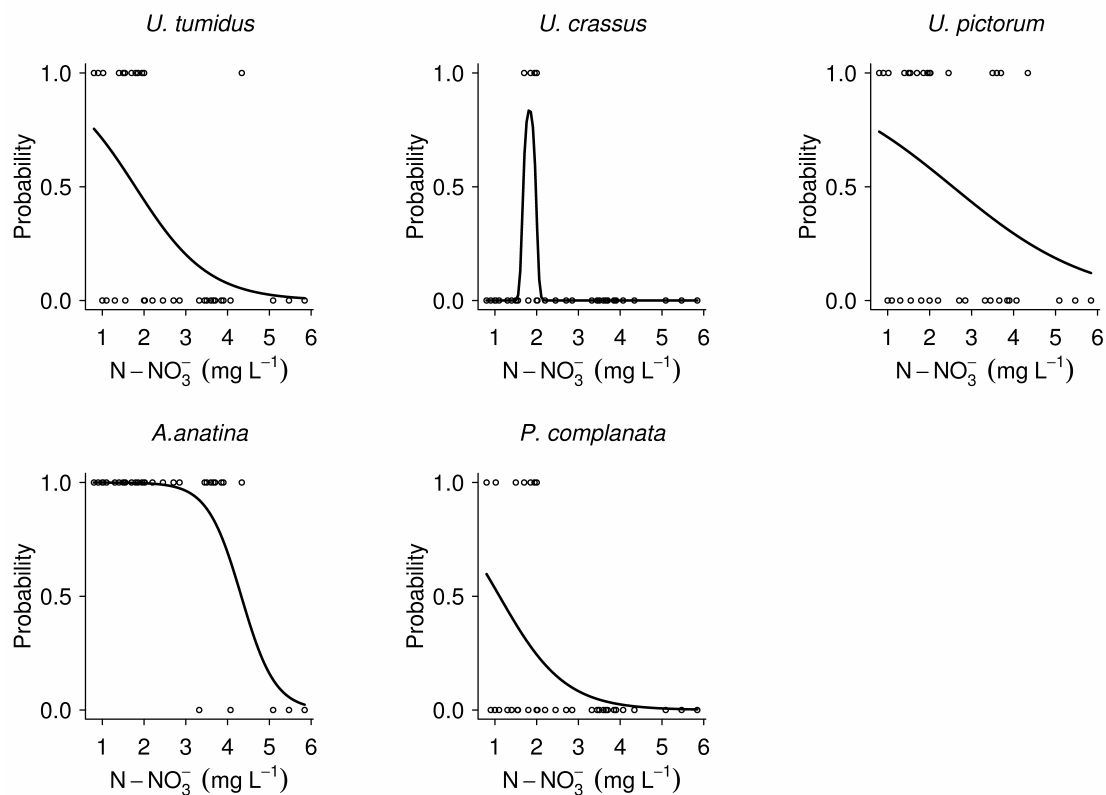
Figure 1.2 Sigmoid and Gaussian logit curve fitted by logistic regression of the presences and absences (o at Probability 1 and 0, respectively) of studied species on N-NO$_3^-$ concentration; Chi-square, p < 0.05, n = 36.
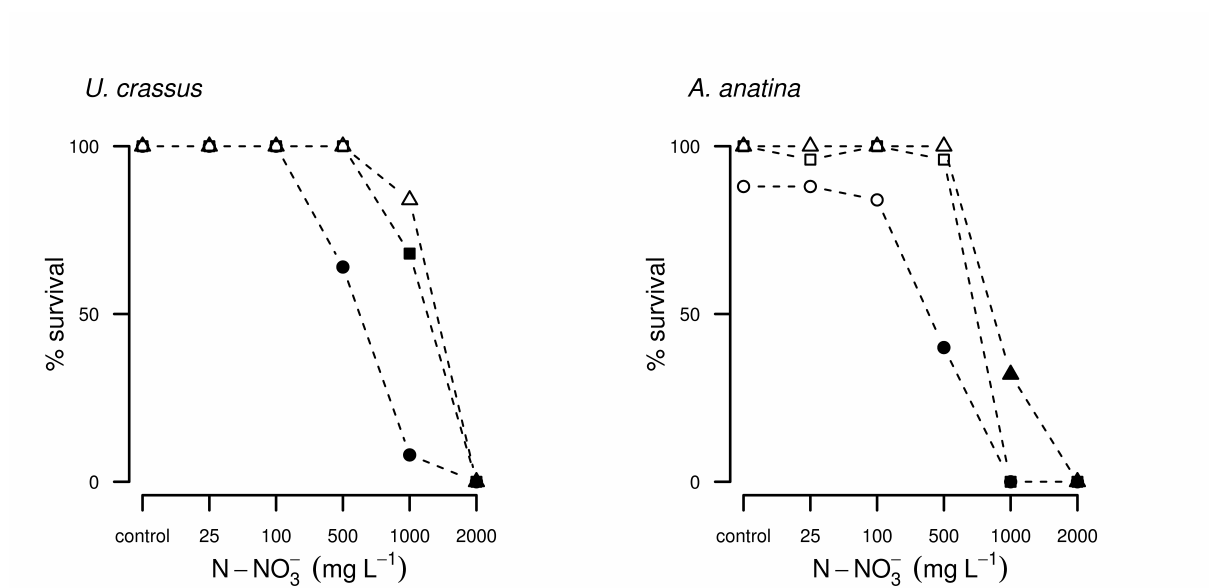
Figure 1.3 Percent survival of juveniles at the end of a 96-hour toxicity test with N-NO₃⁻ at different concentrations, revealed by different test endpoints (□ foot movement, o valve movement, Δ ciliary activity); filled symbols indicate significant reduction in survival of juveniles in the respective treatment relative to control (Fisher's test, $p < 0.01$).

Chapter 2

Water quality requirements of indigenous and alien crayfish in streams of the Czech Republic: identification, threats and conservation implications

Svobodová, J., Douda, K., Štambergová, M., Picek, J., Vlach, P., Fischer, D.

Water quality requirements of indigenous and alien crayfish in streams of the Czech Republic: identification, threats and conservation implications

Jitka Svobodová[a,*], Karel Douda[a], Monika Štambergová[b], Jiří Picek[a], Pavel Vlach[c] and David Fischer[d]

[a]*T.G. Masaryk Water Research Institute, Podbabská 30, 160 62 Prague 6, Czech Republic*
[b]*Agency for Nature Conservation and Landscape Protection of the Czech Republic, Nuselská 39, 140 00 Praha 4, Czech Republic*
[c]*Ekosolution, 5. května 617, 336 01 Blovice, Czech Republic*
[d]*Mining Museum Příbram, Hynka Kličky Place No. 293, 261 01 Příbram VI, Czech Republic*

Abstract

1. Although the noble crayfish (*Astacus astacus* L.) and stone crayfish (*Austropotamobius torrentium* Schr.) are critically endangered European species, their water quality requirements are not sufficiently known.
2. This study aimed to investigate the chemical-physical condition demands of the noble and stone crayfish in the Czech Republic compared to those of the invasive spiny-cheek crayfish (*Orconectes limosus* Raf.). We investigated 18 chemical-physical parameters at 1008 sites at which crayfish were found to be present or absent. We have analyzed the following parameters: dissolved oxygen, pH, BOD_5, COD_{Cr}, ammonia, ammonium ions, nitrate ions, zinc, copper, iron, aluminum, calcium, nitrates, sulphates, chlorides, total phosphorus, suspended solids and conductivity.
3. For the noble and stone crayfish, only minor differences in water quality were found. This result indicated that the water quality requirements of these indigenous crayfish are likely to be very similar. However, significant differences in water quality were observed between locations inhabited by indigenous crayfish and those inhabited by the invasive spiny-cheek crayfish.
4. According to these findings, the latter species was able to survive in locations with lower water quality. Simple logistic regression models were then used to examine relationships between the presence or absence of noble crayfish and each evaluated water quality parameter. The presence of this species was related significantly to the parameters that indicate nutrient enrichment (particularly ammonium, BOD_5, and nitrite) and to iron.
5. Overall, although the indigenous crayfish species were found at several sampling sites that had deteriorated water quality, the statistical analyses indicate that the indigenous species require water of high quality. Improvement in water quality is therefore an important step in sustaining indigenous crayfish populations.

Keyword: river, water quality, invertebrates, pollution, *Astacus astacus, Austropotamobius torrentium, Orconectes limosus*

* Correspondence to: J. Svobodová, T.G. Masaryk Water Research Institute, Podbabská 30, 160 62 Prague 6, Czech Republic

E-mail address: (jitka_svobodova@vuv.cz)

Introduction

Crayfish, one of the largest freshwater invertebrates, play an irreplaceable functional role as important consumers in aquatic ecosystems (Souty-Grosset et al., 2006). They are often designated as "flagship species" and are considered to be part of the cultural heritage of many European countries (Füreder et al., 2003a). Until recently, indigenous crayfish were one of the predominant constituents of the freshwater benthic fauna, however, anthropogenic degradation of many freshwaters and thus crayfish habitats has caused an overall decline in crayfish biodiversity (Souty-Grosset et al., 2006). In addition to their role in producing population declines and loss, all of these negative factors can result in a loss of genetic diversity in indigenous crayfish, thus lowering their evolutionary potential (Weiss, 2005).

At present, a total of five crayfish species are considered to exist in the Czech Republic. However, only the noble crayfish (*Astacus astacus* Linnaeus, 1758) and the stone crayfish (*Austropotamobius torrentium* Schrank, 1803) are indigenous species (Ďuriš, Kozák, 2000; Machino, Holdich, 2005). Both of them are classified as Critically Endangered on the Red List of Threatened Animals of the International Union for Conservation of Nature (IUCN), and the stone crayfish is listed in Annex II of the European Community Habitats Directive 92/43/EEC. The narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823), originally from Eastern Europe, was introduced to the Czech Republic at the end of the 19th century in order to replenish native noble crayfish populations reduced by the crayfish plague (Ďuriš, Horká, 2001). Two North American invasive species, the signal crayfish (*Pacifastacus leniusculus* Dana, 1852) and the spiny-cheek crayfish (*Orconectes limosus* Rafinesque, 1817) have spread widely either due to natural dispersive potential and/or as the result of anthropogenic translocations (Kozák et al., 2004).

Indigenous crayfish are constantly endangered by a range of environmental factors. The pollution of streams by industrial and residential waste, agriculture, and acid rain as well as habitat loss resulting from the straightening, reinforcement and canalization of water courses (e.g., Renz, Breithaupt, 2000; Kettunen, ten Brink, 2006) may all cause declines in crayfish populations (e.g., Holdich, 2002; Füreder et al., 2003a; Seiler , Turner, 2004). Another important factor is the invasion of non-indigenous species causing radical changes in the community structure and thus the extinction of indigenous species (Westman et al., 2002). Moreover, alien species may spread diseases, which may be even more detrimental to native species than direct interspecific competition. A case in point is the crayfish plague in Europe caused by the parasitic oomycete *Aphanomyces astaci* (Schikora 1906) (Unestam, 1965; Kozubíková et al., 2006). Furthermore, strong predatory pressure from introduced mink (*Mustela vison*) (Fischer et al., 2009) and excessive fish farming and breeding, together with unsuitably chosen fish stocks, have negatively influenced crayfish populations (Holdich, 2002; Schulz et al., 2006).

This study focused on water quality, one of the most important environmental parameters influencing the distribution of crayfish populations. Despite significant improvements in water quality over the last two decades, many streams in the Czech Republic are still very highly polluted (Štambergová et al., 2009). Although many studies have been tested the acute toxicity of several potential pollutants (e.g., aluminum, copper, and nitrite) on crayfish under laboratory conditions (e.g., Alexopoulos et al., 2003; Ward et al., 2006; Guner, 2007; Yildiz , Benli, 2004), only a few have considered native (e.g., Bohl, 1987; Foster, 1995; Trouilhé et al., 2007; Pârvulescu et al., 2011) as well as alien crayfish (Rallo et al., 2002) and especially the species investigated here (*A. torrentium, O. limosus*) under natural conditions. There is only scattered knowledge about the physicochemical requirements of these species on a larger scale. It is known that indigenous European crayfish are sensitive to water pollution caused by nutrient enrichment (Favaro, 2010),

that long-term exposure to nitrite reduces crayfish immunity and increases the chance of infection (Yildiz , Benli, 2004), as does also the exposure to heavy metals such as aluminum (e.g., Alexopoulos et al., 2003; Ward et al., 2006). In our study, we hypothesized that invasive crayfish species would have higher tolerance to water pollution and that there would be a significant relationship between water quality conditions and native crayfish occurrence in Central European running waters. To test this hypothesis, we investigated the water quality demands of indigenous and alien crayfish in the Czech Republic through an analysis of the spatial distributions of species in relation to water quality in streams. We focused mainly on determining the differences arising from comparisons between species. Subsequently, we described the relationship between the presence of noble crayfish and the concentration gradients of several water quality parameters.

Materials and Methods

Data compiling and processing

Data on the presence or absence of individual crayfish species covering the total area of the Czech Republic, 78 864 km^2 were compiled as part of the database of the Agency for Nature Conservation and Landscape Protection of the Czech Republic for the period from 2004 to 2006 (Štambergová et al., 2009), which was developed based upon results of detailed crayfish mapping conducted throughout the entire Czech Republic. Selection of reaches for mapping was based on using a line vector layer map of water streams at a scale of 1:50 000. For small and medium streams, one-hundred-meter reaches were identified and mapped, with a distance between reaches of approximately 3.5 km. The mapping itself was performed by wading through the stream channel and thoroughly searching potential crayfish shelters (space under stones, in between roots etc.). For larger rivers and standing waters, baited traps were used, with traps set in the rivers every 4–7 kilometers. Verification of several historical and questionable findings was also performed in addition to regular systematic monitoring. At each monitored sampling reach, the presence or absence of crayfish species was determined. All data and the location of findings were then digitalized. This mapping covered 90 % of the Czech Republic and totaled 11 518 sampling reaches, with crayfish found in 977 of them.

Information on water quality was obtained from a national stream water quality monitoring program, established according to the European Water Framework Directive (Czech Hydrometeorological Institute, 2009). Data from the years 2004–2006 were used. The monitored streams represented a broad range of water quality conditions. Water quality sampling was conducted once a month using standard methods and equipment (ISO EN standard norm protocols). A total of 24 measurements were taken per sampling site. On small 1st–3rd order streams (Strahler, 1957), a database from the T. G. Masaryk Water Research Institute on water quality from monitoring of specially protected, threatened species of water organisms from the years 2006–2007 was used as well. In this data source, the numbers of samples taken over the entire two-year period were 2–10 per site. In all cases, water samples were analyzed by a certified laboratory. We obtained data from a total of 1545 profiles, and we evaluated in total 18 water quality parameters as shown in Table 2.1. Since water quality data were derived from various monitoring programs, complete data on all parameters were not available for all sampling sites Consequently, multidimensional methods were only used on a limited dataset containing complete data. However, the relationship of crayfish to individual parameters was evaluated using separate tests. Sites with crayfish presence or absence data were linked to water quality data using the vector-based map mentioned above. Only those data from sites where the stream corridor distance between the sampling locations for crayfish data and those for water

quality data was closer than 2.5 km were used. The average of water quality values measured at an individual sampling site from 2004–2007 was then used for further analyses. Each profile was therefore represented by one value for each parameter. Finally, we analyzed a total of 1008 sampling sites (out of the 1545 sites initially available) at which the presence or absence of noble crayfish, stone crayfish or spiny-cheek crayfish was known and which were successfully linked to water quality data (Fig. 2.1). *Astacus leptodactylus* and *Pacifastacus leniusculus* were not included in this study because these species are usually restricted to standing waters in the study area.

Statistical analyses

We used canonical correspondence analysis (CCA) to relate the species-occurrence data to water quality parameters in a subset of 103 sampling sites where crayfish occurred (75 sites with *A. astacus*, 9 with *A. torrentium*, and 22 with *O. limosus*) as well a complete data set of seven water quality parameters (dissolved oxygen, pH, BOD_5, COD_{Cr}, ammonium, nitrite, and nitrate) existed. Inclusion of particular parameters in the final model was tested by using a Monte Carlo permutation procedure with 500 permutations. The analysis was performed using CANOCO 4.5 (Ter Braak and Šmilauer 2002).

Pairwise Wilcoxon rank sum tests (with adjusted *p* values using the Bonferroni method) were used to test for differences in water quality at sampling sites at which particular species occurred (Table 2.1). A nonparametric method was used because normality and variance homogeneity assumptions were not satisfied (using the Shapiro-Wilkinson normality test and the Bartlett test of homogeneity of variances). Each site was represented by the arithmetic mean of the measured values.

Simple logistic regression models (Jongman et al., 1995) were fitted to the data to examine the relationship between the presence/absence of noble crayfish and each water quality parameter. This method creates a presence/absence response curve for a given species. The curve describes the probability of occurrence of the species (dependent variable) as a function of the explanatory environmental variable (e.g., Streissl , Hödl 2002; Cruz , Rebelo, 2007; Douda, 2010). Logit functions (g(x) = $\beta_0 + \beta_1 x + \beta_2 x^2$) were plotted against the explanatory variables, and the significance of the model parameters was tested by comparing the deviance reduction (Chi-square, p<0.05) (methods described in Jongman et al., 1995; Peeters , Gardeniers, 1998). The percentage deviance reductions of fitted models were computed to assess the contribution of each variable to the explanation of total variance (Peeters , Gardeniers, 1998). If the estimated parameter β_2 is significantly different from zero, the response curve is symmetrical and bell-shaped, with the highest probability of species occurrence falling within the observed range of values. Analyses were conducted using the R 2.12.0 software package (R Development Core Team, 2010).

Results

Of all 1008 sites, noble crayfish were present at 116 sites, and stone crayfish were present at 19 sites; both species were sympatric at 5 sites. Spiny-cheek crayfish were present at 28 sites. At the remaining 850 sampling sites, no crayfish were found. The mean (± SD) values of water quality parameters measured at the sampling sites where particular crayfish species were found are listed in Table 2.1. The comparison of water quality at sites inhabited by the noble, stone, and introduced spiny-cheek crayfish is shown in Table 2.1. Significant differences between the two indigenous species were recorded only for Zn and oxygen (p<0.05) indicating similar water quality requirements. Conversely, the water quality at sites inhabited by the spiny-cheek crayfish is significantly different (lower quality) from that found at the sites for the noble and stone crayfish for most of the parameters evaluated (p<0.05). However, the parameters pH, Ca, Cu, total phosphorus, SO_4^{2-} and conductivity

showed no significant differences (p>0.05) between the native and the introduced crayfish.

The results of the CCA multivariate analysis showed that five (dissolved oxygen, pH, BOD_5, ammonium, nitrate) of the seven tested water quality parameters were included in the final model (p<0.05; Monte Carlo permutation test). Fig. 2.3 shows an ordination diagram in which species scores, water quality parameters and samples are plotted on the first two CCA axes. The first axis explained 49.3 % and the second axis 10.1 % of the variability in the species data. The species scores showed a negative association between native species occurrence and nitrogen load together with oxygen shortage. The occurrence of native stone crayfish was also positively related to increased pH, noble crayfish needs more oxygenated water, whereas spiny-cheek crayfish occurs in more polluted waters.

More detailed information on the relationship between the occurrence of noble crayfish and water quality gradients was provided by the results obtained from logistic regression. The most important parameters (indicated by the highest deviance reduction for the fitted model) were ammonium, BOD_5, nitrite and iron. Table 2.2 shows the estimated values for the parameters β_0, β_1, and β_2 from the logit function "g" and the percentage reduction in deviance for the fitted models. The estimated parameter β_2 was significantly different from zero for BOD_5, pH, oxygen, and Cu, thus the response curve was symmetrical and bell-shaped, with optima falling within the observed range of values. The calculated optima were as follows: BOD_5 1.16 mg L^{-1}, pH 7.97, dissolved oxygen 9.79 mg L^{-1} and Cu 0.012 mg L^{-1} (Table 2.2, Fig. 2.2). The response curves of seven other tested parameters (COD_{Cr}, ammonium, nitrite, zinc, iron, aluminum, and nitrate) were sigmoid, with an increasing probability of species occurrence at lower values (Fig. 2.2). The remaining tested variables (calcium, sulfate, chloride, total phosphorus, suspended solids and conductivity) were not significant at p=0.05.

Discussion

This study evaluated the relationship between the presence of crayfish and water quality in streams of the Czech Republic. Our results confirmed the high water quality requirements of native crayfish, also found by other authors (Schulz et al., 2006; Pârvulescu et al., 2011). However, our results further refine these requirements for native and non-native crayfish species and specify tolerances to water pollution in the species' natural environment. We compared the species' relative dependence on water quality by contrasting the particular crayfish species as well as sites without crayfish. Significant differences between the noble crayfish and the stone crayfish were identified only for the parameters Zn and oxygen. This pattern of overall similarity in water quality requirements between the indigenous species is also reflected by the sympatric occurrence of these two species at five sites (see also Vlach et al., 2009). In all three species studied, we noted similar water quality requirements for the following parameters: pH, calcium, copper, total phosphorus, sulfate and conductivity (i.e., no significant differences found). For most other parameters, however, the values at sites associated with the presence of spiny-cheek crayfish differed from those found at sites with indigenous crayfish, with spiny-cheek crayfish present in more polluted water. This crayfish is generally found in rivers and larger streams, but it also occasionally migrates to the mouths of smaller inflows with better water quality. Even though these sites often fulfill the water quality requirements of native crayfish species, no sympatric occurrences of native and invasive crayfish were found. This lack of sympatry is most likely caused by the high rates of infection with *Aphanomyces astaci* in populations of spiny-cheek crayfish (Kozubíková et al., 2009). Spiny-cheek crayfish, if healthy, are more resistant to this pathogen and therefore act as potential carriers. Any sympatric occurrence of spiny-cheek crayfish with indigenous species thus usually results

in the extirpation of native, nonresistant populations (Kozubíková et al., 2008). In comparing water quality at sites with and without individual crayfish species, difference could be found mainly for the noble and spiny-cheek crayfish. Differences for the stone crayfish were not as evident, likely because of the limited number of sites and the presence of three sites with extreme chemistry (e.g. Zákolanský potok, see below). Evaluating differences in such localities, however, is also influenced by the presence of many extremely clean sites that are not populated by crayfish, likely limited by the lack of nutrients (as seen for the noble crayfish in the gradient of BOD_5 evaluated by logistic regression).

This study included sites spanning a large range of water quality, from very clean to highly polluted waters. Most average parameter values in sites with noble and stone crayfish were in the ranges found by other authors (Bohl, 1987; Pârvulescu et al., 2011), though some extreme values lie outside of the supposed optimal concentration ranges of the studied indigenous species. This pattern may be explained by the great variability of environmental conditions throughout the studied area, as discussed below in detail.

Generally, this study confirms that physiochemical properties are very important parameters that influence crayfish populations. However, not all studies evaluating data on species distributions have outlined this realationship. The reason for this difference in results is probably that the previous studies did not cover a sufficiently broad scale of water quality conditions, and water pollutants were generally at low concentrations and/or did not significantly differ among sites (Schulz et al., 2006; Trouilhé et al., 2007). For example, in the study of Schulz et al. (2006), the highest ammonium value measured at locations with noble crayfish was 0.15 mg L^{-1}. Trouilhé et al. (2007) reported their highest average value of ammonium as 0.139 mg L^{-1} in streams with white-clawed crayfish (*Austropotamobius pallipes* Lereboullet, 1858). In contrast, we found ammonium to be one of the most fundamental chemical parameters influencing the presence of crayfish. The maximal mean value of ammonium in locations without crayfish was 8.7 mg L^{-1}, whereas among streams with indigenous crayfish, the maximal mean concentration was 1.5 mg L^{-1} NH_4^+ in the Zákolansky brook close to Prague (with stone crayfish). This site also had a low concentration of dissolved oxygen (2.8 mg L^{-1}), a situation that can be unfavorable for aquatic fauna because the toxicity of ammonium increases with declines in oxygen concentration (Svobodová et al., 1987). However, at the pH level measured in the stream (pH 8.1±0.28), only a small amount of NH_4^+ is converted to the toxic non-dissociated molecule NH_3, which easily penetrates the cell membranes of aquatic animals. The favorable pH level may explain why a relatively large indigenous crayfish population exists in such a polluted stream. Another factor that may allow the presence of crayfish at this site is the specific habitat conditions that occur there. Stone crayfish usually use stones for shelters, but in the Zákolansky brook, they inhabit burrows in the clay bottom and stream banks (Vlach et al., 2009) even though sufficient numbers of the appropriate types of stones are available. These burrows may act as refuges during periods of unfavorable conditions, though this has yet to be directly verified.

Similar to the study by Favaro et al. (2010) of the water quality at sites occupied by white-clawed crayfish, our research showed that BOD_5 played very important role in determining the presence versus absence of crayfish at particular locations. Consistently with Pârvulescu et al. (2011) our results suggest that noble crayfish and stone crayfish are sensitive to water eutrophication, as revealed also by the significant negative correlations to ammonium or nitrates. A low degree of tolerance to organic pollution in streams with noble crayfish can be seen in the fitted Gaussian response curve for BOD_5, but this curve also indicates that water with extremely low nutrient enrichment is also inappropriate for crayfish. Foster (1995) demonstrated that crayfish do not prosper in oligotrophic waters but rather need mild nutrient enrichment. Even though some authors have

described crayfish living in oligotrophic water bodies (Westman et al., 2002), in these cases, slightly unfavorable physiochemical water parameters, such as low nutrient enrichment or low calcium concentrations, may be offset by other environmental factors to an extent that allows crayfish to overcome the unfavorable conditions.

Crayfish are also limited by an excess load of nitrite. Though the toxic effects of nitrite are ameliorated by higher chloride concentrations (Beitinger , Huey, 2002; Jeberg , Jensen, 1994), as found for narrow-clawed crayfish by Yildiz , Benli (2004) and for spiny-cheek crayfish by Kozák et al. (2005), research has shown that long-term exposure to nitrite causes a weakening of the immune system and can lead to a higher risk of infectious disease (Yildiz , Benli, 2004). As an example, the highest mean concentration of nitrites recorded in our study at the site occupied by indigenous crayfish was 1.6 mg L^{-1}, however the site had also increased mean chloride concentration of 112 mg L^{-1}, which may have lowered the toxic effects of nitrite. Other authors either did not measure nitrites or have noted considerably lower values.

Other significant parameters that we found to be negatively related to the presence of noble crayfish are iron and aluminum (Table 2.2). According to many authors (e.g., Dalzell, Macfarlane, 1999), iron may cause respiratory disruption by physically clogging the gills of fish, which can lead to mortality by suffocation. Naghshbandi et al. (2007) monitored the bioaccumulation of heavy metals in narrow-clawed crayfish and found that the greatest accumulation of Fe occurred in their gills. In a study of aluminum toxicity, Alexopoulos et al. (2003) found that despite its insolubility at approximately neutral pH, freshly neutralized aluminum is toxic to a variety of freshwater organisms. An Al concentration of approximately 0.5 mg L^{-1} was considered to have sublethal effects on crayfish. Like iron, aluminum can accumulate on the gill surface. This process causes a buildup of mucus and the growth of Al-polymers on the gills and causes respiration to deteriorate (Alexopoulos at al., 2003). According to Ward et al. (2006), this adverse situation produces a decrease in immunocompetence. This decrease is caused by repeated hypoxia and osmoregulatory dysfunction during episodic exposures to sublethal aluminum concentrations rather than by the direct toxic effects of aluminum (Alexopoulos et al., 2003).

The relationship between noble crayfish occurrence and copper is also interesting. Dissolved copper is generally considered to be toxic for many water organisms at concentrations greater than 0.05 mg L^{-1}, but for many organisms, copper is an essential micronutrient (Allinson et al., 2000). Our results suggest that the presence of crayfish may be limited by the insufficiency of trace amounts of copper. The optimal value for copper in sites with noble crayfish was 0.012 mg L^{-1}.

Though experimental studies have shown that a sufficient concentration of calcium is necessary for a crayfish population to successfully survive and reproduce (e.g., Chaisemartin, 1967; Rukke, 2002), our analysis showed calcium to be a nonsignificant parameter. Rukke (2002) has shown experimentally that calcium levels below 5 mg L^{-1} lower the survival of noble crayfish and slow their growth; below this level, complete calcification of their shell is not possible. The relatively low significance of calcium in Czech streams can be explained by the generally high average concentrations of the element throughout the region, in contrast to other areas such as Northern Europe (Westman et al., 2002).

Even though some types of water pollution (characterized by the parameters BOD_5, COD_{Cr}, and suspended solids) in the Czech Republic are declining, there has been an increase in the significance of pollution by substances that are more difficult to eliminate (e.g., specific industrial pollutants). In the future, it will be increasingly important to focus on these dangerous substances because they will have increasing negative impacts on the status of aquatic communities. In addition, other

anthropogenic environmental stressors also often impact the entire ecosystem and therefore cannot be ignored.

The results of this study, in agreement with Füreder , Reynolds (2003b) and Demers et al. (2006), show that crayfish are not necessarily good biological indicators of water quality. Nevertheless, there is a significant connection between the presence of crayfish and water quality. In particular, the probability of the presence of noble crayfish declines in polluted streams. This result indicates that the indigenous crayfish have high requirements for long-term water quality. In contrast, we found a few sites at which these crayfish prosper even in polluted waters, a result that seems partially contradictory. Therefore, investigations into crayfish water quality requirements in the context of other factors (e.g., habitat, stream characteristics, predation and nutrient sources) should be the focus of further research.

Measures leading to the preservation and successful reproduction of indigenous populations of European crayfish in their natural environment are one of the present priorities of water ecosystem protection in Europe (Souty-Grosset et al., 2006). In addition, sustaining indigenous crayfish populations is an important step in preventing an overall decline in the biodiversity of the natural environment. Our results indicate that water pollution is one of the important factors for crayfish populations and that degradation of water quality has particular impacts on the distribution of the indigenous European stone and noble crayfish in streams. If we want to preserve these critically endangered species, a focus on improving water quality will be necessary.

References

Alexopoulos E, McCrohan CR, Powell JJ, Jugdaohsingh R, White KN. 2003. Bioavailability and toxicity of freshly neutralised aluminium to the freshwater crayfish *Pacifastacus leniusculus*. *Archives of Environmental Contamination and Toxicology* 45: 509–514.

Allinson G, Laurenson L, Pistone G, Stagnitti F, Jones PL. 2000. Effects of dietary copper on the Australian freshwater crayfish *Cherax destructor*. *Ecotoxicology and Environmental Safety* 46: 117–123.

Beitinger TL, Huey DW. 2002. Pathogens, Parasites and Commensals . In Holdich, DM (ed.), Biology of Freshwater Crayfish. Blackwell Science Ltd., London, pp. 377–438.

Bohl, E, 1987. Comparative studies on crayfish brooks in Bavaria (*Astacus astacus* L., *Austropotamobius torrentium* Schr.). *Freshwater Crayfish* 7: 287–294.

Braak CJF ter, Šmilauer P. 2002. Program CANOCO for Windows. Version 4.5. Biometris, Plant Research International, Wageningen University and Research Centre, Wageningen, The Netherlands.

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of Europen Communities* L206 :7–50

Chaisemartin C. 1967. Contribution a l'e'tude de l'economie calcique chez les Astacidae. *Thèse de Doctorat Sci. Nat. Université de Poitiers.*

Cruz MJ, Rebelo R. 2007. Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in Southwest Iberian Peninsula. *Hydrobiologia* 575: 191–201.

Czech Hydrometeorological Institute, 2009. Assesment and reference report of water monitoring. http://hydro.chmi.cz/isarrow/index.php   [10 January 2008]

Dalzell DJB, Macfarlane NAA. 1999. The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate. *Journal of Fish Biology* 55: 301–315.

Demers A, Souty-Grosset C, Trouilhé MC, Füreder L, Renai B, Gherardi F. 2006. Tolerance of three European native species of crayfish to hypoxia. *Hydrobiologia*, 560: 425–432.

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.

Ďuriš Z, Horká I. 2001. K výskytu raka bahenního *Pontastacus* (= *Astacus*) *leptodactylus* Eschscholtz na Karvinsku. (Occurrence of the narrow-clawed crayfish *Pontastacus* (= *Astacus*) *leptodactylus* Eschscholtz in vicinity of Karviná.) *Časopis Slezského zemského muzea v Opavě* (A), 50 (supl.): 78–84. (in Czech with English abstract)

Ďuriš Z, Kozák P. 2000. The „Golden Crayfish" in Czech Republic. *Crayfish News* 22: 8–9.

Favaro L, Tirelli T, Pessani D. 2010. The role of water chemistry in the distribution of *Austropotamobius pallipes* (Crustacea Decapoda Astacidae) in Piedmont (Italy). *Comptes Rendus Biologies* 333: 68–75.

Fischer D, Pavluvčík P, Sedláček F, Šálek M. 2009. Predation of the alien American mink, *Mustela vison* on native crayfish in middle-sized streams in central and western Bohemia. *Folia Zoologica* 58: 45–56.

Foster J. 1995. Factors influencing the distribution and abundance of the crayfish *Austropotamobius pallipes* (Lereboullet) in Wales and the Marches, U.K. *Freshwater Crayfish* 8: 78–98.

Füreder L, Oberkofler B, Hanel R, Leiter J, Thaler B. 2003a. The freshwater crayfish *Austropotamobius pallipes* in South Tyrol: Heritage species and bioindicator. *Bulletin Français de la Pêche et de la Pisciculture* 370–371: 81–95.

Füreder L, Reynolds JD. 2003b. Is *Austropotamobius pallipes* a good bioindicator? *Bulletin Français de la Pêche et de la Pisciculture* 370–371: 157–163

Guner U. 2007. Freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823) accumulates and depurates copper. *Environmental Monitoring and Assessment* 133: 365–369.

Holdich DM. 2002. Distribution of crayfish in Europe and some adjoining countries. *Bulletin Français de la Pêche et de la Pisciculture* 367 : 611–650.

Jeberg MV, Jensen FB. 1994. Extracellular and intracellular ionic changes in crayfish *Astacus astacus* exposed to nitrite at two acclimation temperatures. *Aquatic Toxicology* 29: 65–72.

Jongman RHG, Ter Braak CJF, van Tongeren OFR. 1995. Data analysis in community and landscape ecology. Cambridge.

Kettunen M, ten Brink P. 2006. Final report for the European Commission: Value of biodiversity. Institute for European Environmental Policy (IEEP). Brussels, Belgium. 131 pp.

Kozák P, Máchová J, Policar T. 2005. The effect of chloride content in water on the toxicity of sodium nitrite for spiny-cheek crayfish (*Orconectes limosus* Raf.). *Bulletin Français de la Pêche et de la Pisciculture* 376–377: 705–713.

Kozák P, Policar T, Ďuriš Z. 2004. Migratory ability of *Orconectes limosus* through a fishpass and notes on its occurrence in the Czech Republic. Bulletin Français de la Pêche et de la Pisciculture 372–373: 367–373.

Kozubíková E, Petrusek A, Ďuriš Z, Kozák P, Geiger S, Hoffmann R, Oidtmann B. 2006. The crayfish plague in the Czech Republic-review of recent suspect cases and a pilot detection study. *Bulletin Français de la Pêche et de la Pisciculture* 380–381: 1313–1324.

Kozubíková E, Petrusek A, Ďuriš Z, Martín MP, Diéguez-Uribeondo J, Oidtmann B. 2008. The old menace is back: recent crayfish plague outbreaks in the Czech Republic. *Aquaculture* 274: 208–217.

Kozubíková E, Filipová L, Kozák P, Ďuriš Z, Martín MP, Diéguez-Uribeondo J, Oidtmann B, Petrusek A. 2009. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in American invasive crayfishes in the Czech Republic. *Conservation Biology* 5: 1204–1213.

Machino Y, Holdich DM. 2005. Distribution of crayfish in Europe and adjacent countries: updates and comments. *Freshwater Crayfish* 15: 292–321.

Naghshbandi N, Zare S, Heidari R, Razzaghzadeh S. 2007. Concentration of heavy metals in different tissues of *Astacus leptodactylus* from Aras dam of Iran. *Pakistan. Journal of Biological Sciences* 10: 3956–3959.

Pârvulescu L, Pacioglu O, Hamchevici C. 2011. The assessment of the habitat and water quality requirements of the stone crayfish (*Austropotamobius torrentium*) and noble crayfish (*Astacus astacus*) species in the rivers from the Anina Mountains (SW Romania). *Knowledge and Management of Aquatic Ecosystems* 401, 03: DOI 10.1051/kmae/2010036.

Peeters ETHM, Gardeniers JJP. 1998. Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwater Biology* 39: 605–615.

Rallo A, García-Arberas L. 2002. Differences in abiotic water conditions between fluvial reaches and crayfish fauna in some northern rivers of the Iberian Peninsula. *Aquatic Living Resources*. 15 (2): 119–128.

Renz M, Breithaupt T. 2000. Habitat use of the crayfish *Austropotamobius torrentium* in small brooks and in Lake Constance, Southern Germany. *Bulletin Français de la Pêche et de la Pisciculture* 356: 139–154.

R Development Core Team, 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rukke NA. 2002. Effects of low calcium concentrations on two common freshwater crustaceans, 20 *Gammarus lacustris* and *Astacus astacus*. *Functional Ecology* 16: 357–366.

Schulz HK, Smietana P, Schulz R. 2006. Estimating the human impact on populations of the endangered noble crayfish (*Astacus astacus* L.) in North-Western Poland.

Aquatic Conservation: Marine and Freshwater Ecosystems 16: 223–233.

Seiler SM, Turner AM. 2004. Growth and population size of crayfish in headwater streams: individual– and higher-level consequences of acidification. *Freshwater Biology* 49: 870–881.

Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P (eds). 2006. Atlas of Crayfish in Europe. Patrimoines Naturels 64. Muséum National d'Histoire Naturelle, Paris, 187 pp.

Strahler AN. 1957. Quantitative analysis of watershed geomorphology. *American Geophysical Union Transactions* 38: 913–920.

Štambergová M, Svobodová J, Kozubíková E. 2009. Raci v České republice (Crayfish in the Czech Republic). AOPK ČR Praha, 255 pp. (in Czech with English abstract)

Streissl F, Hödl W. 2002. Habitat and shelter requirements of the stone crayfish, *Austropotamobius torrentium* Schrank. *Hydrobiologia* 477: 195–199.

Trouilhé MC, Souty-Grosset C, Grandjean F, Parinet B. 2007. Physical and chemical water requirements of the white-clawed crayfish (*Austropotamobius pallipes*) in western France. *Aquatic Conservation: Marine and Freshwater Ecosystems* 17: 520–538.

Unestam T. 1965. Studies on the crayfish plague fungus *Aphanomyces astaci*: I. Some factors affecting growth in vitro. *Physiologia Plantarum* 18: 483–505.

Vlach P, Hulec L, Fischer D. 2009. Recent distribution, population densities and ecological requirements of stone crayfish. *Knowledge and Management of Aquatic Ecosystems* 394–395, 13: DOI 10.1051/kmae/2010005.

Ward RJ, McCrohan CR, White KN. 2006. Influence of aqueous aluminium on the immune system of the freshwater crayfish *Pacifasticus leniusculus*. *Aquaticic Toxicology* 77: 222–228.

Weiss S. 2005. Keynote address: Conservation genetics of freshwater organisms. *Bulletin Français de la Pêche et de la Pisciculture* 376–377: 571–583.

Westman K, Savolainen R, Julkunen M. 2002. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: A 30-year Study. *Ecography* 25: 53–73.

Yildiz HY, Benli ACK. 2004. Nitrite toxicity to crayfish, *Astacus leptodactylus*, the effects of sublethal nitrite exposure on hemolymph nitrite, total hemocyte counts, and hemolymph glucose. *Ecotoxicology and Environmental Safety* 59: 370–375.
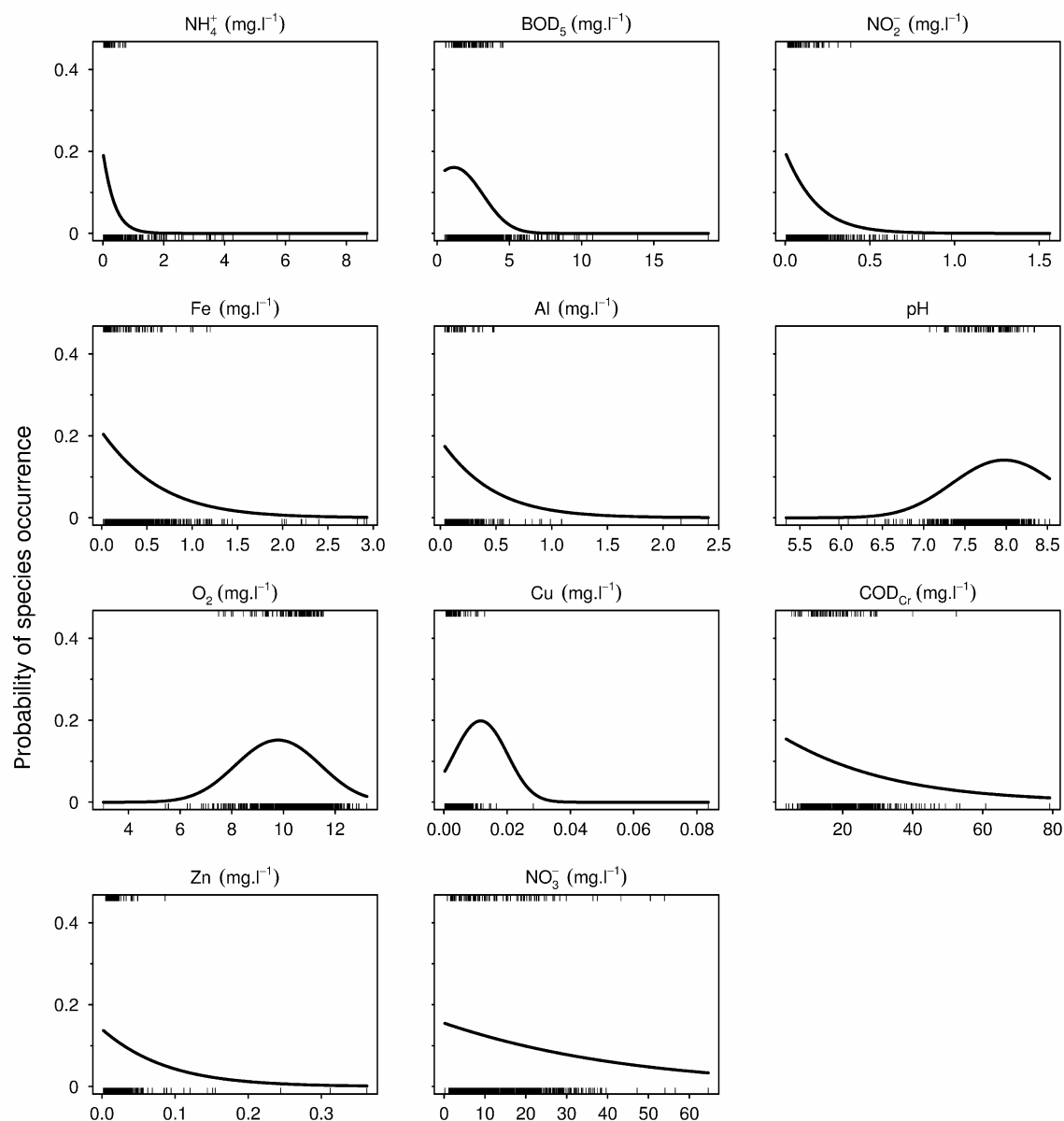
Table 2.1 Mean (±SD) water quality parameters associated with sites where crayfish were present/absent. Superscript letters indicate whether differences between species are significant, where different letters indicate significant differences between species (Pairwise Wilcoxon rank sum tests with adjusted p values using the Bonferroni method, p<0.05). N = number of sites.

Parameter	*Astacus astacus*			*Austropotamobius torrentium*			*Orconectes limosus*			No crayfish		
	N	Mean	±SD	N	Mean	±SD	N	Mean	±SD	N	Mean	±SD
O_2 (mg L^{-1})	112	10.3^A	0.9	19	9.4^B	1.1	27	9.3^B	1.3	799	10.4^A	1.2
pH	110	7.8^A	0.3	19	7.8^{A,B}	0.3	26	7.9^A	0.2	793	7.7^B	0.4
Conduct. (µS cm^{-1})	107	417^A	352	19	446^A	280	26	418^A	144	727	426^A	475
BOD_5 (mg L^{-1})	105	2.1^A	0.9	19	2.4^{A,B}	1.7	26	3.3^C	0.8	789	2.9^B	1.8
COD_{Cr} (mg L^{-1})	80	17.1^A	7.8	10	13.1^A	5.8	25	24.2^B	5.4	718	19.1^A	8.4
NH_4^+ (mg L^{-1})	107	0.150^A	0.139	19	0.212^{A,B}	0.348	26	0.206^B	0.086	784	0.415^B	0.775
NH_3 (mg L^{-1})	99	0.0020^A	0.0022	19	0.0030^{A,B}	0.0053	26	0.0036^C	0.0022	704	0.0050^{B,C}	0,009
NO_2^- (mg L^{-1})	107	0.080^A	0.069	19	0.132^{A,C}	0.221	26	0.136^B	0.056	784	0.143^{B,C}	0.157
NO_3^- (mg L^{-1})	110	12.3^A	10.3	19	11.6^{A,C}	9.1	22	16.7^B	5.6	807	14.2^{B,C}	8.8
Cl^- (mg L^{-1})	96	27.5^A	37.5	17	30.1^{A,B}	34.4	23	26.8^B	10.3	606	36.6^{A,B}	135.9
$P_{total.}$ (mg L^{-1})	70	0.135^A	0.207	17	0.327^A	0.381	20	0.139^A	0.066	332	0.150^A	0.180
SO_4^{2-} (mg L^{-1})	86	77.1^{A,B}	142.9	17	55.7^{A,B}	42.7	22	64.8^A	33.4	588	57.3^B	62.1
Fe (mg L^{-1})	89	0.28^A	0.26	17	0.38^{A,B,C}	0.43	20	0.90^B	0.76	609	0.44^C	0.40
Al (mg L^{-1})	51	0.171^A	0.127	12	0.184^A	0.156	17	0.773^B	0.722	331	0.218^A	0.183
Cu dissol. (mg L^{-1})	95	0.0040^A	0.003	17	0.0049^A	0.0026	23	0.0065^A	0.0038	683	0.0033^B	0.0051
Zn (mg L^{-1})	95	0.015^A	0.013	17	0.006^B	0.001	23	0.016^A	0.007	685	0.019^A	0.028
Ca^{2+} (mg L^{-1})	106	45.2^{A,B}	33.8	17	52.9^{A,B}	40.4	26	55.1^A	24.8	691	45.1^B	37.6
Sus.solids (mg L^{-1})	89	16^A	17	16	19^A	23	27	22^B	8	675	17^A	17

Table 2.2 Results of the logistic regression analysis for *A. astacus*. Estimated values for the parameters β_0, β_1, and β_2 from the logit function "g" and the percentage reduction in the deviance of fitted models; u-optimum (u=-β_1/2β_2); <-optimum lower than observed values; R-deviance reduction against the null model; NS-non-significant deviance reduction versus the null model (Chi, p>0.05).

Parameter	β_0	β_1	β_2	R	u	n $_{total}$	n $_{presence}$
NH_4^+	-1.40	-3.05		5.4	<	931	107
BOD_5	-1.85	0.35	-0.15	4.6	1.16	934	105
NO_2^-	-1.39	-6.30		4.2	<	931	107
Fe	-1.33	-1.86		3.8	<	730	89
Al	-1.46	-2.52		1.9	<	410	51
pH	-94.66	23.29	-1.46	1.6	7.97	943	110
O_2	-22.40	4.22	-0.22	1.5	9.79	952	112
Cu	-2.56	201.08	-8647.02	1.4	0.012	814	95
COD_{Cr}	-1.56	-0.04		1	<	829	80
NO_3^-	-1.70	-0.03		0.7	<	953	110
Zn	-1.82	-12.86		0.6	<	816	95
Sus. solids	NS	NS	NS			802	89
Cl^-	NS	NS	NS			737	96
P_{total}	NS	NS	NS			434	70
SO_4^{2-}	NS	NS	NS			708	86
Ca^{2+}	NS	NS	NS			835	106
Conductivity	NS	NS	NS			874	107

Fig. 2.1 Map of the Czech Republic showing the sampling sites used in this study. Species data and water quality data were recorded from 2004 through 2007.

Fig. 2.2 Sigmoid and Gaussian logit curves fitted by the logit regression to data on the presence of the noble crayfish (*Astacus astacus*) in response to the values of individual parameters. Individual curves indicate the probability of species presence as a function of a given water quality parameter. Vertical lines indicate values of parameters at individual localities without (lines at lower margin) and with (lines at upper margin) the presence of noble crayfish. Chi-square, p<0.05; other characteristics are given in Table 2.2.

31
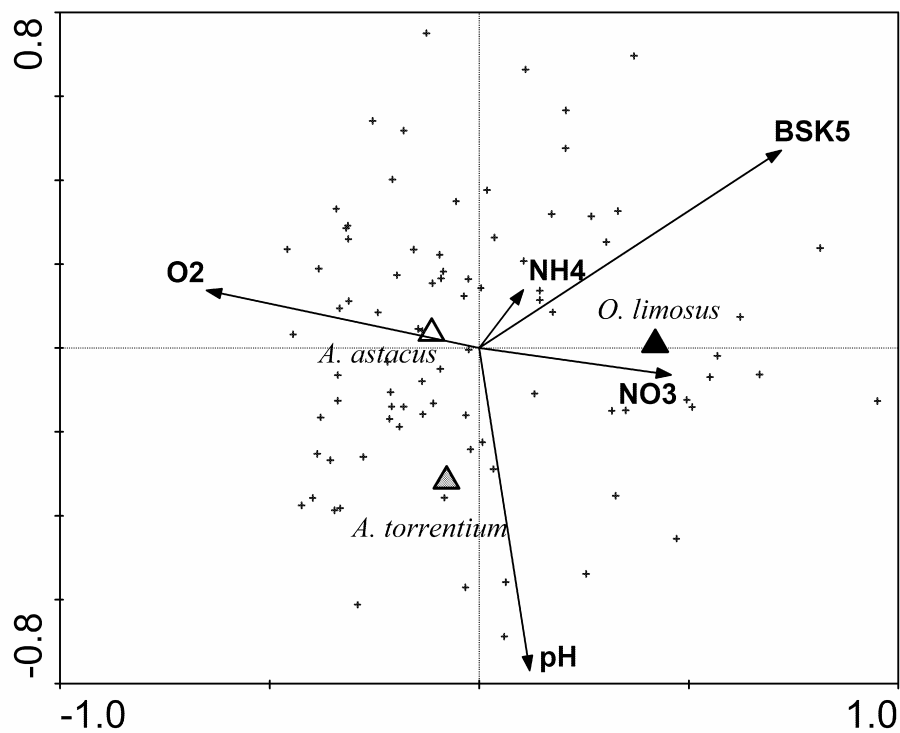
Fig. 2.3 Ordination diagram of crayfish species (triangles), water quality parameters (arrows) and individual sites (crosses) produced by canonical correspondence analysis (CCA). The first two axes are shown (variability in the species data explained: 1st axis 49.3%, 2nd axis 10.1%).

Chapter 3

Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species

Douda, K., Horký, P., Bílý, M.

Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species

Douda, K., Horký, P. & Bílý, M.

Department of Applied Ecology, Water Research Institute TGM, Prague, Czech Republic

Abstract

The conservation of endangered affiliate species is often hindered by a poor understanding of the critical relationships between the interacting species. The parasitic stage of endangered unionid bivalves constitutes a tight host-affiliate linkage between the mussels and their host fishes. However, the threats resulting from potential shortages of the host species are rarely sufficiently quantified and incorporated into conservation strategies. In this paper, we integrated both host quality and availability analyses to assess the potential threats to the endangered thick-shelled river mussel (*Unio crassus*) that result from the impairment of its host resources in Central Europe. The experimental determination of the compatibility of the parasitic larvae (glochidia) of *U. crassus* with its potential host fishes revealed an intermediate level of host specificity. The glochidia successfully developed on 14 of the 27 potential hosts that were evaluated, but the transformation rate was highly variable. Only three fish species (*Scardinius erythrophthalmus, Phoxinus phoxinus,* and *Cottus gobio*) enabled the majority of the attached glochidia to transform successfully. Subsequently, our analysis of host availability at sites inhabited by living or extirpated populations of *U. crassus* in the Czech Republic showed that the local extirpations of *U. crassus* are associated with an altered composition of the fish assemblage and with the absence of the primary host fishes. These results indicate that the availability of host fish resources has played at least an additive role in the present pan-European decline of *U. crassus* and that the evaluation of host limitation without precise data on the host compatibility or host abundance may be ineffective for identifying the threats to particular species. It demonstrates a strong need for more thorough incorporation of host limitation issues into conservation strategies for *U. crassus* and probably also for other species of freshwater mussels that were previously considered safe from host limitation.

Keywords

Bivalvia; Europe; experimental infestation; freshwaters; glochidia; host spectrum; *Unio crassus*; Unionidae

Short title for page headings:

Host limitation of the thick-shelled river mussel

Correspondence: Karel Douda, Department of Applied Ecology, Water Research Institute TGM, Podbabská 30, Prague, CZ 160 00, Czech Republic. Tel: +420 220 197 367; Fax: +420 197 514; Email: douda@vuv.cz

Introduction

The coextinction of species is hypothesised to represent one of the most frequent forms of biodiversity loss (Dunn et al., 2009). The application of knowledge about complex host-affiliate relationships to endangered species conservation is, thus, a challenging issue for conservation biologists. Identifying a species' host-affiliate relationships and the impairment of these relationships facilitates the prioritisation of conservation actions and is important in reducing the effort required to manage threatened species (Koh et al., 2004; Marvier & Smith, 1997; Moir et al., 2010).

The freshwater mussels of the superfamily Unionoidea represent a highly endangered group of affiliate species. Unionids have a short-term larval stage (glochidium) that generally develops as an obligatory parasite of fishes (Kat, 1984). The glochidia of most species can develop successfully into juvenile mussels only on a limited number of host fish species, which influence the reproductive success and dispersal abilities of the mussels (Barnhart, Haag & Roston, 2008; Jansen, Bauer & Zahner-Meike, 2001; Strayer, 2008). After the release from the parent mussel, the glochidia must attach mechanically to the surface of the fish's body, where they become encapsulated within a few hours after attachment through the migration of host cells (Rogers-Lowery & Dimock, 2006). The glochidia remain encapsulated on their host for a period that may last from several days to months, and their internal organs develop into their juvenile form. Fully transformed juvenile mussels escape the capsule and fall to the river bed, where they grow as a free-living mussel (Dillon, 2000). The glochidia that attach to incompatible fish species and also variable proportion of the glochidia attached to functional host fishes do not complete their parasitic stage (e.g., Bauer, 1987; Young & Williams, 1984). They either fail to be encysted or are sloughed off before the transformation is complete (Jansen, Bauer & Zahner-Meike, 2001). These outcomes are attributed to the activity of the fish's immune system: the immune system of the fish determines the compatibility of a given mussel-fish relationship (Rogers-Lowery, Dimock & Kuhn, 2007) and, hence, directly controls the reproductive success of mussel species on a particular host.

An increasing body of literature addresses the effects of host fish availability on freshwater mussel dispersion (Douda et al., 2011; Schwalb, Poos & Ackerman, 2010) and population status (McNichols, Mackie & Ackerman, 2010; Schwalb et al., 2011; Spooner et al., 2011). These studies collectively demonstrate the critical role of the host-parasite relationship for the persistence and distribution of unionid populations. Nevertheless, the effect of potential host limitation on the conservation status of endangered unionid bivalves remains poorly documented, and this lack of information causes uncertainty in the prioritisation of conservation actions (Schwalb et al., 2011). In the present study, we applied a methodological framework integrating both host compatibility and host availability analyses to assess the potential threats to the endangered Central European freshwater mussel, *Unio crassus* (Philipsson, 1788), that are produced by inadequate host resources.

Unio crassus, an endangered European species, inhabits flowing waters, from large rivers to small streams. The species' broad range of habitats has been attributed to its high environmental plasticity (Hochwald, 2001), however, this species declined dramatically in Western and Central Europe during the second half of the 20th century (Bauer, Hochwald & Silkenat, 1991). *U. crassus* is currently listed in annexes II and IV of the European Community's Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (Habitats Directive) and on the International Union for Conservation of Nature Red List (IUCN, 2010). The principal causes of its decline are considered to be water pollution and habitat destruction (Douda, 2010; Hus et al., 2006). The primary strategies to conserve and restore *U. crassus* include the creation of protected areas, the protection of physical habitat, and the elimination of water pollution. It is probable that, at least in some localities,

environmental changes affecting the host fish may be more detrimental than any direct effects on this bivalve (Engel & Wachtler, 1989). *Unio crassus* encounters a wide range of potential host fish species in its habitat. Nevertheless, previous experimental studies showed that successful development into juvenile mussels occurs only on a limited number of species (summarised in Hochwald, 1997). Moreover, the findings of Engel (1990) indicate that the transformation success of *U. crassus* glochidia developing on different host species is highly variable. The observed host specificity may have considerable impact on issues of population dynamics, distribution and conservation. Unfortunately, the previous studies on the host fish compatibility of *U. crassus* generally did not quantify the rate of successful transformation of glochidia developing on particular fish species. Hence, the seriousness of the host limitation of *U. crassus* remains unclear. The availability of fish hosts may or may not play a significant role in the population decline of *U. crassus*. Local extirpations of *U. crassus* might occur independently of the host's availability and result directly from environmental pressure in most cases. Alternatively, the decline of *U. crassus* could be enhanced or even caused by the absence of suitable host fishes at the sites where *U. crassus* occurs. Knowledge of the causes of *U. crassus* limitation may have broad conservation implications that allow a better targeting of the conservation measures for this species.

The aim of this study was to assess the potential threats to *U. crassus* in Central Europe resulting from a lack of host resources by integrating host quality and availability analyses. We examined the development of *U. crassus* glochidia on a variety of freshwater fish species. The success of transformation was evaluated as a critical factor for host fish compatibility. Subsequently, we compared the availability of host species and fish community composition for sites with present and extirpated populations of *U. crassus* in the Czech Republic.

Materials and methods

Host compatibility

Gravid *U. crassus* females were sampled from May through July (2008, 2009, and 2010) in the Vltava River Basin, Czech Republic. The females were collected at two sampling locations (loc. 'A' in 2008 – Lužnice River, N 49°19'24"; E 14°29'22" and loc. 'B' in 2009 and 2010 – Chumava Stream N 49°52'15"; E 14°0'17") due to a population decline of *U. crassus* in the first river caused by a pollution event (Douda, K., Hronek, J. personal observation). Gravid females were identified in the field by observing the swollen outer demibranchs visible through their slightly opened shells (Hochwald, 2001).

The fish species commonly occurring in the natural habitats of the Czech Republic were preferentially used in the analysis of host compatibility, and we also included fish species classified by previous studies as suitable hosts for *U. crassus*. We assessed 27 fish species (Table 3.1) from 8 families during six infestation trials (A–F) (Table 3.2). The number of fishes used for the analyses ranged between 1–8 per species, depending on the number of available *U. crassus* glochidia. We included previously verified suitable host fish species in each trial as a control. In total, we assessed 145 individual fish. All of the fishes used in the analyses originated either from hatcheries or from natural habitats that did not contain freshwater mussels. All of the hatchery-reared fishes were 1+ years old, and sub-adult specimens of the wild-caught fishes were preferentially used (cf. length structure in Table 3.1). The total mortality of the fishes during trials was less than 5% and was not caused by the glochidial infestation.

Gravid females of *U. crassus* were transported to the laboratory and separately held in individual boxes with 10 L of renewed, aerated river water until the spontaneous uniphase release of the egg-glochidial clumps. Immediately after the glochidia were

released, the female mussels were returned to their natural habitats. The fishes in each trial were infested by the glochidia of 2–3 gravid *U. crassus* females that coincidently released their larvae. Glochidia were pooled and used for inoculation within 48 hours after release. The density of the glochidial suspensions used for the inoculation ranged between 2500–5500 living glochidia per litre; this value was assessed by enumerating ten 10-mL subsamples taken during the infestation procedure (Table 3.2). The infestation procedure lasted 30 minutes in a common bath; a volume of 1 L of the glochidial suspension per individual fish was used. Then, the fishes were transferred into a new bath without glochidia for 30 minutes to allow the flushing of the unattached glochidia. Subsequently, fish species were moved to plastic tanks of dechlorinated tap water with a volume of 5 L per individual fish and a 3-mm net on the bottom. Both individual and common fish-holding systems were used during the study (see Table 3.1). The fish were fed daily with commercial fish food (flakes and frozen chironomids). Untransformed glochidia and juvenile mussels were collected one day after infestation and, thereafter, at 2–4 day intervals by filtering all of the water in the tanks and isolating the specimens from the debris using nylon screens (mesh size 139 μm). The water was partially (80%) renewed at the same intervals (methods adapted according to Dodd et al. 2005). The temperature in the monitoring boxes was recorded at 10-min intervals (Hobo dataloggers, Onset).

The offspring collected were classified either as dead (dead glochidia or juveniles, including untransformed glochidia) or as living juveniles and counted under a stereomicroscope (40x magnification). The living juvenile mussels were identified by the presence of foot movements and valve opening (functionality of the adductor muscles). After the completion of the enumeration, the juvenile mussels were released into the natural habitats of the collected female mussels in accordance with the requirements of the local conservation authorities. The data on the time course of the shedding of the glochidia and juveniles were used for the back-calculation of the mean initial number of glochidia per fish and the mean proportion of successfully transformed juveniles (transformation rate). The trials were terminated one week after the last detached glochidium or juvenile was recovered from the box or an examination of the gills revealed no encysted glochidia.

We defined groups of (1) 'host species' that enabled the successful transformation into a juvenile of any attached glochidia and (2) 'primary host species' that exhibited a transformation rate of over 50% of the glochidia. This value corresponds to the typical value of transformation success in the fully functional host relationships of unionid bivalves (Jansen, Bauer & Zahner-Meike, 2001).

Host availability

We compiled the available data on the occurrence of *U. crassus* within the territory of the Czech Republic. We used the results from the monitoring program of *U. crassus* conducted within the framework of the Habitats Directive implementation (database of the Agency for Nature Conservation and Landscape Protection of the Czech Republic), available literature resources (summarised in Beran, 2002) and museum collections (Czech National Museum, Prague) to include the historical (extirpated populations) and present (living populations) occurrence of this species. The river reaches with confirmed *U. crassus* occurrence after the year 2000 were classified as sites with living populations. Field verifications at several questionable sites were also performed during 2003–2010.

A standard electrofishing method (wading along the bank in an upstream direction) was used (between 2007 and 2009) to obtain juvenile fish data from river reaches where *U. crassus* is found and was historically found. A partial sampling procedure was applied by covering all of the types of habitats to obtain a representative sample of the sites. The borders of the sampling areas were determined with the help of a portable GPS receiver

(GPS map 76S, Garmin Ltd., USA). The minimal stream corridor distance between two sampling sites situated on the same river was fixed at 15 km to prevent the spatial pseudoreplication of the data. All of the samples were collected during the late summer to assure the efficiency of young-of-the-year (YOY) sampling (Copp, 1989). We sampled a total of 57 river reaches with both living (n = 17) and extirpated (n = 40) populations of *U. crassus* in the Czech Republic (Fig. 1).

At each site, we determined the fish species composition and calculated the proportion of suitable hosts in a total sample based on the results of our compatibility testing. We calculated the relative proportions of 'host species' and 'primary host species' at each sampling site. We also calculated the European Fish Index (EFI), which predicts the ecological status of running waters based on the fish assemblage composition and attempts to distinguish between (nearly) pristine and disturbed conditions (Pont, Hugueny & Rogers, 2007). Subsequently, we used Fisher's exact test for the count data to analyse the associations between the occurrence of the existing *U. crassus* populations and hosts, both primary and overall. We also used logistical regression analysis (Jongman, Braak & van Tongeren, 1987; Peeters & Gardeniers, 1998) to investigate the dependence of the present occurrence of *U. crassus* in a given reach on the relative abundance of its hosts and on the EFI. All of the analyses were performed using the R 2.12.0 software package (R Core Development Team, 2010).

Results

Host compatibility

A complete metamorphosis of the glochidia into juvenile mussels was recorded to have occurred on 14 of the 27 potential host fish species evaluated (Table 3.1). Even on these suitable host species, the mortality of the glochidia during the parasitic phase was high, reaching 25.3–99.98%. Only three species (*Scardinius erythrophthalmus* L., *Phoxinus phoxinus* L. and *Cottus gobio* L) exhibited a transformation rate of over 50% of the initially attached glochidia and were classified as primary hosts. The sloughing of the unsuccessful glochidia occurred primarily during the first few days after the infestation. Hence, the proportion of the rejected glochidia recorded on the first and fourth days was strongly related to the transformation rate (r_s = 0.58, S = 7121, $P < 0.001$ and r_s = 0.77, S = 7960, $P < 0.001$, respectively). The dropoff rate of the juveniles was unimodal and peaked at 14–40 days after the infestation. After the end of the experiments, no glochidia were found to be attached to the experimental fishes. The glochidia transformation rate did not differ significantly between the trials (Wilcoxon rank sum test, $P \gg 0.05$). Accordingly, we considered the differences between the trial conditions to be low and analysed the data as a single group.

Host availability

A total of 13 287 fishes representing 33 species from 57 sites were used for the analyses of the host availability. Fourteen identified host species were widely distributed throughout the area (at least one host species was present at 55 of 57 localities sampled), and their occurrence was not associated with the occurrence of *U. crassus* (Fisher's exact test, $P > 0.05$). Conversely, the three primary host species were recorded at only 20 of the sites, and their presence was positively associated with the occurrence of *U. crassus* (Fisher's exact test, $P < 0.05$). The primary host species were present at 59% (n = 10) of the sites currently inhabited by living *U. crassus* but only at 25% (n = 10) of the sites with extirpated *U. crassus* populations. Similar results were obtained from an analogous analysis, in which we considered also *Chondrostoma nasus* (transformation rate 33%) as a primary host species (Fisher's exact test, $P < 0.05$).

A more detailed inspection of the data with logistical regression showed a positive association between the relative abundance of the 14 host species in the fish assemblage and the occurrence of *U. crassus* (logistic regression: $P < 0.05$; $\beta_0 = -2.23$, $\beta_1 = 2.46$; $R = 5.62$) (Fig. 3.2a). The probability of *U. crassus* occurrence also increased with the increasing quality of the fish assemblage composition, expressed by the European Fish Index (logistic regression: $P < 0.05$; $\beta_0 = -2.49$, $\beta_1 = 4.49$; $R = 5.9$) (Fig. 3.2b).

Discussion

Our study employed host suitability and availability analyses to examine the host limitation of the pan-European endangered freshwater mussel *U. crassus*. We identified several suitable host fish species for *U. crassus*; nevertheless, the differences in the transformation success of the glochidia demonstrated a considerable variation in the effectiveness among the host species. Moreover, the recorded distributional patterns of *U. crassus* and the fish species sampled indicate that the level of host resources is impaired throughout the historic Central European range of the mussel.

The primary host species observed in our study (*S. erythrophthalmus, P. phoxinus,* and *C. gobio*) have repeatedly been identified as hosts for *U. crassus* glochidia in other drainage basins across Central Europe (Engel, 1990; Hochwald, 1997). This finding is indicative of a general pattern of host fish use that is consistent over the large continental-scale areas. In contrast, we provide evidence of the successful development of *U. crassus* glochidia on several fish species that have been identified by previous studies (summarised in Hochwald, 1997) as unsuitable hosts (e.g., *Tinca tinca* and *Rutilus rutilus*). This result could indicate regional differences in host fish compatibility, as suggested by Engel & Wachtler (1989). However, the greater detection capacity of our methods could also be important because the previous studies of the *U. crassus* host fish spectrum did not include a continuous assessment of the process of the shedding of glochidia and juveniles. In view of the spatial and temporal limitations of host identification studies, we recommend considerable caution when extrapolating host-quality data to different populations for the purposes of direct conservation. Further studies are needed to define the spatial patterns of variation of host compatibility.

Our data suggest that the traditional classification of fishes as suitable and unsuitable hosts, as is frequently used in European conservation practice, does not fully reflect the complexity of the relationship between *U. crassus* and its hosts. We recommend the classification of host fishes as primary and marginal, a practice that is frequently used in the studies dealing with North American unionids (Gray et al., 2002; Haag & Warren, 2003; Khym & Layzer, 2000). Even more precise expressions might be useful because the transition between the non-host and host fishes appears to be continuous rather than categorical. Unfortunately, the data on a qualitative measure of host compatibility are very rare from locations outside of North America.

The results of the host availability analyses showed that the local extirpations of *U. crassus* are associated with an altered fish assemblage composition and, particularly, with the absence of the species' primary host fishes (*S. erythrophthalmus, P. phoxinus*, and *C. gobio*). Nevertheless, we found no relationship between the occurrence of the 14 suitable host species and the presence of *U. crassus*. These 14 fish species were distributed throughout the study region at sites displaying both current and extirpated *U. crassus* populations. Such a result demonstrates that the evaluation of host limitation without precise data on the host compatibility or fish abundance may be ineffective for identifying the risk of host limitation in the field.

Insights into the current and historical ranges of the primary host fishes may help to interpret the observed association of these species with the *U. crassus* populations. *Phoxinus phoxinus* and *C. gobio* usually inhabit cold, well-oxygenated, small- to medium-sized streams with higher slopes (Kottelat & Freyhof, 2007). These species are threatened because of water pollution, habitat modification and the excessive stocking of species of *Salmo* for angling in sub-mountain streams (Kottelat & Freyhof, 2007). These species are considered vulnerable in the Czech Republic (Lusk, Hanel & Lusková, 2004) according to published criteria (IUCN, 2001), and they are protected by the national laws of the Czech Republic: they are listed in the category of endangered 'specially protected species' (Law no. 114/1992 and Intimation no. 395/1992). In contrast, the third primary host species, *S. erythrophthalmus*, occurs primarily in larger lowland rivers with lentic backwaters or side arms (Kottelat & Freyhof, 2007). Despite the fact that it is considered a species of least concern (Lusk, Hanel & Lusková, 2004), its numbers in the natural habitats of large rivers are steeply declining due to channelisation and the isolation of the backwaters from the main channel (Jurajda, 1995). In conclusion, we identified two fish species as primary hosts for *U. crassus* in both small- and medium-sized streams (*P. phoxinus, C. gobio*) and one species as a primary host in larger lowland streams (*S. erythrophthalmus*). These findings are consistent with the previously reported high environmental plasticity of *U. crassus* (Hochwald, 2001). The primary hosts described are sensitive to anthropogenic pressures, and it is probable that their historical ranges and abundances represented crucial host resources for the populations of *U. crassus* in the Central European territory of the Czech Republic. Although it is probable that the parallel declines of *U. crassus* and its primary hosts share a variety of common, directly acting causes (e.g., water eutrophication and channelisation), our data on host fish quality suggest a strong potential for the limitation of *U. crassus* through its hosts' sensitivity to human pressure. This factor may subsequently place general limits on *U. crassus* reproduction in otherwise-suitable habitats because the ability of *U. crassus* to thrive under any type of environmental pressure depends on its own traits and also on the traits of its hosts. Indeed, Strayer (2008) summarises examples suggesting that unionid populations may have expanded or contracted on a local scale following the expansion or contraction of the ranges of their hosts. The host fish assemblage composition and abundance may also modulate mussel distribution and abundance (Haag & Warren, 1998; Vaughn & Taylor, 2000; Watters, 1992). Hence, it could be suggested that the availability of host fish resources plays at least an additive role in the present pan-European decline of *U. crassus*.

Our results agree with the finding that coextirpations of affiliate species represent one of the major causes of the present biodiversity crisis. In particular, parasites with highly specialised life cycles may be vulnerable to extinction in changing host communities (Dunn et al., 2009). The implementation of the European Community's Habitats Directive (92/43/EEC) initiated the designation of many Special Areas of Conservation for *U. crassus* protection across Europe. The member states of the European Community must adopt the necessary management or restoration measures to achieve a favourable conservation status at those sites. Our data demonstrate a strong need for a more thorough incorporation of the host limitation issues into the conservation strategies for *U. crassus*. It is probable that a similar need exists in the case of other species of freshwater mussels that were previously considered safe from host limitation. The present approaches to the conservation of *U. crassus* do not fully reflect the importance of the host limitation of this species that is demonstrated by this study. In the Czech Republic, despite the unfavourable poor conservation status of *U. crassus* (European Environmental Agency, 2007), no systematic monitoring or active conservation measures are applied to host fish assemblages in the special areas of conservation (SAC) established for *U. crassus* protection. Consequently, the stocking activities performed by the angling organisations and the in-stream habitat modifications alter the

community composition of the fish and may, thus, increase the risk of the extirpation of *U. crassus*. The methodological framework presented in this paper may contribute to the identification of the host limitation of unionid bivalves at particular sites. This information is necessary to ensure that host fish availability does not constitute a general limitation on the survival of the mussel population. If such an analysis identifies a potential for host limitation, the conservation authorities should apply strategies that will target both the mussel and the host fishes as their irreplaceable living resource.

. **References:**

Barnhart, M.C., Haag, W.R. & Roston, W.N. (2008). Adaptations to host infection and larval parasitism in Unionoida. *J. N. Am. Benthol. Soc.* **27**, 370–394.

Bauer, G. (1987). The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) III. Host relationship. *Arch. Hydrobiol.* **76**, 413–423.

Bauer, G., Hochwald, S. & Silkenat, W. (1991). Spatial distribution of freshwater mussels – the role of host fish and metabolic rate. *Freshwater Biol.* **26**, 377–386.

Beran, L. (2002). Aquatic molluscs of the Czech Republic – distribution and its changes, habitats, dispersal, threat and protection, red list. *Journal of the Society for Natural Science Uherske Hradiste, Supplementum* **10**, 1–258.

Copp, G.H. (1989). Electrofishing for fish larvae and 0+ juveniles: equipment modifications for increased efficiency with short fishes. *Aquac. Res.* **20**, 453–462.

Dillon, R.T. (2000). The ecology of freshwater molluscs, Cambridge University Press, London.

Dodd, B.J., Barnhart, M.C., Rogers-Lowery, C.L., Fobian, T.B. & Dimock, R.V. (2005). Cross-resistance of largemouth bass to glochidia of unionid mussels. *J. Parasitol.* **91**, 1064–1072.

Douda, K. (2010). Effects of nitrate nitrogen pollution on Central European unionid bivalves revealed by distributional data and acute toxicity testing. *Aquatic Conserv.: Mar. Freshw. Ecosyst.* **20**, 189–197.

Douda, K., Vrtílek, M., Slavík, O. & Reichard, M. (2011). The role of host specificity in explaining the invasion success of the freshwater mussel *Anodonta woodiana* in Europe. *Biol. Invasions.* doi: 10.1007/s10530-011-9989-7.

Dunn, R.R., Harris, N.C., Colwell, R.K., Koh, L.P. & Sodhi, N.S. (2009). The sixth mass coextinction: are most endangered species parasites and mutualists? *P. Roy. Soc. B–Biol. Sci.* **276**, 3037–3045.

Engel, H. (1990), Untersuchungen zur Autökologie von *Unio crassus* (Philipsson) in Norddeutschland, University of Hannover.

Engel, H. & Wachtler, K. (1989). Some peculiarities in developmental biology of 2 forms of the freshwater bivalve *Unio crassus* in Northern Germany. *Arch. Hydrobiol.* **115**, 441–450.

European Environmental Agency. (2007). *Habitats Directive Article 17 Reporting – Unio crassus*. http://eea.eionet.europa.eu (accessed 23.8.11).

Gray, E.V., Lellis, W.A., Cole, J.C. & Johnson, C.S. (2002). Host identification for *Strophitus undulatus* (Bivalvia: Unionidae), the creeper, in the upper Susquehanna River basin, Pennsylvania. *Am. Midl. Nat.* **147**, 153–161.

Haag, W.R. & Warren, M.L. (1998). Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. *Can. J. Fish. Aquat. Sci.* **55**, 297–306.

Haag, W.R. & Warren, M.L. (2003). Host fishes and infection strategies of freshwater mussels in large Mobile Basin streams, USA. *J. N. Am. Benthol. Soc.* **22**, 78–91.

Hochwald, S. (1997). Populationsökologie der Bachmuschel (*Unio crassus*). *Bayreuther forum Ökologie* **50**, 1–171.

Hochwald, S. (2001). Plasticity of life-history traits in *Unio crassus*. in: Bauer, G., Wachtler, K. (Eds.), *Ecology and evolution of the freshwater mussels Unionoida*, Springer-Verlag Berlin Heidelberg, pp. 127–141.

Hus, M., Smialek, M., Zajac, K. & Zajac, T. (2006). Occurrence of *Unio crassus* (Bivalvia, Unionidae) depending on water chemistry in the foreland of the Polish Carpathians. *Pol. J. Environ. Stud.* **15**, 169–172.

IUCN. (2001). *IUCN Red List Categories: Version 3.1*. Prepared by the IUCN Species Survival Commission, IUCN, Gland, Switzerland and Cambridge, UK.

IUCN. (2010). *Unio crassus. IUCN Red List of Threatened Species*. Version, 2010.4. www.iucnredlist.org (accessed 17.2.11).

Jansen, W., Bauer, G. & Zahner-Meike E. (2001). Glochidial mortality in freshwater mussels, in: Bauer, G., Wachtler, K. (Eds.), *Ecology and evolution of the freshwater mussels Unionoida*, Springer-Verlag Berlin Heidelberg, pp. 185–211.

Jongman, R.H.G., Braak, T. & van Tongeren, O.F.R. (1987). *Data analysis in community and landscape ecology*, Pudoc, Wageningen.

Jurajda, P. (1995). Effect of channelization and regulation on fish recruitment in a flood plain river. *Regul. River.* **10**, 207–215.

Kat, P.W. (1984). Parasitism and the Unionacea (Bivalvia). *Biol. Rev. Camb. Philos.* **59**, 189–207.

Khym, J.R. & Layzer, J.B. (2000). Host fish suitability for glochidia of *Ligumia recta*. *Am. Midl. Nat.* **143**, 178–184.

Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C. & Smith, V.S. (2004). Species coextinctions and the biodiversity crisis. *Science* **305**, 1632–1634.

Kottelat, M. & Freyhof, J. (2007). *Handbook of European freshwater fishes*, Kottelat, Berlin.

Lusk, S., Hanel, L. & Lusková, V. (2004). Red List of the ichthyofauna of the Czech Republic: Development and present status. *Folia. Zool.* **53**, 215–226.

Marvier, M.A. & Smith, D.L. (1997). Conservation implications of host use for rare parasitic plants. *Conserv. Biol.* **11**, 839–848.

McNichols, K.A., Mackie, G.L. & Ackerman, J.D. (2010). Host fish quality may explain the status of endangered *Epioblasma torulosa rangiana* and *Lampsilis fasciola* (Bivalvia: Unionidae) in Canada. *J. N. Am. Benthol. Soc.* **30**, 60–70.

Moir, M.L., Vesk, P.A., Brennan, K.E.C., Keith, D.A., Hughes, L. & McCarthy, M.A. (2010). Current constraints and future directions in estimating coextinction. *Conserv. Biol.* **24**, 682–690.

Peeters, E.T.H.M. & Gardeniers, J.J.P. (1998). Logistic regression as a tool for defining habitat requirements of two common gammarids. *Freshwater. Biol.* **39**, 605–615.

Pont, D., Hugueny, B. & Rogers, C. (2007). Development of a fish-based index for the assessment of river health in Europe: the European Fish Index. *Fisheries. Manag. Ecol.* **14**, 427–439.

R Core Development Team. (2010). *R: A language and environment for statistical computing*, R Foundation for Statistical Computing, Viena.

Rogers-Lowery, C.L. & Dimock, R.V. (2006). Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naive and resistant host fish. *Biol. Bull.* **210**, 51–63.

Rogers-Lowery, C.L., Dimock, R.V. & Kuhn, R.E. (2007). Antibody response of bluegill sunfish during development of acquired resistance against the larvae of the freshwater mussel *Utterbackia imbecillis*. *Dev. Comp. Immunol.* **31**, 143–155.

Schwalb, A., Poos, M. & Ackerman, J. (2010). Movement of logperch – the obligate host fish for endangered snuffbox

mussels: implications for mussel dispersal. *Aquat. Sci.* **73**, 223–231.

Schwalb, A.N., Cottenie, K.A.R.L., Poos, M.S. & Ackerman, J.D. (2011). Dispersal limitation of unionid mussels and implications for their conservation. *Freshwater Biol.* **56**, 1509–1518.

Spooner, D.E., Xenopoulos, M.A., Schneider, C.H.R.I. & Woolnough, D.A. (2011). Coextirpation of host-affiliate relationships in rivers: the role of climate change, water withdrawal, and host-specificity. *Glob. Change. Biol.* **17**, 1720–1732.

Strayer, D.L. (2008). *Freshwater mussel ecology: a multifactor approach to distribution and* abundance. University of California Press, Berkley.

Vaughn, C.C. & Taylor, C.M. (2000). Macroecology of a host-parasite relationship. *Ecography* **23**, 11–20.

Watters, G.T. (1992). Unionids, fishes, and the species-area curve. *J. Biogeogr.* **19**, 481–490.

Young, M. & Williams, J. (1984). The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn) in Scotland. II. Laboratory Studies. *Arch. Hydrobiol.* **100**, 29–43.

Table 3.1

Results of the experimental evaluation of the host compatibility between *U. crassus* from the Vltava River Basin (Czech Republic) and its potential host fishes. The column 'Duration of parasitism' represents the length (mean ± SD) of the parasitic period of both the successful and unsuccessful glochidia. The subsequent column refers to the parasitic period of those glochidia that completed their development into juvenile mussels.

| Status	Species	Trial	Number of fish (n)	Body length (mm)	Attached glochidia (n/fish)	Duration of parasitism (days)	Duration of successful parasitism (days)	Transformation rate (%)
Primary hosts	*Phoxinus phoxinus*	E	5	32 ± 2	41	33.4 ± 13.3	39.9 ± 2.2	**78.02**
		F	5*	55 ± 5	620.6	16.6 ± 5.1	18.0 ± 2.7	**74.12**
		A	5	58 ± 19	204	16.2 ± 9.9	23 ± 1.8	**61.35**
		D	4	36 ± 2	12	16.5 ± 10.6	24.7 ± 1.9	**55.48**
	Scardinius erythrophthalmus	C	3*	84 ± 7	739	19.8 ± 5.8	20.7 ± 3.3	**74.7**
	Cottus gobio	A	5	88 ± 8	365	14.8 ± 7.6	19.9 ± 3	**57.26**
Hosts	*Chondrostoma nasus*	A	6	108 ± 5	402	9.3 ± 6.2	16.3 ± 3.8	**32.53**
	Pseudorasbora parva	C	6*	65 ± 5	145	5.4 ± 6.8	19.4 ± 2.8	**13**
	Leuciscus leuciscus	B	3	74 ± 7	94	5.1 ± 5.3	18.4 ± 3.9	**11.39**
	Alburnus alburnus	B	1	71	248	5.4 ± 5.3	22 ± 2.8	**6.46**
	Leuciscus cephalus	B	7	64 ± 7	45	4 ± 3.3	15.6 ± 2.7	**3.47**
	Tinca tinca	A	5	70 ± 5	117	2.3 ± 3.5	22.2 ± 2	**1.87**
	Leucaspius delineatus	D	4	68 ± 6	51	4 ± 2.1	17.3 ± 1.9	**1.48**
	Rutilus rutilus	C	5*	90 ± 9	553	2.6 ± 2.7	13.8 ± 2.3	**0.7**
	Barbus barbus	C	6*	92 ± 8	633	5.9 ± 3.8	16 ± 3.1	**0.4**
	Vimba vimba	A	4	90 ± 2	210	1.6 ± 2	18.7 ± 1.9	**0.36**
	Salmo trutta	E	6	86 ± 5	843	8.6 ± 7	28 ± 0	**0.02**
Non-hosts	*Abramis brama*	A	7	64 ± 5	58	1.3 ± 1	-	**0**
	Barbatula barbatula	A	5	86 ± 19	216	1.2 ± 0.7	-	**0**
	Gobio gobio	A	6	69 ± 3	187	1.1 ± 0.6	-	**0**
	Gymnocephalus cernuus	A	6	86 ± 4	259	1.8 ± 1.4	-	**0**
	Perca fluviatilis	A	8	57 ± 2	42	4.1 ± 2.4	-	**0**
	Cyprinus carpio	B	3	87 ± 12	272	1 ± 0.8	-	**0**
	Acipenser ruthenus	C	6*	136 ± 8	253	2.3 ± 2.5	-	**0**
	Rhodeus amarus	C	1*	36	23	2.4 ± 2.2	-	**0**
	Silurus glanis	C	2*	108 ± 12	708	3.3 ± 2.8	-	**0**
	Anguilla anguilla	D	5	248 ± 8	207	1.7 ± 1.3	-	**0**
	Ctenopharyngodon idella	D	4	38 ± 4	39	3.4 ± 1.3	-	**0**
	Oncorhynchus mykiss	E	6	97 ± 9	947	4 ± 2.8	-	**0**
	Salvelinus fontinalis	E	6	125 ± 10	1095	5.1 ± 3.8	-	**0**

Table 3.2
Conditions of infestation trials with *U. crassus* glochidia (mean ± SD). 'Glochidia density' indicates the number of glochidia per litre of infestation bath; 'Mean temperature' was calculated for the whole period of parasitisation; superscript in 'Number of mussels' indicates source locality of mussels (see Materials and Methods).

Trial	No. of mussels	Length of mussels	Glochidia density	Mean temperature
		(mm)	gloch./l	°C
A	3[A]	69; 69; 56	3666 ± 1614	17.93 ± 1.58
B	3[A]	66; 66; 63	3692 ± 3300	17.93 ± 1.58
C	2[B]	78; 64	4750 ± 951	18.5 ± 0.14
D	3[B]	42; 50; 40	2632 ± 582	14.6 ± 1.13
E	3[B]	42; 61; 49	5498 ± 1795	12.96 ± 0.85
F	2[A]	60; 54	4828 ± 1234	18.44 ± 1.22
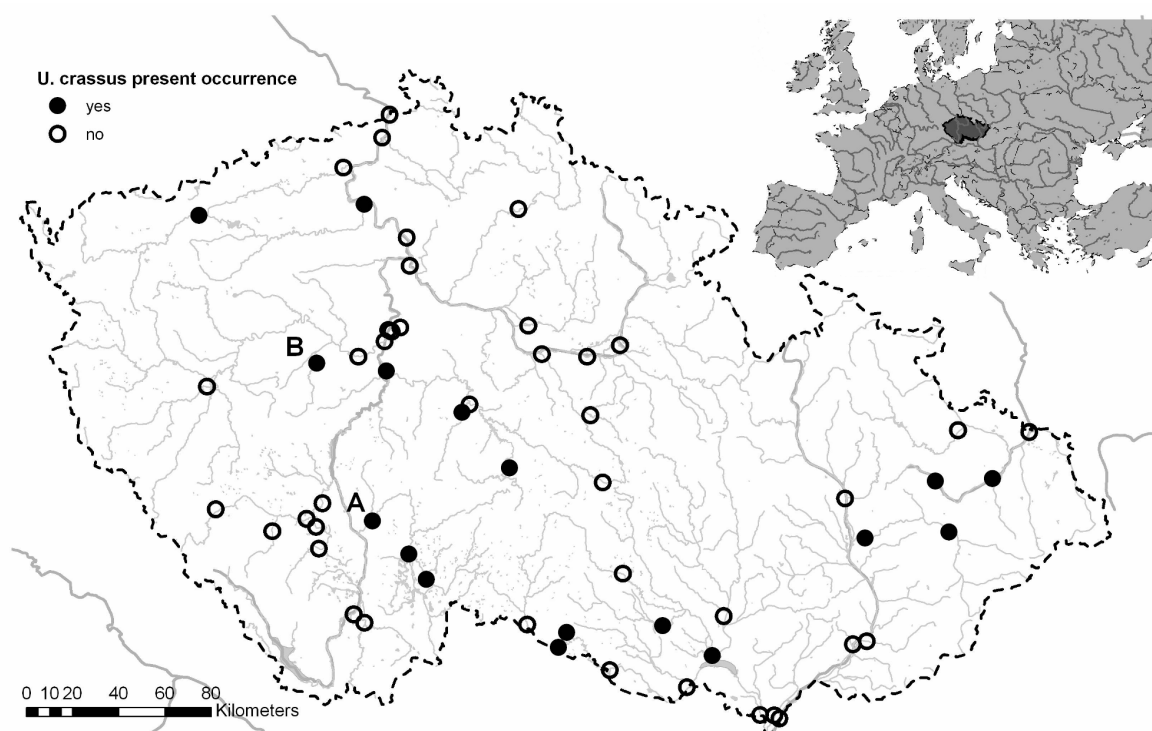
Figure 3.1 Sampling sites for the fish assemblage composition at localities with living (full dots) and extirpated (empty dots) populations of *U. crassus* in the Czech Republic; the sources of the glochidia are marked with A or B (see Materials and Methods).
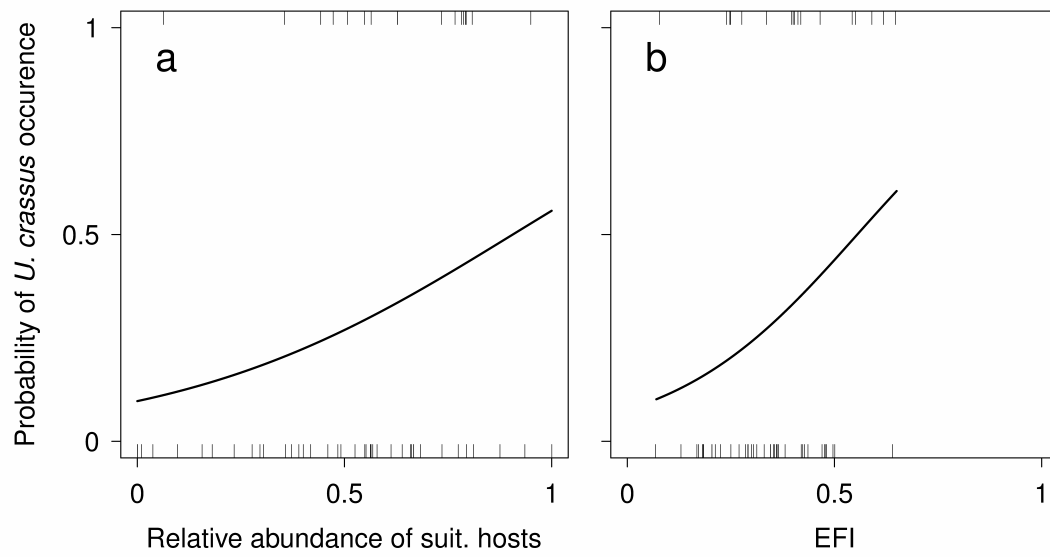
Figure 3.2 The probability of a recent occurrence of *U. crassus* in relation to the relative abundance of all of the suitable hosts (a) and in relation to the European Fish Index (b) fitted by logistic regression (p < 0.05). The presence (dashes at upper margin) and absence of *Unio crassus* (dashes at lower margin) are also shown.

Chapter 4

The role of host specificity in explaining the invasion success of the freshwater mussel *Anodonta woodiana* in Europe

Douda, K., M. Vrtílek, O. Slavík, M. Reichard

The role of host specificity in explaining the invasion success of the freshwater mussel *Anodonta woodiana* in Europe

43

Douda, K., Vrtílek, M., Slavík, O., Reichard, M.

K. Douda (corresponding author), O. Slavík
Water Research Institute T.G.M., Podbabská 30, Prague, CZ 160 00, Czech Republic
e-mail: douda@vuv.cz

K. Douda
Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Kamýcká 1176, Prague, CZ 165 21, Czech Republic

M. Vrtílek, M. Reichard
Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, Brno, CZ 603 65, Czech Republic

Abstract

Several freshwater mussel species represent some of the most problematic invasive species and have considerably altered ecosystems worldwide. Their invasion potential has been partially attributed to their free-living larvae, which have a high dispersal capability. We investigated the invasion potential of *Anodonta* (*Sinanodonta*) *woodiana*, a species of East Asian unionid mussel established worldwide despite having an obligatory parasitic stage (glochidium), which must encyst on host fish. The invasion success of *A. woodiana* has been attributed to the success of worldwide introductions of its sympatric fish hosts. We experimentally found, however, that *A. woodiana* is a broad host generalist, which can complete its development on all eight fish species tested, both co-invasive and native. Subsequently, we used a data on the occurrence and relative abundance of potential hosts in river habitats in the Czech Republic to project scenarios of the effect of host availability on *A. woodiana* invasion. We found that host availability does not constitute a major limit for *A. woodiana* to colonise most aquatic habitats in Central Europe. In addition, we investigated seasonal dynamics of *A. woodiana* reproduction and did not detect any limitations of its reproduction by ambient water temperatures typical of a Central European lowland river. Consequently, we predict that *A. woodiana* may further increase the speed and range of its invasion and we discuss possible consequences to native habitats and communities, especially to the endangered species of unionid mussels.

Keywords: aquatic habitats, Bivalvia, host-parasite relationship, host specificity, Mollusca, Unionidae

Correspondence: Karel Douda, Department of Applied Ecology, Water Research Institute TGM, Podbabská 30, Prague, CZ 160 00, Czech Republic. Tel: +420 220 197 367; Fax: +420 197 514; Email: douda@vuv.cz

Introduction

Several freshwater bivalve species are some of the world's most problematic biological invaders (Higgins and Vander Zanden 2010). For example, recent invasions of *Dreissena polymorpha*, *Corbicula fluminea* and *Limnoperna fortunei* have been particularly problematic. These species have altered entire aquatic environments by their filtration and burrowing activities (Higgins and Vander Zanden 2010; Karatayev et al. 2007b; Sousa et al. 2009; Strayer 2009), and they have severely affected native communities by altering habitats and competing for resources (Karatayev et al. 1997; Sousa et al. 2008b; Ward and Ricciardi 2007). Bivalve invasions in freshwater are becoming increasingly common, but our understanding of their biology, including factors that affect their invasion, is still largely incomplete (Keller et al. 2007; Leung et al. 2004; Stoeckel et al. 1997; Strayer 2009).

The most invasive bivalve species have simple life cycles; they either directly release juveniles (Sousa et al. 2008a), or they produce free living dispersal larvae (Karatayev et al. 2007a; Stoeckel et al. 1997). Life histories among bivalves, however, are diverse and may include an obligatory parasitic stage. Freshwater mussels from the superfamily Unionoidea are sedentary benthic invertebrates, which feed primarily through water filtration. Their life cycle includes a larval stage, termed the glochidium, which attaches generally to freshwater fishes for several days to months, depending on the water temperature. Several thousands glochidia are incubated in a female's modified gill chambers. To complete their development into a juvenile mussel, ripe glochidia are released into the water, where they attach to a host tissue and encyst (Wachtler et al. 2001). This relationship of unionids and fishes is more phoretic than nutritive (Barnhart et al. 2008), although glochidia obtain nourishment from their host (Wachtler et al. 2001). This parasitic stage is the key period for unionid mussel dispersal and invasion.

The host-parasite relationship is one of the most challenging issues in invasion biology (Taraschewski 2006). The geographic range extensions of parasites expose them to novel hosts, which has a direct consequences for establishment success of new parasite populations, their population dynamics and potential for invasiveness (e.g., Lee and Klasing 2004; Prenter et al. 2004). Parasites' levels of host specificity are highly variable (Poulin 1992), which affects their dispersal and their reproduction success within their novel range (Taraschewski 2006). Parasites are not always successful in infecting and exploiting their new hosts (Bakke et al. 2002), but they may capitalise on the 'evolutionary naivety' of novel hosts and exploit them more effectively than hosts that they have co-evolved with (e.g., Reichard et al. 2007; 2010). The degree of host specificity is directly related to the parasite's invasion potential, as the availability of suitable hosts species determine the parasite's ability to reproduce and invade (Shea and Chesson 2002).

We investigated how host specificity affects the invasion success of the Chinese pond mussel, *Anodonta* (*Sinanodonta*) *woodiana* (Lea), a unionid mussel species that has recently been reported as being invasive worldwide. *Anodonta woodiana* is native to south eastern Asia, specifically Indochina and southern China to Korea, Japan, Taiwan, Primorye and the Amur Basin in eastern Russia (Graf 2007; Watters 1997). In their native range, glochidia are released in the summer, attach to fins and gills of host fish and are encysted by the host's tissue. Metamorphosis is complete within several days, and juvenile mussels then release from their host (Dudgeon and Morton 1984). The expansion of *A. woodiana's* range began in the second half of the 20th century; today, *A. woodiana* can be found in the Indonesian islands (Djajasasmita 1982), Central America (Watters 1997), Europe (Kraszewski 2007; Sárkány-Kiss et al. 2000), the Asian part of Turkey (M. Reichard, unpublished data) and North America (Benson 2011). *Anodonta woodiana* range expansion has been attributed to its parasitic stage and the notion that infected host fishes serve as a vector for spreading. Exports

of fish for commercial purposes (mainly carp species from East Asia) are thought to be the main means of human-mediated dispersal (Watters 1997). The establishment of East Asian cyprinid fish populations, such as *Carassius auratus*, *Carrasius gibelio*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*, in non-native areas is believed to enable the persistence of *A. woodiana* populations (Watters 1997). However, direct data concerning host specificity and transformation success of *A. woodiana* glochidia on different host fish species are lacking and it has also been hypothesised that *A. woodiana* may use fish species native to the invaded areas as hosts (Kiss 1995; Sárkány-Kiss et al. 2000; Watters 1997).

The compatibility of a host-parasite combination depends mainly upon the ability of glochidia to survive the host's defensive immune response (Jansen et al. 2001). Most unionid mussel species have a high degree of host specificity and are able to use only one, or a few, host species, though some generalists are reported to use over 30 species (Strayer 2008; Trdan and Hoeh 1982). The parasitic period is critical to unionid mussel population dynamics (Vaughn and Taylor 2000; Watters 1992; McNichols et al. 2011) because host fish directly affect mussel reproductive success (by contact probability and immunological compatibility) and dispersal (by the host movements).

Information on host specificity of *A. woodiana* glochidia will also help in understanding the spatial pattern of *A. woodiana* invasions. Two alternative hypotheses exist to explain *A. woodiana* invasions. First, *A. woodiana* is spread via the intercontinental introductions of its hosts, and *A. woodiana's* high host specificity limits its distribution to the limits of its co-invasive hosts. High local densities of *A. woodiana* may be explained either by the establishment or repeated import of fish species that serve as hosts to *A. woodiana* in its native range. Second, *A. woodiana* initially co-invades with its native hosts, but its low host specificity enables subsequent colonisation of novel host species populations within the novel range. This scenario also includes the possibility that co-invasive hosts are involved in an initial phase of the invasion, and it predicts that there is a more rapid increase in population density and dispersal due to the multiplying effect on the number of potential sites with exploitable hosts and the overall higher density of hosts at each site. The distinction between these two hypotheses is crucial for the proper management of native communities affected by *A. woodiana* invasion, including measures of prevention, control and potential eradication, as well as restoration plans.

The fact that *A. woodiana* is widespread suggests that both juvenile and adult *A. woodiana* can cope with a wide range of environmental conditions. Corsi et al. (2007) showed that *A. woodiana* has particular physiological predispositions (cholinesterase enzymes activity) that probably enable it to tolerate a variety of unsuitable conditions. On the other hand, Kraszewski (2007) suggested that water temperature may play a pivotal role in the variation of *A. woodiana* abundance and biomass and may limit its distribution. The role of water temperature is most likely to be manifested by the failure of gametes to mature (Galbraith and Vaughn 2009). However, details concerning *A. woodiana* environmental tolerance and the role of temperature and other habitat factors on *A. woodiana* survival and reproduction remain unclear.

Potential ecological and economical consequences of an *A. woodiana* invasion likely come from the adult mussels. Despite the existence of the obligatory parasitic stage, host fish are typically not negatively affected (e.g., Treasurer et al. 2006) and rather serve as a vector. In contrast, adult mussels are known to reach a population biomass of up to 25 kg m^{-2} (Kraszewski and Zdanowski 2007), which may have serious consequences for the surrounding ecosystem due to their filtrating capacity (Vaughn and Hakenkamp 2001) and their competition with native mussel species for space and food. Further, native unionid mussels may be threatened by competition for hosts, as the infected hosts often develop cross-specific immunity after the first glochidia infection (Rogers and Dimock 2003). Indeed,

unionid mussels are one of the most threatened animal groups globally (Lydeard et al. 2004) because of their complex life cycles, host specificity and strict environmental condition requirements, which makes them highly vulnerable to the human alteration of freshwater habitats (Bogan 1993; Douda 2010; Vaughn and Taylor 1999).

We investigated potential causes of the recent *A. woodiana* invasion in Central Europe. First, we experimentally tested the host-parasite compatibility of *A. woodiana* with their co-invasive host fish species (sympatric with *A. woodiana* in their natural range) and with several common native European fish species. We compared the dynamics of initial glochidia attachment, the length of the parasitic period and the transformation success of *A. woodiana* glochidia among particular host species. Second, we investigated the potential role of water temperature on *A. woodiana* reproductive success by describing the seasonal dynamics of their reproduction in a Central European lowland river. We discuss our outcomes with an emphasis on the global patterns and consequences of *A. woodiana* invasion.

Material and Methods

Experimental test of host specificity

Gravid females of *A. woodiana* were collected in September 2010 from the Kyjovka River, Czech Republic (N 48°46'45"; E 17°00'60"). Females were transported to the laboratory and held separately in 10-L containers with aerated river water until glochidia clumps were spontaneously released. Eight potential host species (family Cyprinidae) were used for experimental infection by glochidia. Two species were co-invasive (*Pseudorasbora parva* and *Carassius gibelio*, both sympatric with *A. woodiana* in their native range), and five species were of European origin (*Leuciscus cephalus*, *Rutilus rutilus*, *Gobio gobio*, *Barbus barbus* and *Rhodeus amarus*). The origin of *Cyprinus carpio* in Europe is unclear; it is native to Eastern Asia (sympatric with *A. woodiana* populations) and has been hypothesised to be either native to Europe (Kohlmann et al. 2003) or introduced throughout Europe approximately two thousand years ago (Froufe et al. 2002). All species were obtained from a commercial hatchery, except for *R. amarus*, which was collected from a field with a hand net at Štítarský Stream (N 50°16'13"; E 15°11'15"; not populated by *A. woodiana*). Six to ten individual fish per species were used for infections, for a total of 56 individually monitored fish. Ages of experimental fish were either 0 + (*R. amarus*) or 1 + (*B. barbus*, *C. carpio*, *C. gibelio*, *G. gobio*, *L. cephalus*, *P. parva* and *R. rutilus*). Two fish (one *R. rutilus* and one *C. gibelio*) died before the end of juvenile detachment (mortality was not related to experimental infection) and subsequently were not included in the analysis.

Spontaneously released glochidia were removed from boxes containing *A. woodiana* using a pipette and were immediately used for laboratory infections. A random subsample of 30 glochidia from each female was tested for viability with sodium chloride (Wang et al. 2007) to confirm their infection potential. Glochidia from six gravid *A. woodiana* females (mean ± SD of shell length 184 ± 14 mm) with a glochidia viability of over 90% were pooled and used for inoculations. Fish were infected by being placed into a dechlorinated tap water bath containing 4570 ± 1279 viable glochidia L^{-1}. The glochidia were kept in homogeneous suspension through aeration (volume of the suspension was 0.5 L per fish). After 15 min of inoculation, fish were transferred into a bath that did not contain glochidia for 30 min to rinse off non-attached glochidia. Fish were then individually placed into 56 continuously aerated 5-L plastic tanks with dechlorinated tap water that had 3-mm nets on the bottom. Fish remained in these tanks until the end of the experiment. Fish were fed daily with commercial flake fish food. The temperature in the tanks was recorded automatically every 10 min and was 23.3 ± 0.7 C during the experiment; this agrees

with the ambient temperature recorded in the source population during glochidia release (Konečná and Reichard 2011).

Water in individual boxes was partially exchanged (80% of water volume) by siphoning the bottom of the tanks for 26 days, beginning the day after infections and continuing every second day thereafter. Untransformed glochidia and juvenile mussels were isolated from debris in the siphoned water with nylon screens (mesh size 139 and 507 micro m; UHELON). A stereomicroscope with 10 - 40 × magnification was used to count the number of glochidia and transformed juveniles. All individuals that were collected were inspected; if foot activity or valve movement were observed, individuals were classified as live juveniles.

Glochidia infection rate (the number of initially attached glochidia per fish), mean attachment duration of successfully developed glochidia and transformation success were compared between host species with generalised linear models (GLM); host species identity and individual host body weight were used as predictive factors, and the interaction between species and weight was included. Minimal adequate models were constructed using Akaike information criterion. Analysis of deviance tables were computed for fitted model objects. Data for the transformation success of glochidia, expressed as a share between the total number of initially attached glochidia per fish and the total number of living juveniles recovered from the fish, were arcsine-transformed before analyses. Tukey's HSD post-hoc tests were used to examine the pairwise differences between host species. Analyses were done using the R 2.12.0 software package (R Core Development Team 2010).

Host availability under different levels of host specificity

Data from the national fish community monitoring program, established according to the European Water Framework Directive (Czech Hydrometeorological Institute 2010), were used to project scenarios of host availability for *A. woodiana* at two different levels of its specificity (only co-invasive host species, all confirmed host species). A standardised electrofishing method was used to obtain fish data (Jurajda et al. 2010) during 2006 and 2007. Sampling sites were lotic habitats, including upland streams and large lowland rivers, and were evenly distributed throughout the Czech Republic (approximately 78,000 km^2). Lakes were not included as sampling sites because natural lakes only rarely occur outside the active floodplain in Czech Republic (Corine land-cover data, CLC 2000, European Environment Agency). The analysis is based on 49,871 individuals of 40 fish species from 247 sampling sites. First, we calculated the potential availability of the host fish species that are co-invasive with *A. woodiana* in the territory of the Czech Republic as a proportion of sample sites with the presence of at least one of the introduced East Asian species. Second, we calculated the proportion of sample sites that had at least one of the suitable host species for *A. woodiana* experimentally determined by this study. In both scenarios, we also determined the relative abundance of the supposed host species.

Reproduction timing of *A. woodiana* in a Central European lowland river

Anodonta woodiana were sampled monthly between November 2008 and October 2009 in the Kyjovka River to determine the seasonal dynamics of their reproductive cycle. This river sustains a large population of *A. woodiana* that was discovered in 2007 (M. Reichard, pers. observation) but was likely established between 2002 and 2006. At each sampling, 30 individuals were collected by hand, and water temperature was measured. Sampling was conducted at two sites, positioned 7 km apart from each other, because sampling at the first site (48°43'24"N, 16°58'18"E) led to a decrease in local population density. To mitigate the effort needed to collect sufficient number of individuals for analysis, the second sampling site (48°46'44"N, 17°01'00"E), upstream of the first site, was used from June onward. Three hundred sixty one individual *A. woodiana* were collected over 12 sampling occasions. Adult mussels were targeted.

Mussels were transported alive to the Institute of Vertebrate Biology in Brno, Czech Republic. Mussels were dissected to determine their sex and the developmental phase of their glochidia, which were incubated in marsupia. Individuals were sexed by the appearance of their demibranchs, which is a reliable method for sex determination in mature *Anodonta* mussels (Mackie 1984). Females had swollen outer gills, whereas the outer gills of males did not differ from their inner gills. A sample of the swollen outer gill tissue was dissected from each female, the state of glochidia development was identified under a microscope and glochidia were assigned to three groups: eggs (indiscernible shell structure), immature glochidia and ripe glochidia (amber shells with hook). If glochidia were absent in swollen demibranchs, they were assumed to be recently released.

Results

Host specificity and its effect on host availability

Anodonta woodiana glochidia successfully developed on all host species tested (Fig. 4.1). Mean (± SD) transformation success of glochidia ranged from 17.6 ± 12.4% in *G. gobio* to 52.4 ± 8.4% in *B. barbus*. Mean duration time of successful glochidia development ranged from 6.3 to 7.2 days (Table 4.1).

The infection rate varied among host species ($F_{7,46}$ = 123.6; $P < 0.001$) and was positively affected by fish body mass ($F_{7,45}$ = 96.8; $P < 0.001$). The relationship between host body mass and infection rate varied across species ($F_{7,46}$ = 2.5; $P < 0.05$). Mean duration of successful development ($F_{7,45}$ = 4.4; $P < 0.001$) and transformation success ($F_{7,46}$ = 5.0; $P < 0.001$) also differed among species but were not related to host body mass ($P > 0.05$). The transformation success for two co-invasive host species (*P. parva* and *C. gibelio*) was not higher than those for novel hosts (Table 4.1). One of the East Asian host species, *P. parva*, had lower transformation success than one of the host species indigenous to Central Europe, *B. barbus* (Table 4.1, Tukey's HSD tests: $P < 0.05$).

At least one of the two co-invasive species (*P. parva* and *C. gibelio*) occurred at 19.4% of the sample sites but had a relative abundance of only 1.4%. In contrast, all host species confirmed as suitable hosts in the host specificity experiment were found in 75.3% of sample sites, with a relative abundance of 39.5%.

Seasonal dynamics of *A. woodiana* reproduction in Central Europe

Mature glochidia were detected from April to September, with maximum numbers detected in July, when 69% of females had ripe glochidia in their outer demibranchs. Fertilised eggs and non-mature glochidia were observed in demibranchs from December to August (Fig. 4.2). Glochidia maturation was positively associated with increased water temperature; production of the first ripe glochidia coincided with a temperature of 15.6 °C. The sex ratio did not differ from parity at any sampling date or across samples (binomial tests, all $P > 0.05$).

Discussion

We have demonstrated that *A. woodiana*, an invasive unionid species with an obligatory parasitic stage, is a broad generalist; it successfully developed on all eight freshwater fish host species we tested, regardless of whether they were co-invasive or native species. Projection of the invasive potential of *A. woodiana* onto a detailed fish distribution dataset demonstrated that, should dispersal limitation come from host availability, *A. woodiana* has the capability to colonise the majority of river habitats in the Czech Republic. There was also no apparent limitation on *A. woodiana* reproduction due to the ambient temperature range typically found in a Central European lowland river.

Invasion potential of *A. woodiana*

Invasions of several freshwater bivalve species (such as *Dreissena polymorpha* or *Limnoperna fortunei*) have caused considerable alterations to ecosystems worldwide (e.g., Sousa et al. 2009), and their invasion potential has been attributed particularly to the existence of their free-living larvae that have high dispersal capabilities (Johnson and Padilla 2010; Stoeckel et al. 1997). We have shown that other species of freshwater mussels may be similarly able to invade rapidly, despite having more complex life cycles. *Anodonta woodiana* has an obligatory parasitic stage, in which the larva must attach and encyst to a freshwater fish host. We found that host specificity in *A. woodiana* was low, allowing for successful glochidia transformation on a variety of host fish, including a species (*R. amarus*) that has been shown to resist glochidia infection of native unionids (Reichard et al. 2006; 2010).

Interspecific differences in transformation success of *A. woodiana* among host species were low; even the least suitable species had a transformation success over 17% and may be considered a functional host. Natural mortality of glochidia often reaches more than 50% of the initially attached glochidia, even in their most compatible mussel-fish relationships (e.g., Jansen et al. 2001; Strayer 2008). In their native range, a Hong Kong population of *A. woodiana* successfully attached to all four host species examined (*Gambusia affinis* - Poeciliidae and *Puntius semifasciolatus*, *Metzia takakii* and *Rhodeus sinensis* - Cyprinidae), with the highest abundance observed on the non-native *G. affinis* (Dudgeon and Morton 1984). In Japan, Fukuhara et al. (1986) recorded attached glochidia of *A. woodiana* on five host species (*Rhodeus ocellatus*, *Pseudorasbora parva* - Cyprinidae; *Oryzias latipes* - Adrianichthyidae; *Rhinogobius brunneus* - Gobiidae and non-native *Lepomis macrochirus* - Centrarchidae), with the highest density found on *R. brunneus*. In addition, Kiss (1995) successfully bred juveniles of *A. woodiana* on several cyprinid fishes, most of which were native to East Asia. Although it is the crucial measure of host suitability, transformation success was not unfortunately measured in those studies. Nevertheless, collectively, those data document that *A. woodiana* is able to exploit a wide range of host species.

The generalist behaviour observed in *A. woodiana* plays a critical role in its invasive potential, as host community structure fundamentally influences the establishment and prevalence of parasites (Holt et al. 2003). A generalist parasite strategy is advantageous during changes in host availability during an invasion, and generalist parasites may quickly become independent of their native hosts. For example, a fish-parasitising cestode from East Asia, *Bothriocephalus acheilognathi*, was introduced globally by the intercontinental export of carp species and now has exploited many native fish species in its novel range, causing significant economic losses in fisheries worldwide (e.g., Bean and Bonner 2010; Brouder and Hoffnagle 1997). We predict that *A. woodiana* has the potential to invade areas outside the range of their co-invasive native hosts, even if their initial invasion was likely facilitated by the presence of their native hosts. Hence, the present commercial transportation of many fish species used in aquaculture will likely contribute to the further spread of *A. woodiana* and the establishment of new *A. woodiana* populations.

Our host availability projection quantified the potential of *A. woodiana* to encounter suitable host species at over 75% of lotic habitats in the Czech Republic. This number is the most conservative estimate, as all eight experimentally infected species were suitable hosts. Many host species migrate within the rivers and across the floodplain (Lucas and Baras 2001), further facilitating the spatial dynamics of an *A. woodiana* invasion. Suitability of environmental conditions for adult mussels and glochidia maturation, however, may substantially decrease the proportion of habitats accessible to *A. woodiana*. Data on the water temperature regime for the sites used in the projection were not available and thus could not be incorporated in the analysis.

Water temperature governs the timing of reproduction and gamete maturation in freshwater mussels (Galbraith and

Vaughn 2009; Hruška 1992; Watters and O'Dee 2000), and the first reports of *A. woodiana* occurrence in Europe were restricted to the south, or to the thermally-polluted water systems (Kraszewski 2007). Our data from the Kyjovka River indicate that *A. woodiana* reproduction was not constrained by the temperature regime as strongly as previously believed, and its seasonal dynamics are similar to those observed in south-eastern Asia (Dudgeon and Morton 1983). We demonstrated that ripe glochidia were present for 6 months, when ambient temperature ranged from 15 to 27°C (Fig. 4.2). During that period, the proportion of females with ripe glochidia was high (Fig. 4.2). *Anodonta woodiana* has recently been reported in colder areas within Europe, such as southern Sweden (von Proschwitz 2008) and the subalpine lake, La Garda, in Northern Italy (Cappelletti et al. 2009). Hence, low water temperature is likely a smaller constraint to *A. woodiana* invasion than previously believed and might be even further relaxed with increasing thermal pollution, global change and evolutionary response of invasive populations to novel conditions.

Potential consequences of *A. woodiana* invasion

Given that neither host availability nor ambient temperatures appear to impede basically *A. woodiana* invasions, we predict that *A. woodiana* may rapidly spread throughout European water bodies. As far as we are aware, no one has examined the foraging ecology of *A. woodiana*, and it is possible that *A. woodiana* may be limited by food availability in nutrient-poor habitats. Freshwater mussel communities are often structured by their metabolic rate (Bauer et al. 1991; Bauer 2001), and *A. woodiana* seems to be more abundant in nutrient-rich habitats, such as in lowland floodplains and in aquaculture ponds (e.g., Paunovic et al. 2006), although a report from a subalpine Lake, La Garda (Cappelletti et al. 2009) imply that *A. woodiana* may be able to exploit a wider range of habitats.

An invasion by *A. woodiana* has three potential negative consequences for the native unionid mussel species. First, *A. woodiana* may directly compete with native mussels for food. High population densities of this species suggest that it may have a capacity to reduce food resources in some habitats. This is supposed to be the primary mechanism of exploitative competition between bivalves, which depends on the minimum food level at which each bivalve can thrive (Strayer 1999). Indeed, competition for food is considered to be the main factor threatening the native populations of North American unionids in areas that have been invaded by *D. polymorpha* (Haag et al. 1993). Second, *A. woodiana* is expected to compete with native unionids for hosts. Direct competition by glochidia for space on hosts or via the activation of innate host tissue responses, such as hyperplasic reaction, may be expected between *A. woodiana* and native species of genus *Unio* because European *Unio* spp. also release their glochidia in the European summer (Blazek and Gelnar 2006; Mackie 1984). Third, glochidia infections can trigger an adaptive immune response by hosts, making them immunised against repeated infections after contact with glochidia (Rogers and Dimock 2003). Such cross-resistance is expected to significantly decrease the proportion of hosts available to native mussels.

Anodonta woodiana abundance increased dramatically within the first two generations it was observed in the Czech Republic. The first report of *A. woodiana* in the Czech Republic comes from 1996 (Beran 1997); however, *A. woodiana* had become a dominant bivalve species in some locations by 2008, with several population outbreaks documented in the southern (warmer) part of the Czech Republic (Beran 2008). This is similar to dynamics documented in other European countries (Cianfanelli et al. 2007; Kraszewski 2007; Paunovic et al. 2006; Popa et al. 2007; Pou-Rovira et al. 2009). Despite an increasing number of invaded regions, there are no comprehensive data documenting effects on native communities by *A. woodiana* invasion, though the first signs of native unionids impairment by *A. woodiana* have been observed in Italy (summarised in Cianfanelli et al. 2007).

The invasion of *A. woodiana* may directly affect an environment by means of physical habitat alternation. In particularly suitable habitats, *A. woodiana* may reach extremely high population densities; a population biomass of up to 25 kg m^{-2} (Kraszewski and Zdanowski 2007), and a density of over 30 individuals m^{-2} was observed in parts of the Kyjovka River (M. Reichard, M. Vrtílek, personal observation). Other invasive freshwater bivalves can seriously affect physical habitat properties (Darrigran 2002; Higgins and Vander Zanden 2010; Sousa et al. 2009; Strayer 2009), and filtration and burrowing activities of unionid mussels may affect organic matter pathways within the sediment and the water column (Vaughn and Hakenkamp 2001).

In conclusion, we have demonstrated that *A. woodiana* has predispositions to be a successful invader in Europe and also probably in other continents. These predispositions include employing a generalist strategy in using its hosts during the parasitic stage and being able to successfully reproduce in environmental conditions previously rendered as suboptimal. We have shown that the spread of *A. woodiana* is not limited by the availability of its hosts and that the temperature conditions in a typical Central European lowland river are suitable for completion of its life cycle. We have also identified potential threats to native habitats and communities, mainly the endangered species of unionid mussels. Given that genetic constraints limiting invasion may be gradually relaxed during the initial establishment (due to adaptive evolution; Sakai et al. 2001), it is possible that tolerance of lower water temperatures will be selected in invasive *A. woodiana* populations, further increasing their ability to colonise new areas.

Literature
Bakke TA, Harris PD, Cable J (2002) Host specificity dynamics: observations on gyrodactylid monogeneans. Int J Parasitol 32:281–308
Barnhart MC, Haag WR, Roston WN (2008) Adaptations to host infection and larval parasitism in Unionoida. J N Am Benthol Soc 27:370–394
Bauer G (2001) Framework and driving forces for the evolution of naiad life histories. In: Bauer G and Wachtler K (eds) Ecology and evolution of the freshwater mussels Unionoida. Springer-Verlag, Berlin, pp 233–255
Bauer G, Hochwald S, Silkenat W (1991) Spatial distribution of freshwater mussels: the role of host fish and metabolic rate. Freshwater Biol 26:377–386
Bean MG, Bonner TH (2010) Spatial and temporal distribution of the Asian fish tapeworm *Bothriocephalus acheilognathi* (Cestoda: Bothriocephalidea) in the Rio Grande (Rio Bravo del Norte). J Aquat Anim Health 22:182–189
Benson AJ (2011) *Sinanodonta woodiana*. USGS nonindigenous aquatic species database. http://nas.er.usgs.gov/queries/FactSheet aspx?speciesID=2824. Accessed 18 January 2011

Beran L (1997) First record of *Sinanodonta woodiana* (Mollusca: Bivalvia) in the Czech Republic. Acta Soc Zool Bohem 61:1–2

Beran L (2008) Expansion of *Sinanodonta woodiana* (Lea, 1834) (Bivalia: Unionidae) in the Czech Republic. Aquat Invasions 3:91–94

Blazek R, Gelnar M (2006) Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca : Unionidae) on host fishes. Folia Parasit 53:98–106

Bogan AE (1993) Freshwater bivalve extinctions (Mollusca: Unionoida): A search for causes. Am Zool 33:599–609

Brouder MJ, Hoffnagle TL (1997) Distribution and prevalence of the Asian fish tapeworm, *Bothriocephalus acheilognathi*, in the Colorado River and tributaries, Grand Canyon, Arizona, including two new host records. J Helminth Soc Wash 64:219–226

Cappelletti C, Cianfanelli S, Beltrami ME, Ciutti F (2009) *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae): a new non-indigenous species in Lake Garda (Italy). Aquat Invasions 4:685–688

Cianfanelli S, Lori E, Bodon M (2007) Non-indigenous freshwater molluscs and their distribution in Italy. In: Gherardi F (ed) Biological invaders in inland waters: Profiles, distribution, and threats. Springer, Dordrecht, pp 103–121

Corsi I, Pastore AM, Lodde A, Palmerini E, Castagnolo L, Focardi S (2007) Potential role of cholinesterases in the invasive capacity of the freshwater bivalve, *Anodonta woodiana* (Bivalvia : Unionacea): A comparative study with the indigenous species of the genus, *Anodonta* sp. Comp Biochem Phys C 145:413–419

Darrigran G (2002) Potential impact of filter-feeding invaders on temperate inland freshwater environments. Biol Invasions 4:145–156

Djajasasmita M (1982) The occurrence of *Anodonta woodiana* Lea, 1837 in Indonesia (Pelecypoda, Unionidae). Veliger 25:175

Douda K (2010) Effects of nitrate nitrogen pollution on Central European unionid bivalves revealed by distributional data and acute toxicity testing. Aquat Conserv 20:189–197

Dudgeon D, Morton B (1983) The population dynamics and sexual strategy of *Anodonta woodiana* (Bivalvia, Unionacea) in Plover Cove Reservoir, Hong-Kong. J Zool 201:161–183

Dudgeon D, Morton B (1984) Site selection and attachment duration of *Anodonta woodiana* (Bivalvia, Unionacea) glochidia on fish hosts. J Zool 204:355–362

Froufe E, Magyary I, Lehoczky I, Weiss S (2002) mtDNA sequence data supports an Asian ancestry and single introduction of the common carp into the Danube Basin. J Fish Biol 61:301–304

Fukuhara S, Nagata Y, Yamada T (1986) Glochidium parasitic period, host-fish and parasitic site of *Anodonta woodiana* in small pond. Venus 45:43–52

Galbraith HS, Vaughn CC (2009) Temperature and food interact to influence gamete development in freshwater mussels. Hydrobiologia 636:35–47

Graf DL (2007) Palearctic freshwater mussel (Mollusca: Bivalvia: Unionoida) diversity and the Comparatory Method as a species concept. P Acad Nat Sci Phila 156:71–88

Haag WR, Berg DJ, Garton DW, Farris JL (1993) Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can J Fish Aquat Sci 50:13–19

Higgins SN, Vander Zanden MJ (2010) What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecol Monogr 80:179–196

Holt RD, Dobson AP, Begon M, Bowers RG, Schauber EM (2003) Parasite establishment in host communities. Ecol Lett 6:837–842

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 Hydrobiol 126:181–197

Jansen W, Bauer G, Zahner-Meike E. (2001) Glochidial mortality in freshwater mussels. In: Bauer G and Wachtler K (eds) Ecology and evolution of the freshwater mussels Unionoida. Springer-Verlag, Berlin, pp 185–211

Johnson LE, Padilla DK (2010) Geographic spread of exotic species: Ecological lessons and opportunities from the invasion of the zebra mussel *Dreissena polymorpha*. Biol Conserv 78:23–33

Jurajda P, Slavik O, White S, Adamek Z (2010) Young-of-the-year fish assemblages as an alternative to adult fish monitoring for ecological quality evaluation of running waters. Hydrobiologia 644:89–101

Karatayev AY, Boltovskoy D, Padilla DK, Burlakova LE (2007a) The invasive bivalves *Dreissena polymorpha* and *Limnoperna fortunei*: Parallels, contrasts, potential spread and invasion impacts. J Shellfish Res 26:205–213

Karatayev AY, Burlakova LE, Padilla DK (1997) The effects of *Dreissena polymorpha* (Pallas) invasion on aquatic communities in eastern Europe. J Shellfish Res 16:187–203

Karatayev AY, Padilla DK, Minchin D, Boltovskoy D, Burlakova LE (2007b) Changes in global economies and trade: the potential spread of exotic freshwater bivalves. Biol Invasions 9:161–180

Keller RP, Drake JM, Lodge DM (2007) Fecundity as a basis for risk assessment of nonindigenous freshwater Molluscs. Conserv Biol 21:191–200

Kiss A (1995) The propagation, growth and biomass of the Chinese huge mussel (*Anodonta woodiana woodiana* Lea, 1834) in Hungary. Dissertation, University of Agricultural Sciences of Gödöllo

Kohlmann K, Gross R, Murakaeva A, Kersten P (2003) Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. Aquat Living Resour 16:421–431

Konečná M, Reichard M (2011) Seasonal dynamics in population characteristics of European bitterling *Rhodeus amarus* in a small lowland river. J Fish Biol 78:227–239

Kraszewski A (2007) The continuing expansion of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in Poland and Europe. Folia Malac 15:65–69

Kraszewski A, Zdanowski B (2007) *Sinanodonta woodiana* (Lea, 1834) (Mollusca) - a new mussel species in Poland: occurrence and habitat preferences in a heated lake system. Pol J Ecol 55:337–356

Lee KA, Klasing KC (2004) A role for immunology in invasion biology. Trends Ecol Evol 19:523–529

Leung B, Drake JM, Lodge DM (2004). Predicting invasions: Propagule pressure and the gravity of Allee effects. Ecology 85:1651–1660

Lucas MC, Baras E (2001) Migration of Freshwater Fishes. Blackwell Science, Malden

Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG (2004) The global decline of nonmarine mollusks. Bioscience 54:321–330

Mackie GL (1984) Bivalves. In: Tompa AS, Verdonk NH, van den Biggelaar JAM (eds) The Mollusca Vol.7: Reproduction. Academic Press, London, pp 351–418

McNichols KA, Mackie GL Ackerman JD (2011) Host fish quality may explain the status of endangered *Epioblasma torulosa rangiana* and *Lampsilis fasciola* (Bivalvia: Unionidae) in Canada. J N Am Benthol Soc 30:60–70

Paunovic M, Csányi B, Simic V, Stojanovic BCP (2006) Distribution of *Anodonta* (*Sinanodonta*) *woodiana* (Rea, 1834) in inland waters of Serbia. Aquat Invasions 1:154–160

Popa OP, Kelemen BS, Murariu D, Popa LO (2007) New records of *Sinanodonta woodiana* (Lea, 1834) (Mollusca: Bivalvia: Unionidae) from Eastern Romania. Aquat Invasions 2:265–267

Pou-Rovira Q, Araujo R, Boix D, Clavero M, Feo C, Ordeix M, Zamora L (2009) Presence of the alien chinese pond mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae) in the Iberian Peninsula. Graellsia 65:67–70

Poulin R (1992) Determinants of host-specificity in parasites of freshwater fishes. Int J Parasitology 22:753–758

Prenter J, MacNeil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasions. Trends Ecol Evol 19:385–390

R Development Core Team (2010). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Reichard M, Ondrackova M, Przybylski M, Liu H, Smith C (2006) The costs and benefits in an unusual symbiosis: experimental evidence that bitterling fish (*Rhodeus sericeus*) are parasites of unionid mussels in Europe. J Evol Biol 19:788–796

Reichard M, Polacik M, Tarkan AS, Spence R, Gaygusuz O, Ercan E, Ondrackova M, Smith C (2010) The bitterling-mussel coevolutionary relationship in areas of recent and ancient sympatry. Evolution 64:3047–3056

Reichard M, Przybylski M, Kaniewska P, Liu H, Smith C (2007) A possible evolutionary lag in the relationship between freshwater mussels and European bitterling. J Fish Biol 70:709–725

Rogers CL, Dimock RV (2003) Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia : Unionidae) after multiple infections. J Parasitol 89:51–56

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Sárkány-Kiss A, Sirbu I, Hulea O (2000) Expansion of the adventive species *Anodonta woodiana* (Lea,1834) (Mollusca, Bivalvia, Unionoidea) in central and eastern Europe. Acta Oecol Univ Sibiu 7:49–57

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends Ecol Evol 17:170–176

Sousa R, Antunes C, Guilhermino L (2008a) Ecology of the invasive Asian clam *Corbicula fluminea* (Muller, 1774) in aquatic ecosystems: an overview. Ann Limnol-Int J Lim 44:85–94

Sousa R, Nogueira AJA, Gaspar M, Antunes C, Guilhermino L (2008b) Growth and extremely high production of the non-indigenous invasive species *Corbicula fluminea* (Müller, 1774): possible implications for ecosystem functioning. Estuar Coast Shelf S 80:289–295

Sousa R, Gutierrez JL, Aldridge DC (2009) Non-indigenous invasive bivalves as ecosystem engineers. Biol Invasions 11:2367–2385

Stoeckel JA, Schneider DW, Soeken LA, Blodgett KD, Sparks RE (1997) Larval dynamics of a riverine metapopulation: implications for zebra mussel recruitment, dispersal, and control in a large-river system. J N Am Benthol Soc 16:586–601

Strayer DL (1999) Effects of alien species on freshwater mollusks in North America. J N Am Benthol Soc 18:74–98

Strayer DL (2008) Freshwater mussel ecology: a multifactor approach to distribution and abundance. University of California Press. Berkley, California

Strayer DL (2009) Twenty years of zebra mussels: lessons from the mollusk that made headlines. Front Ecol Environ 7:135–141

Taraschewski H (2006) Hosts and parasites as aliens. J Helminthol 80:99–128

Trdan RJ, Hoeh WR (1982) Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda, Unionidae, Anodonta). Am Midl Nat 108:381–388

Treasurer JW, Hastie LC, Hunter D, Duncan F, Treasurer CM (2006) Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank-reared Atlantic salmon (*Salmo salar*). Aquaculture 256:74–79

Vaughn CC, Hakenkamp CC (2001) The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biol 46:1431–1446

Vaughn CC, Taylor CM (1999). Impoundments and the decline of freshwater mussels: A case study of an extinction gradient. Conserv Biol 13:912–920

Vaughn CC, Taylor CM (2000) Macroecology of a host-parasite relationship. Ecography 23:11–20

von Proschwitz T (2008) The Chinese giant mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae): an unwelcome addition to the Swedish fauna. Basteria 72:307–311

Wachtler K, Dreher-Mansur MC, Richter T (2001) Larval types and early postlarval biology in naiads (Unionoida). In: Bauer G and Wachtler K (eds) Ecology and evolution of the freshwater mussels Unionoida. Springer-Verlag, Berlin, pp 93–125

Wang N, Augspurger T, Barnhart MC, Bidwell JR, Cope WG, Dwyer FJ, Geis S, Greer IE, Ingersoll CG, Kane CM, May TW, Neves RJ, Newton TJ, Roberts AD, Whites DW (2007) Intra- and interlaboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). Environ Toxicol Chem 26:2029–2035

Ward JM, Ricciardi A (2007) Impacts of *Dreissena* invasions on benthic macroinvertebrate communities: a meta-analysis. Divers Distrib 13:155–165

Watters GT (1992) Unionids, fishes, and the species-area curve. J Biogeogr 19:481–490

Watters GT (1997) A synthesis and review of the expanding range of the Asian freshwater mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia:Unionidae). Veliger 40:152–156

Watters GT, O'Dee SH (2000) Glochidial release as a function of water temperature: beyond bradyticty and tachyticty. Proceedings of the conservation, captive care, and propagation of freshwater mussels symposium. Ohio Biological Survey, Ohio, pp 135–140

Table 4.1 Results of host compatibility test (mean ± SD). Attached glochidia represent the sum of glochidia and juveniles recovered from fish; transformation success indicates % of attached glochidia that were recovered as live juveniles; the duration of successful parasitism indicates the mean number of days from attachment to excystment of live juveniles. Means denoted by the same superscript letter are not significantly different (Tukey's HSD test at $P < 0.05$)

Species	Number of fish (n)	Fish length (mm)	Attached glochidia (n)	Transformation success (%)	Duration of successful parasitism (days)
Pseudorasbora parva	10	66.7 ± 4.5	170 ± 55 [a]	20.8 ± 20.4 [b]	6.3 ± 0.4 [a]
Carassius gibelio	5	82.7 ± 9.7	828 ± 250 [c]	33.8 ± 14.2 [a,b]	7.2 ± 0.1 [c]
Cyprinus carpio	6	68.8 ± 7.4	428 ± 268 [b]	40.7 ± 6.7 [a,b]	7.0 ± 0.5 [c,b]
Leuciscus cephalus	6	49.0 ± 0.9	179 ± 36 [a]	31.8 ± 10.1 [a,b]	6.6 ± 0.4 [a,c]
Rhodeus amarus	10	28.7 ± 1.8	22 ± 8 [a]	21.7 ± 13.1 [b]	6.7 ± 0.6 [a,c]
Gobio gobio	6	75.7 ± 3.4	697 ± 113 [c]	17.6 ± 12.4 [b]	6.7 ± 0.2 [a,c]
Barbus barbus	6	97.8 ± 8.2	705 ± 123 [c]	52.4 ± 8.4 [a]	7.0 ± 0.4 [c,b]
Rutilus rutilus	5	97.6 ± 5.5	654 ± 73 [b,c]	28.1 ± 8.7 [a,b]	6.3 ± 0.2 [a,b]
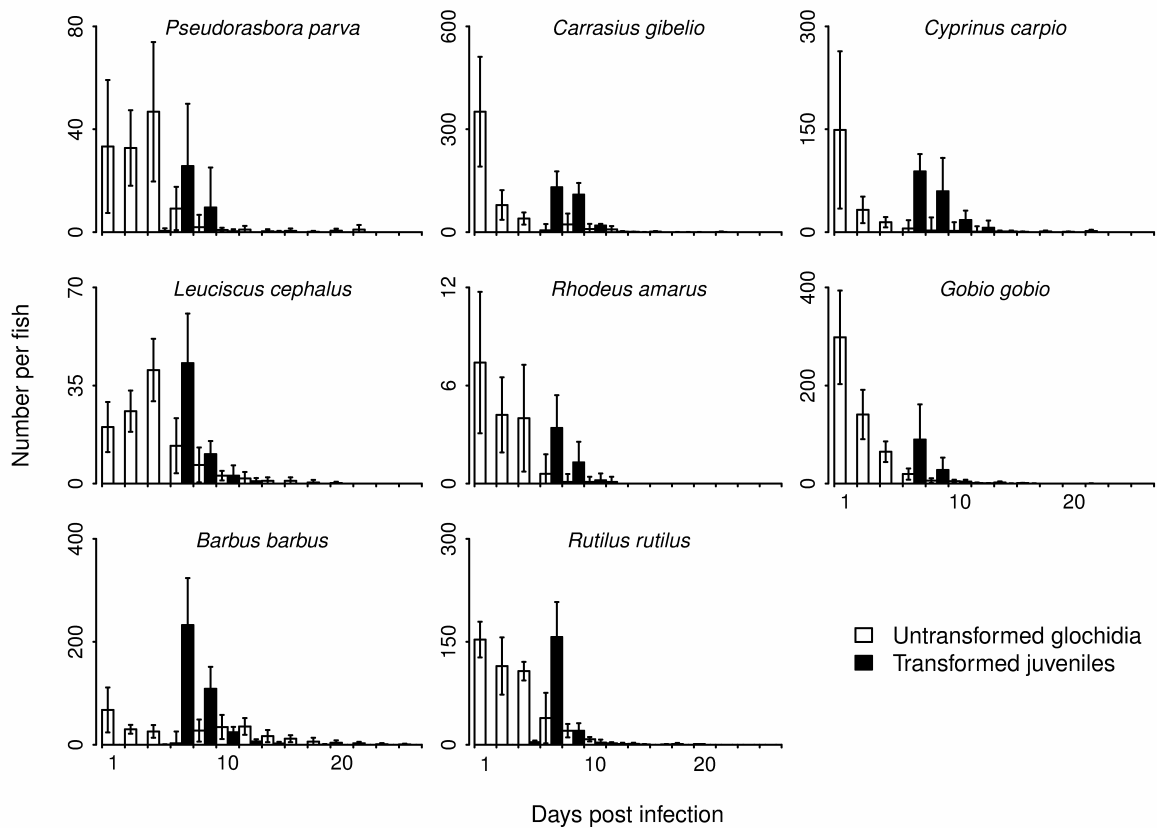
Fig. 4.1 Developmental dynamics of *A. woodiana* glochidia on different host species. Bars indicate the mean ± SD of the number of untransformed glochidia (white bars) or live juveniles (black bars) recovered per host species in the respective day after attachment
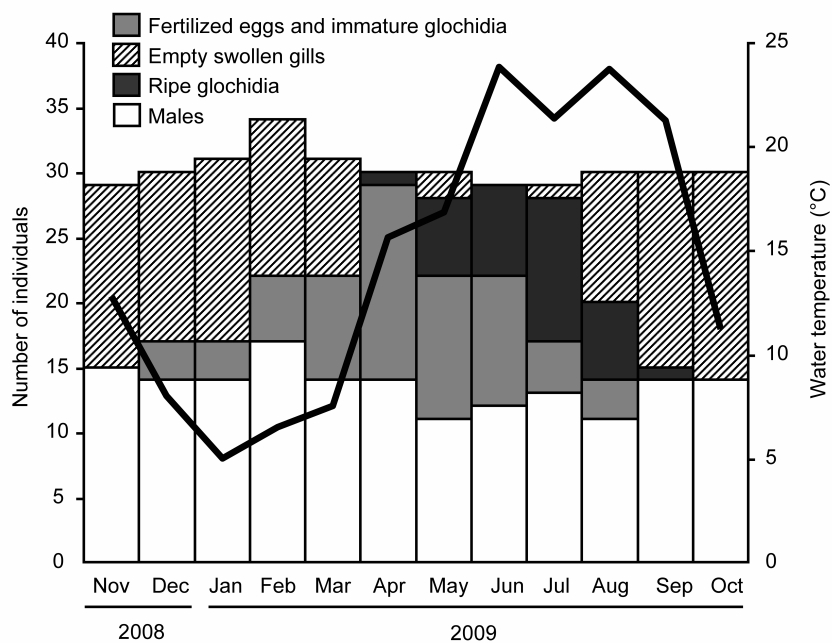
Fig. 4.2 Seasonal dynamics in sex ratio and glochidia maturation in *A. woodiana* population from the Kyjovka River in 2008 – 2009, with total numbers of mussels in particular categories. Water temperature recorded at time of mussel collection is indicated by a solid line

50

Principal Conclusions of the Thesis

The increasing knowledge of the processes that govern the distribution and dynamics of freshwater biodiversity has enabled functional conservation strategies to be established for many threatened species. Nevertheless, there still exist very few examples of successful restoration programs for freshwater invertebrate species, a group that has recently suffered the threat of a disproportionate loss of biodiversity worldwide. Surprisingly, even the conservation of the large Central European macroinvertebrate species, the centre of attention for many freshwater biologists for decades, is still insufficiently supported by appropriate knowledge of their biology and of the environmental relationships of these species. The studies presented in this thesis demonstrate the need for the cautious selection of approaches to be used for the identification of the factors affecting the viability and persistence of threatened species. Specifically, there is a need for studies that directly address the causal mechanisms through which environmental pressure operates. Moreover, indirect methods that build species-environment models based on species occurrence data may be very useful if appropriate scales are used in the study. An equally important, if often-overlooked aspect of biodiversity conservation is the issue of resource availability for threatened species. Integrative assessment of both resource quality and availability should be used to identify possible effects on species persistence. Finally, the recent onset of global biotic homogenisation is creating novel interactions between many native species and alien species. The ability of invasive species to spread and the potential threats posed by invasive species should be examined as soon as possible to allow for the protection of local native biodiversity through appropriate prevention, control and eradication measures.

English Summary

This dissertation addresses the environmental biology and conservation of stream macroinvertebrate species. It focuses on two flagship groups: large freshwater mollusks of the family Unionidae and crayfish. These organisms may serve as surrogate species for the protection of the freshwater environment. Various approaches were used for the identification of threats resulting from both the impairment of abiotic environmental conditions (water pollution) and threats resulting from biotic interactions (host availability). Specifically, this thesis provides novel information on the following topics: (1) pathways of causality in the effects of nitrate pollution on freshwater bivalves; (2) water quality issues in the protection of freshwater crayfish; (3) the detection of host limitation in unionid bivalves; and (4) the role of host specificity in the spread of an invasive freshwater bivalve. Collectively, the thesis demonstrates the need for the use of more thorough approaches for the identification of threats that limit the viability and persistence of the endangered freshwater fauna.

Czech Summary

Disertační práce se zabývá vlivem faktorů prostření na vybrané skupiny vodních bezobratlých z hlediska jejich ochrany. Zaměřuje se na skupiny tzv. vlajkových druhů vodních bezobratlých - velké mlže čeledi Unionidae a raky, které mohou být považovány za zaštiťující druhy pro ochranu sladkovodního prostředí. Práce využívá různé přístupy pro identifikaci hrozeb pramenících z narušení abiotických podmínek prostředí (znečištění vody) i biologických interakcí (dostupnost hostitelských druhů). Práce poskytuje nové informace v oblastech: (1) otázka kauzality v působení dusičnanů na sladkovodní mlže; (2) problematika jakosti vody pro ochranu sladkovodních raků; (3) detekce limitace velkých mlžů prostřednictvím nedostupnosti hostitelů; (4) význam hostitelské specificity pro šíření invazního druhu mlže. Práce ukazuje na potřebu využití důkladnějších přístupů pro identifikaci vlivů, které limitují životaschopnost a přežití ohrožené sladkovodní fauny.

List of publications including submitted papers:

Peer reviewed journals with IF

o Douda, J., Čejková, A., **Douda, K.**, Kochánková, J. (2009): Development of alder carr after the abandonment of wet grasslands during the last 70 years. *Annals of forest science* 66, 712. (IF 2009: 1.441)

o **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. (IF 2010: 1.968)

o **Douda, K.**, M. Vrtílek, O. Slavík, and M. Reichard (in press 2011). The role of host specificity in explaining the invasion succes of the freshwater musel *Anodonta woodiana* in Europe. *Biological Invasions*, DOI: 10.1007/s10530-011-9989-7. (IF 2010: 3.474)

o Tichá, K., Simon, O.P., **Douda, K.**, Kubíková, L. (2011 accepted). Detrital components in submontane organogenic springs in relation to their morphology, microhabitats and macroinvertebrates. Polish Journal of Ecology. (IF 2010 = 0.542)

o **Douda, K.**, Horký, P., Bílý, M.: Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. (September 2011, submitted in *Animal Conservation*)

o Svobodová, J., **Douda, K.**, Štambergová, M, Picek, J., Vlach, P., Fischer, D.: Water quality requirements of native and invasive crayfish in streams in the Czech Republic. (October 2011, submited to *Aquatic Conservation: Marine and Freshwater Ecosystems*)

o Reichard, M., Vrtílek, M., **Douda, K.**, Smith, C. An invasive species reverses the roles in a host-parasite relationship. (October 2011, submitted to *PNAS*)

Peer reviewed journals without IF

o **Douda, K.** (2007): The occurrence and growth of *Unio crassus* (Mollusca: Bivalvia: Unionidae) in Lužnice River basin in respect to water quality. *Acta Universitatis Carolinae, Environmentalica* 21: 57-63.

o **Douda, K.**, Hronek, J., Beran, L., Bílý, M. Vodní měkkýši Vltavy v Praze – habitatové nároky a populační dynamika v prostředí silně modifikovaného vodního toku. (january 2011, submitted to *Bohemia Centralis*)

o Svobodová, J., **Douda, K.**, Vlach, P. (2009): Souvislost mezi výskytem raků a jakostí vody v České republice. *Bulletin VÚRH Vodňany* 45. s. 100-109.

o Svobodová, J., Štambergová, M., Vlach, P., Picek, J., **Douda, K.**, Beránková, M. (2008): Vliv jakosti vody na populace raků v České republice – porovnání s legislativou ČR. *VTEI, příloha Vodního hospodářství* 12, roč. 50 (6): 1-5.

Popular and technical papers:

o **Douda, K.**, Beran, L.(2009): Ochrana velevruba tupého v České republice. *Ochrana přírody* 2009/2. s. 16-19.

o Beran, L., **Douda, K.** (2009): Bečva - nejvýznamnější moravská lokalita velevruba tupého. *Ochrana přírody* 2. s. 19-21.

o **Douda, K.** (2010): Příběh velevruba tupého z pošumavských potoků a řek. Šumava 3/2010.

o VÚV T.G.M., v.v.i.. Detritový kontinuální vzorkovač pro proudící vody tlakový. Původce vzoru: Simon, O., **Douda, K.**, Fricová, K., Kubíková, L.. Int. 20173. ÚPV ČR. 23.8.2009

o VÚV T.G.M.. užitný vzor PUV 18924 -2008 Kontinuální vzorkovač DDG. Původce vzoru: Simon, O., **Douda, K.**. Int. 18924/2008. ÚPV ČR. 29.5.2008

Abstracts and conference proceedings

o **Douda, K.**, Horký, P., Bílý, M. (2011): Threats to the endangered freshwater mussel *Unio crassus* caused by the impairment of its host fish resources in the Czech Republic. 6TH CONGRESS OF THE EUROPEAN MALACOLOGICAL SOCIETIES. Vitoria-Gasteiz. Portugal.(**First prize for the best oral communication**)

o **Douda, K.**, Vrtílek, M., Slavík, O., Reichard, M. (2011): Chinese pond mussel (*Anodonta woodiana*) in Europe - generalist strategy of host fish use as a crucial trait driving its invasiveness. 6TH CONGRESS OF THE EUROPEAN MALACOLOGICAL SOCIETIES. Vitoria-Gasteiz. Portugal.

o **Douda, K.** (2009): Water quality criteria for protection of endangered Unionid Bivalves from nitrate nitrogen pollution. 2nd European congress of conservation biology, Prague.

o Fricová, K., Simon, O., **Douda, K.**, Baudišová, D.(2008): Sources and transformation processes of fine particulate organic matter (FPOM) in oligotrophical spring areas (Bohemian Forest, Czech Republic). Proceedings of 5th International Meeting on Plant Litter Processing in Freshwaters, Coimbra, Portugalsko, 23 - 26.7.2008, 64.

o **Douda, K.**, Simon, O., Bílý, M., Vejmelková, J., Spisar, O. (2007): The Influence of Water Quality on the Occurence of Endangered Freshwater Mussels (Unionoida) in Selected Protected Areas of the Czech Republic. In Nakic, Z. Proceedings of Second International Conference on Waters in Protected Areas. Dubrovnik, Croatia, 24.4.2007. Zagreb, Croatia : Croatian Water Pollution Society, 2007, s. 204—207.

o **Douda, K.**, Bílý M. (2010) Hostitelské druhy ryb pro glochidie velevruba tupého (*Unio crassus*) v České republice (předběžné výsledky). Zoodny 2010, Praha.

o Fricová, K., Simon,O., **Douda, K.** (2007) Detritové toky v oligotrofních povodích – metoda kontinuálního vzorkování. In Měkotová, J., Stěrba,O. Říční krajina 5. Olomouc, 17.10.2007. Olomouc : Universita Palackého Olomouc, 2007, s. 59—66. ISBN 978-80-24418-90-2.

o **Douda, K.** (2006): Autekologie druhu Unio crassus (Mollusca: Bivalvia: Unionidae) v povodí řeky Lužnice. In Sacherová, V. Sborník příspěvků 14. konference České limnologické společnosti a Slovenskej limnologickej spoločnosti. Nečtiny, 26.6.2006. Praha : Česká limnologická společnost, 2006, s. 35—35.

o **Douda, K.** (2006): Vliv faktorů prostředí na rozšíření mlžů čeledi Unionidae ve vodních tocích povodí řeky Lužnice. In Bryja, J. & Zukal, J. Zoologické dny Brno 2006. Brno, 9.2.2006. Brno : AV Ústav biologie obratlovců, 2006, s. 31—31.

o **Douda, K.**, Simon, O., Bílý. M., Kladivová, V. (2010) Monitoring of in-stream nitrate levels as a tool in freshwater mussels conservation – interpretation and application. In Šustr,P. ed. Research actualities in Bohemian/Bavarian forest, Srní, Czech Republic, 19.-20. October 2010.

o Fricová, K., Simon, O., **Douda, K.**, Kubíková,L. (2010) Comoposition of detritus in submontane organogenic springs. In Šustr,P. ed. Research actualities in Bohemian/Bavarian forest, Srní, Czech Republic, 19.-20. October 2010.

o Simon, O., Fricová, K., Kubíková, L., Kladivová, V., **Douda, K.**, Dort, B. (2010) Spring complexes in the Bohemian Forest (Blanice catchment) In Šustr,P. ed. Research actualities in Bohemian/Bavarian forest, Srní, Czech Republic, 19.-20. October 2010.