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Defining the ecological niche of the thick-shelled river mussel *Unio crassus* – implications for the conservation of an endangered species

Katharina Bernadette Stöckl

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”The important thing is not to stop questioning. Curiosity has its own reason for existing.”

Albert Einstein

Preface

This study aims at contributing to the development of effective conservation strategies for native European freshwater mussels. Using the example of the endangered thick-shelled river mussel *Unio crassus*, habitat factors as well as potential bottlenecks for freshwater mussels were analyzed in order to define important components of ecological niche of this species. Those abiotic and biotic components are necessary to deduce appropriate and effective conservation measures.

The introduction of this thesis illustrates the global threat of freshwater mussel biodiversity, the ecological function of freshwater mussels in aquatic ecosystems and specific life-history traits of the study species. Following a description of the objectives of this thesis, three distinct research topics dealing with the importance of abiotic and biotic habitat variables for the functionality of mussel populations are presented. Each topic has been published as an autonomous research paper in a slightly modified form (following different journal requirements).

The thesis ends with a general discussion on the importance of autecological information in species conservation, the need for identifying species-specific threats and a critical discussion on the suitability of *U.crassus* as an indicator species. The new findings on the ecology of this species as well as the conclusions drawn from those results are finally used to propose a systematic conservation approach for this endangered mussel species that may be also useful for other freshwater mussel species.

Abstract

Freshwater mussels of the order Unionoidea are among the most threatened biota worldwide. Their life-cycle involves a parasitic larval phase on a suitable host fish before a subsequent metamorphosis into a juvenile stage. This unique life-history trait as well as the sessile adult life make freshwater mussels highly dependent on the prevailing conditions of the surrounding environment. Identification of abiotic and biotic habitat requirements is therefore a crucial prerequisite for establishing appropriate conservation strategies.

The main goal of this thesis was to define important components of the ecological niche of the thick-shelled river mussel *Unio crassus*, a native European freshwater mussel, as a basis to develop a systematic conservation strategy for this species. In field studies, key aspects of habitat quality including flow regime, substrate and water quality and host fish status were analyzed and linked to the functionality of the mussel population, i.e. the natural recruitment of juvenile mussels.

In case study one, flow regimes and substrate characteristics, such as shear stress, flow velocity and penetration resistance were studied in relation to mussel presence/absence data. The findings contradict previous assumptions on the microhabitat use of the species. Areas with high mussel densities were characterized by low flow velocities ($< 0.3\,\mathrm{m\,s^{-1}}$), low mean penetration resistances ($0.36 \pm 0.52\,\mathrm{kg\,cm^{-2}}$) as well as by low near-bed shear stress ($1.06 \pm 0.33 \text{ x}10^{-6}\,\mathrm{N\,cm^{-2}}$) compared with non-colonized sites. Mussel density was significantly negatively correlated with near-bed shear forces. The results suggest that lentic microhabitats are critically important for mobile species such as *U.crassus* that change position for glochidial release because mussels in those habitats have a lower risk of being displaced at high flows.

The following study focused on the identification of potential impairments of juvenile habitat. Physicochemical habitat parameters, including nutrient concentrations, fine sediment deposition and redox potential in the substrate, were measured at sites with recent population recovery and compared with non-colonized sites. The results revealed that *U.crassus* is able to persist and successfully reproduce at sites with elevated nutrient loads and high fine sediment deposition with up to $19.4\,\mathrm{kg\,m^{-2}\,month^{-1}}$, indicating that the species is more tolerant to eutrophication than previously assumed.

The key aspect of the third study was to determine the importance of host fish densities for the functionality of *U.crassus* populations. The status of host fish communities was assessed in nine streams and linked to the recruitment patterns of *U.crassus* populations. Despite a high variability in species composition and

richness, functional streams had significantly higher densities of primary host fish species, i.e. fish species that reveal highest rates of mussel metamorphosis, than non-functional streams.

No relationship was found between the numbers and densities of juvenile mussels and densities of poor hosts as well as of non-hosts. Concluding from this study, host fish limitation is a major threat for *U.crassus* populations and plays an important role in the lack of juvenile recruitment in populations of this species. In conclusion, the case studies revealed that *U.crassus* has a broader habitat spectrum and a broader ecological niche width than previously defined. Host fish limitation was identified as an important bottleneck that limits population recovery. Thus, assessment and management of the co-occurring fish community need to be incorporated into conservation strategies for *U.crassus.* Based on the new findings, a step-wise approach for the conservation of *U.crassus* is proposed. Determination of the status quo, subsequent identification of threats and prioritization of populations represent core elements that should be addressed before conservation action takes place. Following this concept, the next steps in the conservation of *U.crassus* should include the standardization of monitoring guidelines and assessment of populations in large streams, the identification of other bottlenecks, for example on a population genetic level, and the prioritization of populations. This systematic approach may help to make conservation action more effective and sustainable in the future, serving as an example for other freshwater mussel species.

Zusammenfassung

Süßwassermuscheln der Ordnung Unionoida zählen zu den am stärksten gefährdeten Tierarten weltweit. Ihr Lebenszyklus schließt eine parasitäre Larvalphase an einem geeigneten Wirtsfisch ein, an dem eine Metamorphose zum Juvenilstadium stattfindet. Aufgrund des komplexen Lebenszyklus sowie der sessilen Lebensweise im Adultstadium sind Süßwassermuscheln stark von den jeweils vorherrschenden Umweltbedingungen abhängig. Die Identifikation von abiotischen und biotischen Lebensraumansprüchen stellt daher eine Voraussetzung für die Etablierung von geeigneten Schutzstrategien dar.

Das übergeordnete Ziel dieser Arbeit war es, wichtige Komponenten der ökologischen Nische der Kleinen Flussmuschel *Unio crassus* zu definieren, die als Basis zur Entwicklung einer systematischen Schutzstrategie für diese Art dienen. In mehreren Feldexperimenten wurden Schlüsselkomponenten der Habitatqualität analysiert – einschließlich der hydrologischen Bedingungen, der Substrat- und Wasserqualität sowie des Wirtsfischstatus – und anschließend mit der Funktionalität von Populationen verknüpft. In der ersten Studie wurden verschiedene Variablen zur Beschreibung der Strömungsverhältnisse und spezifischer Substrateigenschaften in Abhängigkeit von dem Vorkommen von Muscheln analysiert. Die Ergebnisse widersprechen bisherigen Annahmen zur Eignung bestimmter Mikrohabitate für *U.crassus*. Bereiche mit den höchsten Besiedelungsdichten wiesen geringe Fließgeschwindigkeiten ($< 0.3\,\mathrm{m\,s^{-1}}$), geringe Penetrationswiderstände (0.36 ± 0.52 kg $\mathrm{cm^{-2}}$) als auch eine geringe Sohlschubspannung in Bodennhähe (1.06 ± 0.33 x10^{-6} $\mathrm{N\,cm^{-2}}$) auf. Die Muscheldichte war signifikant negativ mit der Sohlschubspannung korreliert. Die Ergebnisse der Studie lassen darauf schließen, dass strömungsberuhigte Bereiche für mobile Muschelarten wie *U.crassus* von hoher Wichtigkeit sind, da dort die Gefahr der Verdriftung während der Phase der Glochidienabgabe geringer ist.

Die zweite Studie konzentrierte sich auf die Identifikation von potentiellen Beeinträchtigungen des Juvenilhabitats. Physikochemische Habitatparameter, wie die Nährstoffkonzentrationen in der freien Welle und die Feinsedimentdeposition im Substrat, wurden zwischen funktionalen und nicht besiedelten Bereichen verglichen. Die Ergebnisse zeigten, dass *U.crassus* in Bereichen überleben und sich erfolgreich reproduzieren kann, die hohe Nährstoffkonzentrationen und eine hohe Feinsedimentdeposition mit bis zu $19.4\,\mathrm{kg\,m^{-2}\,Monat^{-1}}$ aufweisen. Die Art ist demnach toleranter gegenüber Eutrophierung als bisher angenommen.

Den Schlüsselaspekt der dritten Studie stellte die Untersuchung der Bedeutung von Wirtsfischdichten für die Funktionalität von *U.crassus* Populationen dar. Der

Status der Wirtsfischgemeinschaft wurde in neun Gewässern untersucht und in Abhängigkeit von dem Aufkommen von Jungmuscheln in den Populationen analysiert.

Trotz einer insgesamt hohen Variabilität hinsichtlich der Fischartenzusammensetzung und der Anzahl an Fischarten wurde in funktionalen Gewässern eine signifikant höhere Dichte an primären Wirtsfischen– nachgewiesen als in nicht funktionalen Gewässern. Dagegen wurde kein signifikanter Zusammenhang zwischen der Dichte von weniger geeigneten und ungeeigneten Wirtsfischarten und der Anzahl und Dichte der Juvenilmuscheln festgestellt. Somit spielen geringe Wirtsfischdichten eine wesentliche Rolle für das geringe Aufkommen von Jungmuscheln in *U.crassus* Populationen.

Schlussfolgernd zeigten die Fallstudien dieser Arbeit, dass *U.crassus* ein breiteres Habitatspektrum besiedeln kann und somit eine größere ökologische Nische besetzt als bisher angenommen. Ebenso konnte mit dem Mangel an geeigneten Wirtsfischen ein Faktor identifiziert werden, der den Erhalt und die Erholung von Populationen erheblich einschränkt. Die Bewertung und das Management der Fischartengemeinschaft in den Muschelgewässern muss demnach einen höheren Stellenwert bei der Entwicklung von Schutzstrategien für *U.crassus* erhalten.

Basierend auf den neuen Erkenntnissen wird ein systematischer Ansatz für den Schutz von *U.crassus* vorgeschlagen. Die Erfassung des Status quo, die Identifizierung möglicher Gefährdungsfaktoren sowie eine Priorisierung der Populationen stellen dabei Kernelemente dar, die vor Umsetzung von Schutzmaßnahmen adressiert werden sollten. Die nächsten Schritte für die Etablierung einer Schutzstrategie sollten folgende Elemente mit einschließen: die Standardisierung von Bewertungsverfahren sowie die Erfassung von Populationen in großen Fließgewässern, die Identifizierung von weiteren Gefährdungsfaktoren, zum Beispiel auf Ebene der genetischen Struktur von Populationen, sowie die Priorisierung von Populationen nach definierten Kriterien. Dieser systematische Ansatz soll dabei helfen, die Bestände von *U.crassus* zukünftig effektiver und nachhaltiger zu schützen, und dient als Beispiel für die Entwicklung von Schutzstrategien für andere Süßwassermuscheln.

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1 Introduction

1.1 Global decline of freshwater mussel biodiversity

Freshwater ecosystems have undergone severe changes in the last decades. As a result of cumulative effects of overexploitation, flow modification, water pollution, degradation of habitats and introduction of invasive species, dramatic population declines and a decrease of distribution areas have been observed for freshwater biodiversity worldwide (Dudgeon et al., 2006). Freshwater organisms thus are considered one of the most threatened biota (Ricciardi and Rassmussen, 1999), with an estimate of 10.000 up to 20.000 freshwater species already being extinct (Vörösmarty, 2010), resulting from human activities (Strayer, 2006).

Among all taxonomic groups living in freshwater, freshwater mussels of the Order Unionoida are probably one the most imperiled organisms (Bogan, 1993; Lydeard et al., 2004, Strayer, 2004; Neves et al., 2007) that show high extinction rates with mean species losses up to 4 % per decade (Geist, 2010).

Due to their relatively low mobility compared to other freshwater taxa, ranging from migration rates of less than one to a maximum of 100 m per year (Gum et al., 2014; Allen and Vaughn, 2009) as well as due to their complex life cycle including a host fish phase, freshwater mussels are particularly susceptible to any kind of habitat disruption or degradation. Since mussels feature a late reproductive maturity from 6 (Hochwald, 1997, Vaughn, 2010) to 20 years (Geist, 2010) and long life spans ranging from 10 to 400 years (Geist, 2010), they cannot recover in short periods of time from habitat disruptions through reproduction and growth.

It is widely recognized that significant declines of unionids will result in important alterations of aquatic ecosystems, given their important role in ecosystem functioning (Vaughn and Hakenkamp, 2001; Howard and Cuffey, 2006). For example, mussels substantially affect nutrient cycling by filter–feeding on seston and by excretion and deposition of faeces or pseudofaeces, thus having both top down as well as bottom up effects (Atkinson et al., 2013; Allen et al., 2012; Vaughn et al., 2008). Declines in mussel biodiversity will also affect functional redundancy and thus reduce the resilience of aquatic ecosystems (Vaughn, 2010). Due to their important functions in aquatic ecosystems, implementation of appropriate conservation measures is urgently needed to maintain the remaining populations.

1.2 The importance of defining the ecological niche for the conservation of freshwater mussels

Development of effective conservation strategies requires fundamental knowledge on the biology as well as on the ecology of the target species (Lee and Jetz, 2011). For example, for successful restoration of suitable habitats, tolerance ranges of the target freshwater mussel species for abiotic and biotic factors need to be known. The total requirements of a species for certain environmental conditions determine its distribution and abundance, which can be referred to as "ecological niche". Following the definition of Hutchinson (1957), the ecological niche is a n-dimensional hypervolume in a multi-dimensional space of environmental variables in which a species is able to maintain a viable population. In this context, the fundamental niche defines the complete range of environmental conditions and resources a species can possibly occupy and use in the absence of competition from other species. The realized niche represents the set of environmental conditions that are actually used by the species after limiting factors, especially interaction with other species, have been taken into account. Concerning freshwater mussels, abiotic variables such as water chemistry and temperature, substrate composition and texture as well as hydological conditions may be important components in determining the fundamental niche of a mussel species. Distribution and availability of suitable host fish may influence the realized niche width of a mussel species. Despite an increasing number of research devoted to freshwater mussel ecology in the last years and a comprehensive number of published literature (Lopes-Lima et al., 2014), information on habitat requirements is still incomplete for the majority of mussel species. For example, in the thick-shelled river mussel *Unio crassus*, an endangered European mussel species listed in Annexes II and IV of the Habitats Directive (Council of the European Communities, 1992), there is a mismatch between the habitats that are occupied by the species and the habitat needs described in currently available literature. Defining the ecological niche of this species therefore appears to be necessary to delineate its habitat requirements.

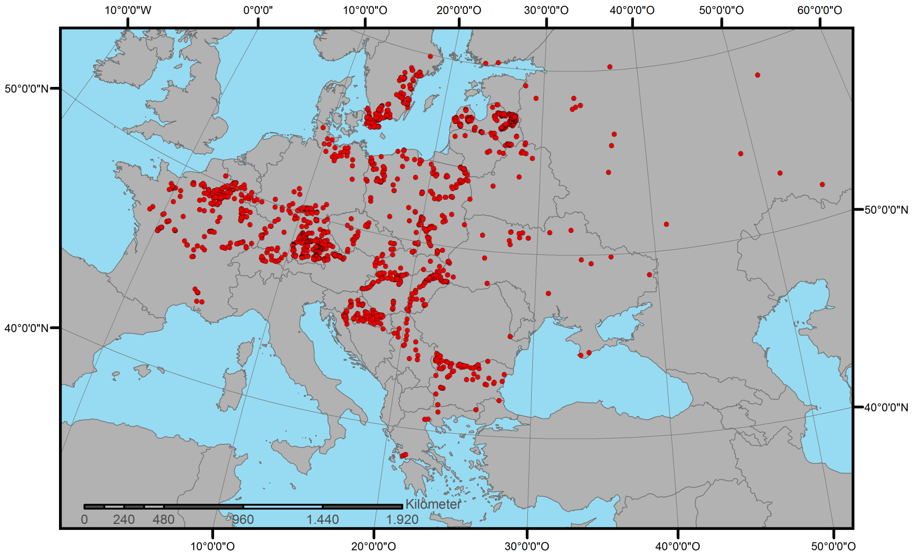

Figure 1.1: Current distribution of *U.crassus* in Europe.(Geographical data obtained from naturalearthdata.com, distribution data provided by Manuel Lima, Coordinator of the IUCN Red List Authority on Freshwater Bivalves within the Mollusk Specialist Group; Map created with ArcGIS 2010, ESRI, USA)

1.3 Current knowledge on the autecology of the thick-shelled river mussel *Unio crassus*

The thick-shelled river mussel *Unio crassus* is a widespread unionid species in Europe. Its natural distribution area covers central, south-eastern and northern Europe and ranges from France in the west to Russia in the east (see Figure 1.1).

Species identification based on shell characteristics is challenging in *U.crassus*, because shell morphology is variable within and between streams (Hochwald, 2001) as well as between different regions (Nesemann, 1994; Glöer et al., 2005). Modern taxonomic research additionally uses molecular data for species identification and only recently, changes in the taxonomy of the Unio genus were made. For example, Iberian populations of *U.crassus* now have been recognized as a distinct endemic species called *Unio tumidiformis* Castro 1885 (Reis and Araujo, 2009).

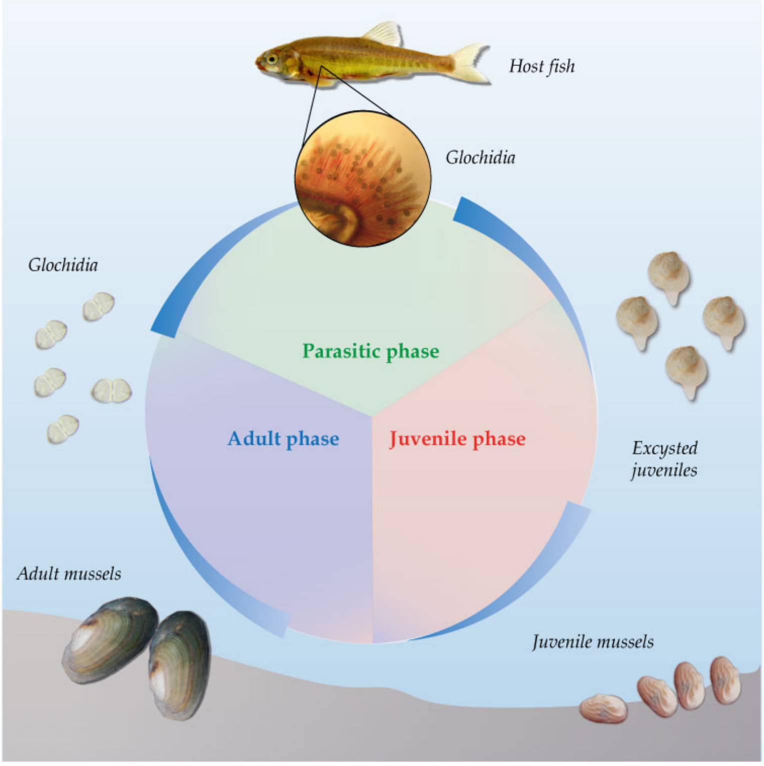

Figure 1.2: Life cycle of the thick-shelled river mussel *Unio crassus*. As in all species of the Unionoidea, the life cycle of *U.crassus* involves a parasitic stage on a suitable host fish (Parasitic phase). Upon completion of metamorphosis, juvenile mussels drop off from the host fish and live completely buried for up to three years in the streambed (Juvenile phase). Adult female and female mussels live as filter feeders on the substrate surface (Adult phase).

Ongoing taxonomic work may further divide the crassus lineage into several species and consequently, the distribution range of may need to be adapted accordingly in the near future.

As for all species of the Unionoida, the life cycle of *U.crassus* is closely linked to the associated fish community, because it involves a parasitic life stage on a suitable host fish. The species is strictly dioecious (Bauer, 2001), i.e. males release sperm into the water, which are taken up by females. During that time, mussels show increased horizontal movements (Schwalb and Pusch, 2007), probably bringing animals of the opposite sex closer together (Amyot and Downing, 1998) and thus increasing the fertilization success.

Eggs are fertilized in the suprabranchial chambers of the females and released upon completion of glochidia development to viable glochidia. Resulting from an adaption to the parasitic life style (Watters, 2001), Unionidae exhibit high fecundity/fertility rates, e.g. *Unio crassus* produces up to 100.000 glochidia per reproductive season.

High variations of larval production are assumed to result from different levels of fitness of each individual rather than by size/age relations (Bauer, 1998). The proportion of undeveloped eggs in gravid females is at least 10 % both at high and low mussel densities and might be the result of natural failure rates during egg development or low sperm concentrations (Hochwald and Bauer, 1990). Spawning is reported to occur asynchronously and multiple times during the reproductive season (Hochwald, 2001), extending the time span for larvae to infest potential host fish. Gravid females then crawl to the shoreline, expose the excurrent aperture and release glochidia by spurting jets of water into the center of the water course. This unusual behavior has been known for a long time (Israel, 1913; Mentzen, 1926), yet it was only recently that Vicentini (2004) showed that ejected water contains glochidia; it is assumed that the water jet attracts potential host fish and enhances the sinking time of glochidia in the water column, thus increasing the probability infecting host fish and mussel dispersal (Vicentini, 2004).

Several fish species serve as suitable hosts for *U.crassus*, with chub (*Squalius cephalus*), European minnow (*Phoxinus phoxinus*) and stickleback (*Gasterosteus aculeatus*) serving as primary hosts in the upper Danube drainage (Taeubert et al. 2012 a, b); in other regions of the mussels' range different fish species may be the most important hosts; for example in Eastern Europe, *P.phoxinus*, common rudd (*Scardinius erythrophthalmus*) European bullhead (*Cottus gobio*) are considered the primary hosts of *U.crassus.* Since temperature affects metabolism of poikilothermic animals, duration of the host fish phase as well as metamorphosis success of the mussels is also strongly influenced by this parameter: Taeubert et al.(2013) found highest metamorphosis rates as well as lowest host mortalities at 17 °C. Duration of mussel development is negatively correlated to water temperature and excystment of living juvenile mussels was reported to occur between 2 and 5 weeks post infection. After metamorphosis, juvenile mussels excyst from the host and bury themselves into the river-bed substratum, where they live up to 3 years as pedal feeders (Hochwald, 2001) until they appear at the substrate surface and live as filter-feeding adults.

Until the middle of the last century, *U.crassus* was considered the most abundant unionid species in Central Europe, reaching high densities up to several hundred individuals per square metre of stream bed (Tudorancea and Gruia, 1968). The species typically occurs in lotic environments (Lopes-Lima et al., 2016), including brooks, headwater streams (Denic et al., 2014) as well as large rivers (Bodis et al., 2015). Within the last 70 years however, *U.crassus* experienced drastic population losses throughout its natural range, with an estimated decline of up to 90 % in the most industrialized and highly populated areas in Europe (Lopes-Lima et al., 2014). Today, *U.crassus* is considered endangered and even prone to extinction in several European countries. *U.crassus* is also described as endangered in the IUCN Red List of threatened species (Van Damme, 2011), and is included in Annex II and Annex IV of the European Habitats Directive (Council of the European Communities, 1992).

In the early 1990s, when ecologists became aware of those significant population declines, a search for causes began and several fundamental studies on the biology and ecology of *U.crassus* were conducted (Engel, 1990, Hochwald and Bauer, 1990, Bauer et al., 1991, Hochwald, 1997, Hochwald, 2001). The results of those studies suggested that *U.crassus* a) requires high water quality similar to the freshwater pearl mussel *Margaritifera margaritifera* and occurs in oligotrophic streams (Hochwald, 1990) b) prefers streams or areas with moderate to high flow velocity (Zettler, 1996; Hus et al., 2006) c) is particularly sensitive to eutrophication (Hochwald, 1997, Zettler and Jueg, 2007) and fine sediments (Buddensiek, 1993; Zettler, 1996). Because of its proposed high sensitivity to water pollution, *U.crassus* was added to the list of indicator species, being indicative for oligosaprobic streams. Furthermore, it was proposed that the rapid decline of populations resulted, like in *M.margaritifera* populations, from a lack of juvenile recruitment, leading to superannuated populations (Hochwald, 1990).

In recent years, information about the occurrence and the status of *U.crassus* populations significantly improved in Europe, mainly as a result of new legal frameworks. For example, with the implementation of the European Habitats Directive in 1992, EU member states have to monitor protected species in a 6-year interval. Furthermore, revision of national laws, e.g. in Germany, has led to increased protection of the environment, which also includes monitoring work. This development contributed to the discovery of new, previously unknown *U.crassus* populations, partly in highly degraded streams. Furthermore, populations that had been predicted to go extinct by the end of the 20th century (Hochwald, 1990), could be successfully recovered. At the same time, results of more recent studies are in marked contrast to the previous findings on *U.crassus* habitat requirements. For example, Lewin (2014) detected juvenile recruitment of *U.crassus* in a highly eutrophic stream in Poland, that had high nitrate concentrations of up to $87{,}71\,\mathrm{mg\,dm^{-3}}$ NO_3^-. In an ecotoxicological experiment, Douda et al. (2010) showed that juvenile *U.crassus* were less sensitive to nitrate than most freshwater macroinvertebrates.

1.4 Objectives

Due to the high imperilment of this freshwater mussel species, the implementation of appropriate conservation measures is urgent to be able to successfully protect the remaining populations and its natural habitats. One of the main prerequisites for an effective conservation strategy is to integrate knowledge of the ecological information of the endangered species (Geist, 2011). Because of the striking discrepancy between currently established knowledge of the ecology of *Unio crassus* and the more recent contradicting results and observations, a validation of the habitat requirements of *U.crassus* appears to be necessary.

In a systematic approach, this thesis aimed at analyzing the most crucial factors for functional mussel populations, including abiotic as well as biotic characteristics of habitat. In detail, the main objectives of this thesis were:

1. Analysis and validation of current assumptions on hydrological and substrate preferences of *U.crassus*
2. Assessment of physicochemical conditions in functional *U.crassus* habitats
3. Quantitative analysis of associated fish communities and host fish densities in *U.crassus* streams

2 Hydrological and substrate requirements of the thick-shelled river mussel *Unio crassus* (Philipsson 1788)

A similar version of this chapter was published: Stoeckl K, Geist J. 2016. Hydrological and Substrate Requirements of the Thick-Shelled River mussel *Unio crassus* (Philipsson 1788). Aquatic Conservation: Marine and Freshwater Ecosystems. Published online DOI:10/1002/aqc2598

2.1 Abstract

Conservation and restoration strategies require knowledge on the autecology of target species. The thick-shelled river mussel *Unio crassus* is an endangered freshwater bivalve with largely unknown habitat preferences. In this study, hydrological and substrate parameters including shear stress, flow velocity and penetration resistance of substrate were examined in relation to mussel presence/absence data in six streams with recruiting and self-sustaining populations to derive information on the species' hydrological and substrate habitat requirements. Mean flow velocities between 0.11 and $0.31\,\mathrm{m\,s^{-1}}$ and absolute maximum values in the study streams ranging from 0.22 to $0.95\,\mathrm{m\,s^{-1}}$ indicate that the species is tolerant of a wide range of hydrological conditions. Areas with high mussel densities were characterized by low flow velocities ($< 0.3\,\mathrm{m\,s^{-1}}$), low mean penetration resistances ($0.36 \pm 0.52\,\mathrm{kg\ cm^{-2}}$) as well as by low near-bed shear stress ($1.06 \pm 0.33\,\mathrm{x}\,10^{-6}\,\mathrm{N\,cm^{-2}}$) compared with non-colonized sites. Maximum near-bed flow velocity at mussel beds was $0.33\,\mathrm{m\,s^{-1}}$ and $0.35\,\mathrm{m\,s^{-1}}$ at 60 % depth. Higher flows with maximum velocities of $0.52\,\mathrm{m\,s^{-1}}$ and $0.95\,\mathrm{m\,s^{-1}}$ at 60 % depth were measured at non-colonized sites. Mussel density was significantly negatively correlated with near-bed shear forces. The results indicate that the hydrological and substrate characteristics of *U.crassus* habitat are broader than expected. In contrast to the assumption that it depends on moderate to high flows ($> 0.3\,\mathrm{m\,s^{-1}}$), streams with low water flow and soft substrate were also identified as suitable habitats. Functional characteristics of the substrate, especially stability and areas with low shear stress

($<1.5\ \mathrm{x}^{-6}\,\mathrm{N\,cm^{-2}}$), seem to be of great importance for the persistence of the species, and monitoring protocols used at present for habitat assessment of *U.crassus* should be updated.

2.2 Introduction

Diversity and abundance of freshwater mussels severely declined worldwide over the past century (Bogan, 1993; Lydeard et al., 2004). As a result of human impacts that lead to degradation, alteration and fragmentation of mussel habitats (Williams et al., 1993; Vaughn and Taylor, 1999), many freshwater mussel species are at present threatened or close to extinction, and most populations are listed as vulnerable or endangered. Direct threats such as harvesting, dredging and predation by alien invasive species such as crayfish (Strayer, 1999 a; Klocker and Strayer, 2004) or muskrats (Neves and Odom, 1989; Zahner-Meike and Hanson, 2001) also affect mussel populations at a local scale. A lack or decline of host fish populations (Watters, 1996; Douda et al., 2012, Taeubert et al., 2012, Stoeckl et al., 2015) and a series of additional interferences with the chemistry, biology, hydrology and geomorphology of streams as well as alterations in catchments (Moorkens, 2010) may also have contributed to the present imperilment of many freshwater mussel species (Geist, 2010).

The thick-shelled river mussel *Unio crassus* formerly was a widespread and highly abundant unionid species in Central Europe. Under favourable conditions, this stream-dwelling mussel species occurred in high densities up to several hundred individuals per square metre of stream bed (Tudorancea and Gruia, 1968; Zettler and Jueg, 2007). Today, *U.crassus* is considered endangered and even prone to extinction in several European countries, including Germany.

As a result of drastic population losses throughout its natural range, the species has now become a priority for conservation: *U.crassus* is described as endangered in the IUCN Red List of threatened species (Van Damme, 2011), as well as being included in Annex II and Annex IV of the European Habitats Directive (Council of the European Communities, 1992). The aim of the Habitats Directive is to protect species and habitats of European interest, following particular criteria given in the Directive. For example, species on Annex II require designation of Special

Areas of Conservation, whereas Annex IV lists species in need of strict protection. The Member States are required to report the species' conservation status every six years based on assessments of mussel population and habitat surveys.

One of the main prerequisites for an adequate assessment of habitat quality is a profound understanding of the autecological requirements of each species. Although the specific habitat requirements are well studied for many other target species, habitat requirements of *U.crassus* are still largely unknown. For example, a recent study found that the species is more tolerant of adverse habitat conditions and high fine sediment deposition (Denic et al., 2014) than previously postulated: juvenile recruitment occurred in a stream with high loads of fine sediment and nutrients, exceeding the previously proposed threshold value for nitrate concentrations (Hochwald, 2001; Zettler and Jueg, 2007; Douda et al., 2012). Another key knowledge gap is the species' requirements concerning hydrological conditions, which remains controversial in national conservation practice (Pfeiffer and Nagel, 2010).

Following the German assessment protocol for *U.crassus* (Planungsbuero fuer angewandten Naturschutz GmbH and Institut fuer Landschaftsoekologie, AG Biozoenologie, 2010), evaluation of habitat quality also includes analyses of the flow regime. According to the protocol, habitats in favourable condition for *U.crassus* (category A) must have high flow velocities between 0.3–1 $\mathrm{m\,s^{-1}}$, whereas lower flow velocities automatically result in a classification of the habitat as 'poor' (category B, 0.1–0.3 $\mathrm{m\,s^{-1}}$) or 'bad' (category C, $< 0.1\,\mathrm{m\,s^{-1}}$). However, despite the wide use of this assessment method, no further details are given on the methodological procedure for measuring of flow velocities in *U.crassus* streams, such as the sampling position in the stream or in the water column. Moreover, the validity of the assumptions concerning the flow requirements of *U.crassus* remains to be tested. In other European countries hosting *U.crassus* populations, evaluating the hydrological conditions of the habitat is not a compulsory part of the monitoring program (Bergengren et al., 2012).

Hardizon and Layzor (2001) suggested that the hydrological conditions most relevant to the distribution of freshwater mussels are probably those that characterize flows near the stream bed where the mussels live. Therefore, Moorkens and Killeen (2014) measured near-bed velocities as the most ecologically relevant for the stream-dwelling freshwater pearl mussel Margaritifera margartifera, in addition to the usual velocity measurements taken at 60 % depth from the water surface. Results of recent mussel population surveys in Germany indicate that many streams with functional *U.crassus* populations – i.e. self-sustaining and recruiting populations–do not fall within the current classification system of the German survey protocol. For example, it is known that *U.crassus* often occurs in areas near the bank (Pfeiffer and Nagel, 2010), where flow velocities are lower than in the middle of a stream.

To our knowledge, there have been no scientific studies investigating the hydrological requirements of *U.crassus* including flow velocities or near-bed shear stress. Thus, baseline data for an adequate assessment of *U.crassus* habitats with regard to the optimum flow conditions are still missing to aid the conservation and restoration of habitats for this species. This study is the first step towards a better understanding of flow regimes in functional *U.crassus* habitats. The results constitute an initial contribution to defining of the species' hydrological and substrate requirements, to the validation of evaluation criteria of the survey protocol for *U.crassus* habitats, and to developing recommendations concerning habitat requirements of *U.crassus*.

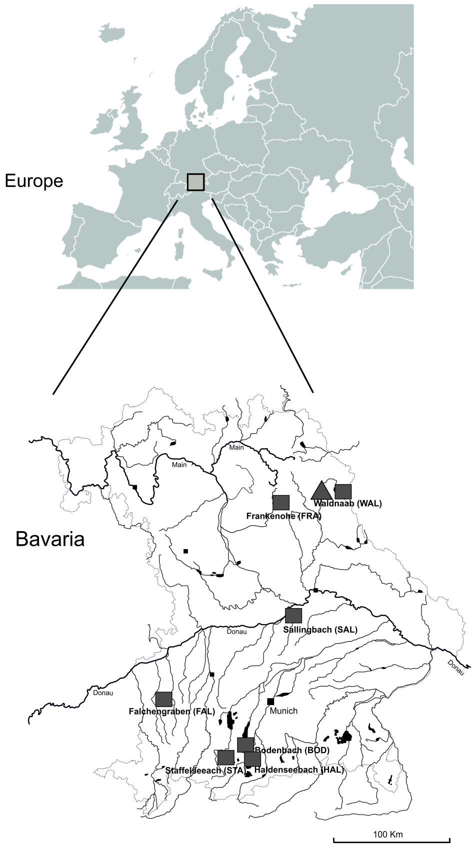

Figure 2.1: Map and names of study streams with *U.crassus* populations (quadrats) and the *M. margaritifera* population (triangle). In the study stream Waldnaab, both mussel species co-occur as indicated by quadrat and triangle.

2.3 Material and Methods

2.3.1 Study streams

The study was carried out at six streams in southern Germany containing self–sustaining *U.crassus* populations. All streams investigated belong to the upper Danube drainage system (Figure 2.1). In addition, physical and hydrological conditions were assessed in one of the few streams where *U.crassus* co-occurs with the freshwater pearl mussel *M.margaritifera*, a species in which the flow requirements have previously been described by Moorkens and Killeen (2014). This population, comprising more than 10,000 individuals, is the largest freshwater pearl mussel population in the state of Bavaria. For the representativeness of the study, streams of different size were selected: their average widths ranged between 1.0 and 9.5 m and the total lengths varied between 1.1 and 17.0 km (Table 2.1). The catchment areas of all streams are characterized by agricultural land-use, forests and settlements. The streams represent typical habitats of *U.crassus* in southern Bavaria, including small brooks and medium-sized upland streams. Sampling was carried out between July and September 2014, which falls within the optimum monitoring period for *U.crassus* (May-October).

2.3.2 Sampling design

The selection of sampling sites for quadrat sampling was based on a combination of a systematic and random sampling strategy. A sampling quadrat (area of quadrat: 50 x 50 cm) was placed on to the stream bed from downstream to upstream at intervals of 1 in 10 in the main distribution area of the mussel population. Depending on the presence or absence of mussels, the quadrat was classified a posteriori as ‘colonized’ (UC = colonized with *U.crassus*, MC = colonized with *M.margaritifera*) or ‘not-colonized’ (UNC = quadrat in *U.crassus* stream without mussels, MNC = quadrat in *M.margaritifera* stream without mussels). Where the desired total number of UC, MC (n = 5) and UNC, MNC (n = 5) sites was not reached at the end of each study area, sampling continued at additional transects within a lower frequency of 1:20 to each other until a sufficiently large number of replicates was obtained. In small streams with a width of $\leq$ 1m, the quadrat was placed in the middle of the stream. For the other study streams, one quadrat was positioned near to the bank as well as in the middle of the stream at each sampling site, alternating consistently between true left, true right banks and the stream centre. Sampling was conducted in the main distribution area of *U.crassus*. Thus, the total sampling area differed between streams and varied between lengths of 100 and 500 m. (Falchengraben and Bodenbach: 100 m, Haldenseebach: 200 m, Frankenohe: 350 m, Sallingbach: 400 m, Staffelseeach: 500 m).

Table 2.1: Study streams. Calculation of Minimum water discharge NQ $m^3\ s^{-1}$) , Mean low water discharge MNQ ($m^3\ s^{-1}$), Mean annual discharge MQ ($m^3\ s^{-1}$), Mean flood discharge MHQ ($m^3\ s^{-1}$) and maximum discharge HQ ($m^3\ s^{-1}$) is based on measurements performed by the state water management authorities over the last 30 years (data available online at www.hnd.de). Due to their small tributaries, measuring points for three streams (Falchengraben, Haldenseebach, Sallingbach) only exist further downstream in the main stem. In this case, discharge on the sampling day was calculated using the Gauckler-Manning-Strickler Formula. NQ, MNQ, MQ, MHQ and HQ were calculated in relation to the data from the main stem.

Code	Stream	Sub-Drainage	Mean width [m]	Total length [km]	Minimum water discharge NQ [$m^3\ s^{-1}$]	Mean low water discharge MNQ [$m^3\ s^{-1}$]	Mean annual discharge MQ [$m^3\ s^{-1}$]	Mean flood discharge MHQ [$m^3\ s^{-1}$]	Maximum water discharge HQ [$m^3\ s^{-1}$]	Discharge during sampling date [$m^3\ s^{-1}$]	*U.crassus* population size [n]	Max. Mussel density [$\mathrm{Ind\,m^{-2}}$]
SA	Sallingbach	Abens	2.0 ± 0.5	8.8	0.03	0.05	0.08	0.84	3.12	0.07	2,500	44
FG	Falchengraben	Günz	1.0 ± 0.1	1.5	0.001	0.002	0.005	0.05	0.08	0.004	10,000	164
HA	Haldenseebach	Ammer	1.1 ± 0.5	1.1	0.0001	0.003	0.01	0.14	0.43	0.009	2,000	120
BO	Bodenbach	n.a.	4.7 ± 1.2	1.6	0.11	0.36	1.02	5.27	11	0.75	500	32
ST	Staffelseeach	Ammer	9.3 ± 1.7	15	0.3	0.88	2.7	20.1	51.7	3.5	6,000	24
FR	Frankenohe	Vils	2.4 ± 0.2	17	0.01	0.11	0.33	5.25	10.1	0.25	500	36

2.3.3 Physical parameters

Physical parameters were sampled at low discharge levels since this is the most common time period during which national assessments of mussel populations in wadeable streams are carried out, ensuring comparability of data. In each stream, sampling of physical parameters was done in the same quadrats in which mussels were sampled. Hydrological and stream substrate measurements were made in the centre of each quadrat. Assessment of habitat quality, e.g. in the monitoring programmes for the European Habitats Directive, is usually done at the same time as the mussel population surveys, which are mostly conducted at low discharge levels.

Flow velocity was measured with a hand-held flow meter (HFA, Höntzsch, Waibling, Germany). Measurements of flow velocity were made in the centre of each 0.25 m^2 quadrat. In each plot, flow velocity was measured 2 cm above the stream substrate, since near-bed flow velocity is regarded as ecologically most relevant to freshwater mussels (Moorkens and Killeen, 2014). According to common techniques for measuring flow velocities in hydrological studies, flow velocity was also measured at 60 % depth from the water surface (mid–column), as well as 2 cm below the water surface. At plots with low water levels ($< 5\,\mathrm{cm}$), flow velocities were measured three times in the same water depth within the plot. Near-bed shear stress was measured using Fliesswasserstammtisch (FST) hemispheres following the method developed by Statzner and Müller (1989).

The FST method is considered a suitable tool to analyse the distribution of benthic invertebrates (Borchardt, 1993; Dittrich and Schmedtje, 1995), including mussels (Hardison and Layzer, 2001). Compared with other methods for measuring shear stress, e.g. the DuBoys equation, which is only strictly applicable under uniform flow conditions, even bed topography and regular channel geometry in wide channels (Schwendel et al., 2010), FST hemispheres can be used to analyse hydraulic microhabitats in streams. The 24 FST hemispheres (KC Denmark A/S, Silkeborg, Denmark) are of uniform size but differ in their densities. Each hemisphere represents an estimate of the minimum near-bed shear stress in $\mathrm{dyn\,cm^{-2}}$ and $\mathrm{N\,cm^{-2}}$ respectively. Hemispheres are numbered in ascending order, with hemispheres with higher numbers having a higher density. For example, hemispheres number 0 and 12 represent a minimum near-bed shear stress of $7.7*10^{-6}\,\mathrm{N\,cm^{-2}}$ and $1.1*10^{-4}\,\mathrm{N\,cm^{-2}}$ respectively. In the field, a platform was placed into the substrate flush with the stream bed. Individual hemispheres were placed on the platform one after the other, and the hemisphere with the greatest density that drifted downstream was recorded. In this study, the range of measured near-bed shear force was between hemisphere number 0 (low near–bed shear stress) and 12 (high near–bed shear stress).

Following Geist and Auerswald (2007), the redox potential (Eh) was measured in the free-flowing water and at 5 cm substrate depth using a hand-held voltmeter (315i pH meter, WTW, Weilheim, Germany). In short, the electric potential was measured between a Platin electrode and $Ag/AgCl_2$ electrode and Eh was obtained by correcting for $Ag/AgCl_2$ and temperature. High redox potentials indicate an oxidizing environment as a result of high concentrations of dissolved oxygen (O_2). Oxygen is one of the primary determinants of the redox potential (Dodds and Whiles, 2010). In the study of Geist and Auerswald (2007), loss of redox potential clearly separated functional (i.e. recruiting) from non-functional (non-recruiting) freshwater pearl mussel populations. Values of Eh < 300 mV indicate reduced oxygen conditions (Schlesinger 1991).

Water temperature (°C), dissolved oxygen concentration ($\mathrm{mg\,L^{-1}\,cm^{-1}}$), conductivity ($\mu\mathrm{S\,cm^{-1}}$) and pH values were measured in the free-flowing water using a hand-held voltmeter (Multi350i WTW, Weilheim, Germany). To examine the interface between the water column and the hyporheic zone, resistance of the substrate was measured using a hand-held penetrometer (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Low resistance values (0.001–0.1 $\mathrm{kg\,cm^{-2}}$) are indicative of areas with high fine sediment deposition, whereas high values (0.1–5 $\mathrm{kg\,cm^{-2}}$) are observed in colmated areas of the stream bed or in areas of coarse substrate (Geist and Auerswald, 2007). For the measurement, a metal adapter disc (diameter 25 mm) was used and the readings were corrected according to the adapter surface area. A minimum value of $0.001\,\mathrm{kg\,cm^{-2}}$ was assigned to areas with extremely soft mud (Geist and Auerswald, 2007).

2.3.4 Mussel sampling

After physical measurements, including flow velocity, redox potential, pH, conductivity, dissolved oxygen, penetration resistance and shear stress, mussels within sampling quadrats were collected by tactile detection from the surface as well as from the subsurface to a depth of 10 cm, and counted. The mussels were immediately released to their original habitat.

2.3.5 Statistical analyses

Quadrats were grouped according to the presence (C = colonized) or absence (NC = non–colonized) of mussels (UC and UNC = *U.crassus*, MC and MNC = *M.margaritifera*). Differences in hydrological parameters (near–bed flow velocity $\mathrm{m\,s^{-1}}$, mid-column flow velocity $\mathrm{m\,s^{-1}}$, surface flow velocity $\mathrm{m\,s}\ ^{-1}$, loss of redox potential Δ mV, penetration resistance $\mathrm{kg\,cm^{-2}}$ and shear stress $\mathrm{N\,cm^{-2}}$) between groups (UC, UNC, MC, MNC) and differences in mussel abundance between streams were tested using the non-parametric Kruskal-Wallis sum of ranks test and post hoc pairwise Wilcoxon rank sum test for non-normally distributed data. Bonferroni correction was applied to correct for multiple testings. Correlations between shear stress, redox potential, penetration resistance, flow velocities and mussel densities were assessed using non-parametric Spearman's rank correlation.

Principal component analysis (PCA) was performed in PRIMER (Clarke and Warwick, 2001) to identify quadrats exhibiting similar hydrological characteristics and to test for differences between UC and UNC sites. Before analysis, data were normalized to account for different measurement scales. A non-parametric Kruskal-Wallis test was used to compare principal component factors between UNC and UC. Univariate statistical analyses were performed in R version 3.0.2 (2014, R Foundation for Statistical Computing), multivariate analyses were conducted using PRIMER (Clarke and Warwick, 2001).

2.4 Results

In a total, 265 mussels were found at an average density of 35 mussels m^{-2} in UC quadrats. Mean mussel densities did not differ significantly between the streams studied (Kruskal-Wallis, $\mathrm{p} > 0.05$), but varied considerably between 4 and 164 mussels m^{-2}. Water depths were different between streams and ranged between 4 and 80 cm, with an average of 8.2 ± 5.5 cm (mean ± SD) (Haldenseebach) and 41.9 ± 19.6 cm (Frankenohe) (minimum and maximum average values are given, for values for the other streams see Table 2.2). Mean mid-column (60 % water depth from surface) flow velocities in the six *U.crassus* streams ranged between $0.11 \pm 0.06\,\mathrm{m\,s^{-1}}$(Falchengraben) and $0.31 \pm 0.32\,\mathrm{m\,s^{-1}}$ (Staffelseeach) for all study sites (UC and UNC) and did not differ significantly between streams (Kruskal-Wallis; $\mathrm{p} = 0.08$). The average near bed-flow velocities were lower and ranged between $0.07 \pm 0.03\,\mathrm{m\,s^{-1}}$ (Falchengraben) and $0.18 \pm 0.17\,\mathrm{m\,s^{-1}}$ (Staffelseeach). The maximum near-bed flow velocity at mussel beds was $0.33\,\mathrm{m\,s^{-1}}$ and $0.35\,\mathrm{m\,s^{-1}}$ at 60 %, while considerably higher flows with a maximum of $0.52\,\mathrm{m\,s^{-1}}$ and $0.95\,\mathrm{m\,s^{-1}}$ at 60 % depth recorded at non-colonized sites.

Table 2.2: Mean values, standard deviation and ranges of physical habitat parameters (n = 10) in *U.crassus* streams. Δ Redox potential refers to the difference in redox between open and interstitial water at 5 cm.

Stream	Water depth [cm]	Δ Redox potential [mV]	O2-concentration [mg L^{-}1]	Flow velocity surface [m s^{-1}]	Flow velocity mid-column [m s^{-1}]	Flow velocity near-bed [m s^{-1}]	Shear stress [N cm^{-2}]	Penetration resistance [kg m^{-}2]	Conductivity [µS cm^{-}1]	Water temperature [℃]	pH
Staffelseeach	30.5 ± 15.3	171 ± 68	8.4 ± 0.1	0.32 ± 0.31 (0.05-0.90)	0.31 ± 0.32 (0.05-0.95)	0.18 ± 0.17 (0.02-0.52)	4.4x10^{-}5	0.36 ± 0.32	374 ± 0.7	20.5 ± 0.6	8.4 ± 0.1
Falchengraben	20.1 ± 6.0	218 ± 23	7.9 ± 0.2	0.10 ± 0.06 (0.04-0.20)	0.12 ± 0.06 (0.05-0.22)	0.07 ± 0.03 (0.02-0.11)	8.0x10^{-}6	0.00 ± 0.00	360 ± 3.5	16.7 ± 0.5	7.6 ± 0.1
Haldenseebach	10.4 ± 6.6	305 ± 75	7.3 ± 0.4	0.13 ± 0.07 (0.03-0.26)	0.11 ± 0.06 (0.01-0.23)	0.11 ± 0.08 (0.01-0.24)	8.0x10^{-}6	0.66 ± 0.70	944 ± 8.0	17.4 ± 0.4	7.2 ± 2.2
Bodenbach	39.4 ± 13.6	244 ± 32	8.3 ± 0.0	0.25 ± 0.17 (0.09-0.60)	0.24 ± 0.13 (0.09-0.53)	0.13 ± 0.11 (0.00-0.36)	1.0x10^{-}5	0.44 ± 0.43	407 ± 0.0	17.4 ± 0.4	7.2 ± 2.2
Sallingbach	24.7 ± 8.4	187 ± 106	8.7 ± 0.2	0.18 ± 0.10 (0.03-0.31)	0.20 ± 0.13 (0.01-0.53)	0.18 ± 0.12 (0.02-0.42)	2.0x10^{-}5	0.32 ± 0.34	656 ± 29.0	14.7 ± 0.6	8.0 ± 0.1
Frankenohe	40.0 ± 14.0	269 ± 75	7.2 ± 2.5	0.17 ± 0.07 (0.03-0.28)	0.20 ± 0.09 (0.02-0.36)	0.13 ± 0.10 (0.00-0.36)	1.0x10^{-}5	0.94 ± 0.50	250 ± 8.0	16.2 ± 0.1	7.4 ± 0.1

Similar values for flow velocities at all three depths (surface, mid-column, near-bed) were measured at colonized and non-colonized sites (Figure 2.2).

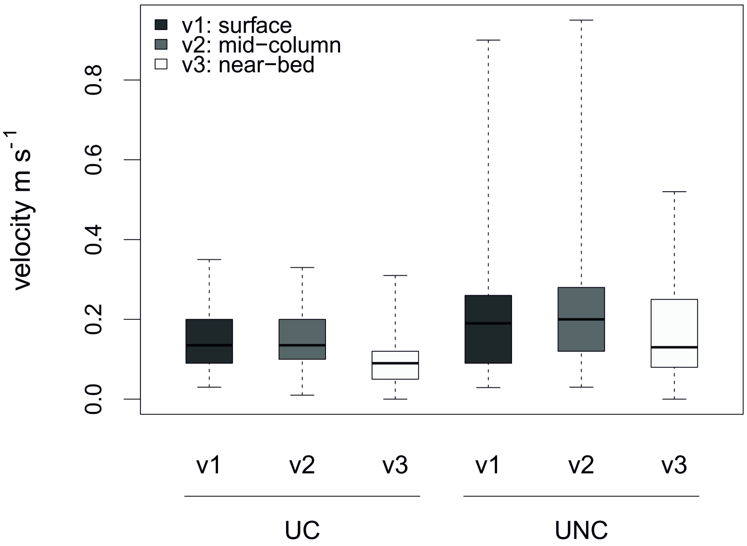

Figure 2.2: Comparison of flow velocities measured 2 cm below water surface (v1), at 60 % water depth, i.e. mid-column (v2) and near-bed (v3) ($m\,s^{-1}$) at UC and UNC sites (Kruskall-Wallis, Mann-Whitney-U). Letters above whiskers (a, b) indicate significant differences between flow velocities (v1, v2, v3) and sites (comparisons between UC and UNC groups) ($p < 0.05$; p-values of single comparisons were adjusted using Bonferroni correction, i.e. multiplying them with the number of comparisons). Whiskers represent the data range.

When comparing only colonized areas, a trend of low flow velocities at both near-bed and mid-column level was noticeable, and values ranged between $0.00\,m\,s^{-1}$ and $0.31\,m\,s^{-1}$. In areas with mussel densities of more than 40 individuals m^{-2}, the mean near-bed velocity was $0.09 \pm 0.05\,m\,s^{-1}$ ($n = 8$). In comparison with UC streams, flow velocities in the stream with co-occurring *M.margaritifera* were higher and colonized sites differed significantly from UC sites (Figure 2.3): mean mid-column flow velocities ranged between 0.37 ± 0.16m s^{-1} (colonized) and $0.54 \pm 0.34\,m\,s^{-1}$ (non-colonized). Maximum mid-column flow velocity at colonized sites (MC) was $0.96\,m\,s^{-1}$.

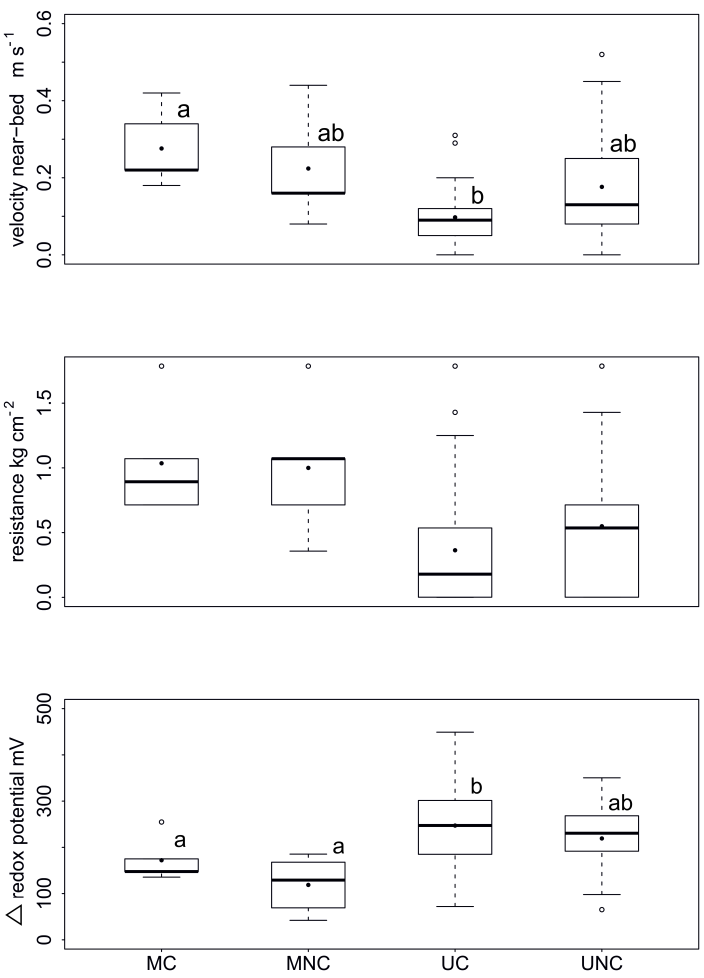

Figure 2.3: Comparison of flow velocity near-bed ($\mathrm{m\,s^{-1}}$), penetration resistance ($\mathrm{kg\,cm^{-2}}$) and loss of redox potential (mV) at sites colonized with *U.crassus* (UC) and *M.margaritifera* (MC) versus non-colonized sites (UNC, MNC) at all study streams (n = 30 for UC and UNC, n = 5 for MC and MNC; Kruskal Wallis, Mann-Whitney-U). Mean values of groups are shown as black dots. Significant differences between groups are indicated by different letters (a, b).

Mean penetration resistance in the *U.crassus* streams ranged between 0.01 ± 0.01 $\mathrm{kg\,cm^{-2}}$ (Falchengraben) and 0.94 ± 0.50 $\mathrm{kg\,cm^{-2}}$ (Frankenohe). Mean penetration resistance at colonized sites was lower than at non-colonized sites and averaged 0.36 ± 0.52 $\mathrm{kg\,cm^{-2}}$ (UC) and 0.61 ± 0.53 $\mathrm{kg\,cm^{-2}}$ (UNC), although no significant difference between the two groups was detected owing to the great variability in both groups. It is notable that a penetration resistance of 0.001 $\mathrm{kg\,cm^{-2}}$ was recorded for 43 % of all colonized sites, and 78 % of colonized sites revealed values below 0.6 $\mathrm{kg\,cm^{-2}}$. Penetrometer readings at colonized sites in the *M.margaritifera* stream differed significantly from UC sites, averaging a higher mean of 1.16 ± 0.49 $\mathrm{kg\,cm^{-2}}$ (Mann-Whitney-U, $P < 0.05$).

Principal component analysis extracted three principal components (PCs) with an Eigenvalue of > 1, accounting for 81.2 % of the variance in the dataset. The variables flow at surface, flow at mid-column, flow at near-bed, as well as shear stress were strongly correlated with the principal component 1 (48.3 %), indicating that hydrologic stability increases along this component. Factor score of PC1 significantly separated colonized (UC) and non-colonized sites (UNC) (Mann-Whitney-U, $P < 0.05$). A noticeable overlap of data points from both groups (Figure 2.4) did not allow a clear separation of UC and UNC sites, suggesting that hydraulic conditions of some UNC sites would be suitable for colonization by mussels. Variables describing the substrate characteristics (penetration resistance and difference in redox potential) contributed to PC 2, which explained 18.5 % of the variance. The variable water depth loaded on the principal component 3 (factor score 14.3 %).

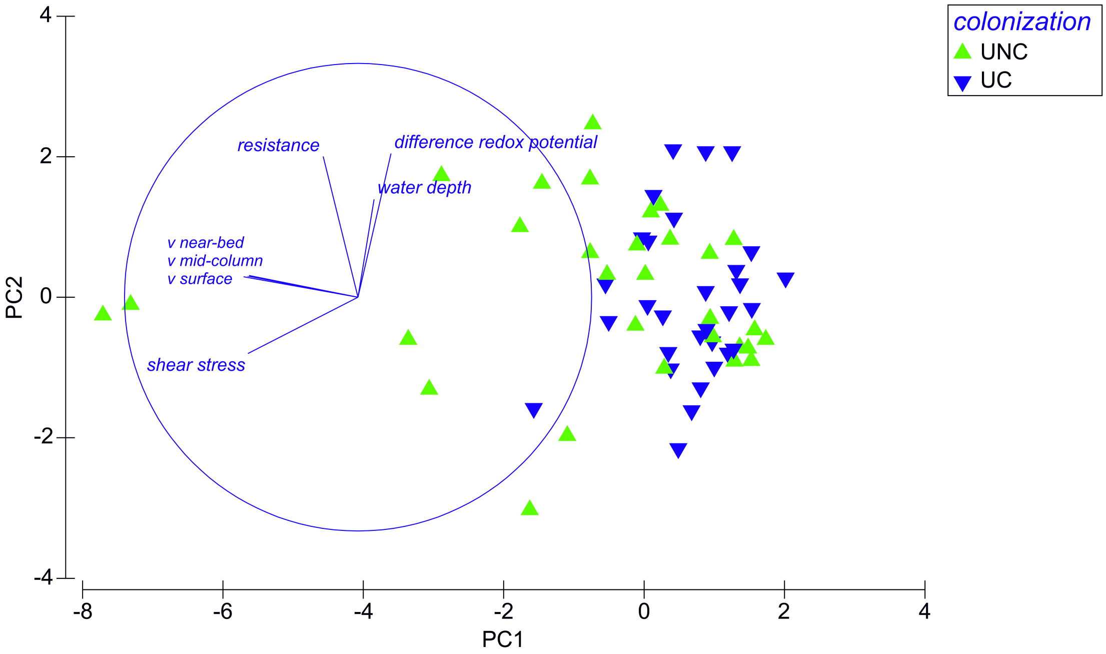

Figure 2.4: Two-dimensional PCA ordination plot of hydrological parameters at colonized and non-colonized sites (green and blue triangles) (n = 60). Factor scores were 48.3 % (PC1), 18.5 % (PC 2) and 14.3 % (PC3, not shown). PC 1 and PC 2 account for 66% of the total variance. Significant difference between UNC and UC were identified for component 1 (Mann-Whitney-U, p <0.05).

In line with the finding that highest *U.crassus* densities occurred at sites with low flow velocity, mussel density in UC streams was negatively correlated with near-bed shear bed forces ($\mathrm{N\,cm^{-2}}$) (Spearman's rank correlation, $P < 0.05$, Spearman's Rho: − 0.34) (Figure 2.5); correlations between surface, mid-column, and near-bed flow velocities were highly significant (Spearman's rank correlation, $P < 0.01$, Spearman's Rho > 0.65), but no significant correlation was found between mussel density and flow velocities, loss in redox potential in 5 cm substrate depth as well as penetration resistance (Spearman's rank correlation, $P > 0.05$).

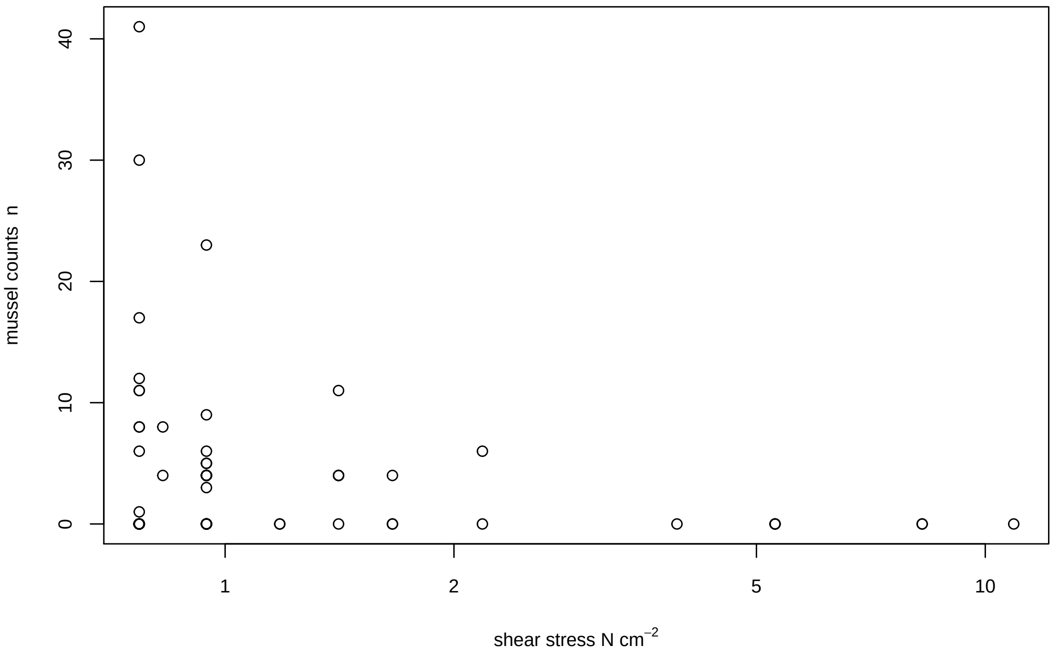

Figure 2.5: Correlation between shear stress (N cm^{-2}) and mussel abundance (Individuals quadrat^{-1}) in UC quadrats (n = 30) (Spearman's rank correlation, $p < 0.05$, Spearman's Rho:–0.34).

2.5 Discussion

Knowledge on the habitat requirements of endangered species is crucial for the implementation of effective conservation strategies which typically include habitat restoration. This study identified low shear stress and low flow velocities as key variables explaining the spatial distribution of the thick-shelled river mussel *U.crassus*.

In contrast to the current perception that *U.crassus* populations depend on high flow velocities, the species was instead mostly found in areas with low or stagnant water flow and high concentrations of fine sediment, as indicated by low penetration resistance. Although this study does not show that this species cannot also persist in streams with high flow currents, the present view that streams with low flow velocities and soft substrate are poor habitats clearly has to be rejected. Thus, currently valid monitoring protocols for habitat assessment of *U.crassus* need to be updated. Since the status of *U.crassus* populations and its habitats have to be assessed regularly in several European countries in implementing the Habitats Directive, some common European guidelines for monitoring populations and habitats would increase comparability of the data and assessments. The standard for monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations and habitats, which is being developed at present by the European Committee for Standardization (CEN), could serve as a model.

Areas with highest mussel densities in this study were characterized by low flow velocities $< 0.3\,\mathrm{m\,s^{-1}}$, low penetration resistance $< 0.1\,\mathrm{kg\,cm^{-2}}$ as well as by low near-bed shear stress $< 1.0 \text{ x } 10^{-6}\,\mathrm{N\,cm^{-2}}$. Together with these observations, the negative correlation between near-bed shear stress and mussel density suggests that adult *U.crassus* occur preferably in areas with low flows which eventually may prevent the mussels from being dislocated from the substrate in which they are anchored. Yet, it is important to note that the results presented here are only valid for adult mussels, since ideal sites were identified based on the presence of adult mussels and since a search for juveniles was not possible because of the resulting disturbance of the substrate. It should also be noted that the observed range in environmental variables associated with *U.crassus* is representative of the species' tolerance rather than of the narrower optimum habitat. Layzer and

Madison (1995) and Hardison and Layzer (2001) also found negative relationships between shear stress and mussel density or occurrence. The authors suggested that high shear stresses may prevent juvenile mussel settlement. However, Strayer (2008) argued that high shear stress is unlikely to be the main limiting factor for settlement of juvenile mussels since this would not match the observed similar spatial distribution patterns of different species with different spawning and settlement seasons. For example, an alternative interpretation of Strayer (1999 b) is that mussels occur in flow refuges, which are areas where shear stress during floods is too low to wash out the mussels from the stream substrate.

In accordance with the findings of those studies, the results presented here suggest that hydrological variables such as low shear stress play a key role in the distribution of *U.crassus* in streams. Since shear stress was not measured at high flows in this study, it is recommended that future studies should investigate this parameter in areas with high mussel densities during periods with elevated discharge, and include extreme conditions which may be more important for mussel distribution patterns than average conditions. Densely colonized patches may be characterized by stable substrate. The importance of substrate stability for stream-dwelling freshwater mussel species has become apparent in recent studies (Quinlan et al., 2015). This hypothesis may also at least partly explain the distribution patterns of *U.crassus*, with mussel beds often being found near stream banks in eddies (Engel and Waechtler, 1989; Zajac and Zajac, 2011) or in areas with a high bank roughness, whereas areas in the central channel of a stream are rarely colonized. In general, the deep central channel of a stream, which is regularly scoured by floods, has significantly higher flow velocities (Newbury and Bates, 2006) and higher bed-load dynamics than shallow areas and back eddies at the margins of the channel.

Based on the observation that *U.crassus* declined substantially throughout its distribution range in the last century and is now prone to extinction in many areas, it was assumed that the species is a habitat specialist. However, as shown for many other endangered freshwater mussel species (Haag and Warren, 1998), the results of this study support the hypothesis that *U.crassus* may have a broader niche for habitat variables related to flow and substrate than previously expected. Mean flow velocities in the study streams ranging between $0.11\,\mathrm{m\,s^{-1}}$ and $0.31\,\mathrm{m\,s^{-1}}$ (mid-column) and highly variable maximum values ($0.22\,\mathrm{m\,s^{-1}}$ to $0.95\,\mathrm{m\,s^{-1}}$) indicate that *U.crassus* is able to colonize and reproduce successfully in a wide range of different stream habitat types with diverse flow regimes, including small brooks and medium-sized upland streams (Table 1). The mean flow velocities recorded are in line with those reported elsewhere: Engel (1990) measured mean flow velocities of $0.16 \pm 0.04\,\mathrm{m\,s^{-1}}$ using drift units in a stream with *U.crassus* in Northern Germany. Undoubtedly, flow velocities will be higher at times during elevated water discharge and therefore higher flows will be also measurable at colonized sites, presumably reaching, at least at some sites, the recommended values $> 0.3\,\mathrm{m\,s^{-1}}$ for optimal habitats. Furthermore, it is possible that streams with higher flows and discharge that were not studied herein, are also populated. However, assessments of habitat quality are usually made at the same time as the mussel population surveys, which are typically only possible during periods of low flow. Because of the high variability of flow velocities, fixed values for this parameter in habitat assessments seem to be unsuitable for classifying an optimal habitat for *U.crassus*. Ideally, assessment of habitat quality, and in particular of flow dynamics, should thus be made at a range of discharges.

It is interesting to note that pronounced low penetration resistances at colonized sites indicate that *U.crassus* is able to persist in areas characterized by high deposition of fine sediments and an extremely soft substrate, similar to previous findings by Denic et al. (2014). To date, it was assumed that the species has a similar narrow niche like the endangered freshwater pearl mussel *M.margaritifera*, because the two species have a slight distribution overlap, populate similar habitats, and both have had comparable population declines in the 20th century resulting in similar conservation status. In contrast to *U.crassus*, habitat requirements of *M.margaritifera* are better known: river beds ideal for *M.margaritifera* are stable enough to protect adults and juveniles from drifting, but current velocity is high enough to prevent sedimentation of fine sediments that eventually lead to colmation of the substratum (Moorkens and Killeen, 2014). The resulting oxygen depletion in the substrate is considered a major threat for the recruitment of *M.margaritifera* populations, because juvenile mussels that live buried in the intersitital zone for up to several years are highly sensitive to adverse habitat conditions

(Hastie et al., 2000, Geist and Auerswald, 2007, Österling et al., 2010). The present results show that *U.crassus* has a broader ecological amplitude in its abiotic habitat requirements including stream substrate quality, than *M.margaritifera*.
This observation may be mechanistically explained by the life-history strategy of this freshwater mussel species. First, compared with *M.margaritifera*, the post-parasitic phase of *U.crassus* in the interstitial zone is significantly shorter and lasts only up to two years (Hochwald, 2001) compared with five or more years for *M.margaritifera*. For successful recruitment of juveniles, substrate quality and stability are therefore required over a significantly shorter period of time. Second, *U.crassus* has a broader host-fish range than *M.margaritifera*, including common and widespread species such as three-spined stickleback (Gasterosteus aculeatus), European chub (*Squalius cephalus*), as well as species that require specific habitat conditions such as European minnow *Phoxinus phoxinus* or bullhead (*Cottus gobio*). *Phoxinus phoxinus* and *C. gobio* are adapted to oligotrophic, cool and well-oxygenated streams (Freyhof, 2013) with stable gravel beds for spawning and successful development of eggs and larvae (Bless, 1992). *Squalius cephalus* is typical of the barbel zone and occurs both rheophilic and lithophilic habitats. Arlinghaus and Wolter (2003) showed that this fish species even reproduces in an artificial still-water-like canal. In the upper Danube region, *S. cephalus* is the most important host fish in *U.crassus* streams (Taeubert et al., 2012; Stoeckl et al., 2015). Thus, the thick-shelled river mussel as well as some of its most important host fish species are less affected by fine sediments and have a broader habitat range than *M.margaritifera* and its salmonid hosts. Also, in contrast to other mussel species, *U.crassus* exhibits a unique spurting behaviour during the reproductive cycle, enhancing the probability of host fish infection (Vicentini, 2005). During the time of glochidial release, gravid mussels move to the river banks and take on a distinct position at right angles to the river bank. The glochidia are then released in several spurts, which hit the water surface in the centre of the watercourse. Vicentini (2005) assumed that the spurts might help to attract potential host fish. This behaviour requires a fine enough substrate for *U.crassus* to move.

2.6 Implications for Conservation

The results of this study indicate that *U.crassus* populations persist in various types of habitats with variable hydrological conditions. High mussel densities and mussel beds were predominantly found in areas with low flow velocities ranging between $0.00\,\mathrm{m\,s^{-1}}$ and $0.31\,\mathrm{m\,s^{-1}}$. Thus, the classification of streams with slow currents and soft substrate as poor habitat for *U.crassus* must be questioned critically, and consequently, the monitoring protocols for habitat assessment in this species need to be updated. The results of this study suggest that the occurrence of low flow areas is important for the sustainability of *U.crassus* populations. These areas are presumably most crucial during the reproductive cycle, where gravid mussels change their location towards the stream bank to release their glochidia and thus have a higher risk of being displaced at high flows. This species-specific behavior should be considered in restoration projects that often aim at modifying hydrological conditions to improve habitat quality in degraded streams. Since many stream restoration projects have been unsuccessful in re-establishing natural flow regimes in streams (Roni et al., 2002; Palmer et al. 2010), we recommend that models should be included in the planning process of conservation projects to predict the effects of restoration on the hydrological, morphological and ecological conditions, as described by Jungwirth et al. (2002). The application of evidence-based restoration approaches (Pander and Geist, 2013; Geist, 2015) as well as the application of different restoration measures in smaller representative areas that are only used at larger scales if they are successful (Geist, 2015) is advisable in the context of habitat restoration for *U.crassus*.

In order to define optimal habitat requirements of this species, further studies should consider hydrological conditions over a range of discharges. One of the major challenges in mussel conservation research is to define the requirements for an endangered species in streams that have experienced major changes in recent years. It is of great importance to consider all factors that may have led to significant habitat changes in the past so as to interpret correctly the distribution and colonization patterns of mussels. The complex interactions between biotic and abiotic factors that may explain the downward trend of many populations are still poorly understood. For example, the requirements of juvenile mussels and the prerequisites for successful recruitment are still largely unknown for this species, as for many other unionoid species. Further research should focus, therefore, on identifying key factors for the recruitment of juvenile *U.crassus*, i.e. following physical measurements, the quality of reproductive success should be investigated by an intensive search for juvenile mussels.

In the last decade, research in freshwater mussel ecology in Europe has focused on a restricted number of species, with freshwater pearl mussel *M.margaritifera* being the most frequently studied. Using the example of *U.crassus*, we suggest that conclusions on the autecological requirements of mussel species drawn by analogy with other species are not appropriate. However, methods and techniques that have been successfully used in *M.margaritifera* studies, can be also applied for analysing *U.crassus* streams. For example, transect studies across the river helped to understand how adult and juvenile *M.margaritifera* densities vary over short differences in relation to physical differences in these sites (Hastie et al., 2000). These results help in the ecological interpretation of the physical differences between sites that control life history success and failure.

3 Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation

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3.1 Abstract

The abundance of *Unio crassus* (Philipsson 1788) has declined over the last decades. Despite the high conservation status of this species, knowledge on its ecological requirements is scarce. The objective of this study was to identify key habitat characteristics in areas with recent recruitment of *Unio crassus* in a small upland stream. Furthermore, we investigated stretches where the species is presently absent. Sediment deposition, redox potential, flow velocity, water depth, nitrogen and phosphorus load were investigated. Fine sediment deposition was high with 19.4 $\mathrm{kg\,m^{-2}\,month^{-1}}$ at colonized and 13.3 $\mathrm{kg\,m^{-2}\,month^{-1}}$ at non-colonized stretches of the Sallingbach. At all study stretches redox potentials in the interstitial zone varied around 300 mV, which constitutes the boundary value between oxic and anoxic conditions. Results of chemical water analyses indicated high nitrogen loads. Nitrate nitrogen concentrations averaged between 4.1 and 6.5 $\mathrm{mg\,L^{-1}}$ NO_3–N at all study stretches, significantly exceeding the currently proposed threshold value of 2.0 $\mathrm{mg\,L^{-1}}$ for functional *U.crassus* streams.The results in this study suggest that *U.crassus* is more tolerant to eutrophic habitat conditions than previously expected. Our findings show that currently considered physicochemical parameters and high fine sediment deposition cannot mechanistically explain the occurrence of *U.crassus* in the stream.

3.2 Introduction

Worldwide, freshwater ecosystems experience high biodiversity losses (e.g. Dudgeon, 1992; Abell, 2002; Dirzo and Raven, 2003; Geist, 2011), with species extinction rates significantly exceeding those of terrestrial ecosystems (Sala et al., 2000; Revenga and Kura, 2003). Non-marine mollusks are particularly affected, withspecies extinction rates exceeding 40 % during the last millennium (Lydeard et al., 2004). According to literature from the beginning of the 20th century, the thick-shelled river mussel (*Unio crassus*) was regarded as the most common unionoid species in Central Europe (Israel, 1913; Geyer, 1927), reaching densities > 700 individuals m^2 (Tudorancea andGruia, 1968). Due to significant declines within the last 50 years (e.g. Bless, 1980; Jungbluth et al., 1988; Reischuetz and Sackl, 1991), the species is now strictly protected and considered “critically endangered” in several European countries, since only few successfully reproducing populations remain (von Proschwitz and Lundberg, 2004; Zettler and Jueg, 2007). Although potentially harmful effects of anthropogenic activities, including water pollution and flow modification, have been reduced in Central Europe over the last decades (Aarts et al., 2004; Bogan, 2008), *U.crassus* populations are not recovering accordingly.

The sensitivity of *U.crassus* to unfavorable conditions is presumably closely linked to the complex life cycle, during which different life stages have specific ecological requirements. After maturation, adult mussels release glochidial larvae, which need to attach to the gills of suitable host fish (Waechtler et al., 2001; Taeubert et al., 2011). After their parasitic life stage, juvenile mussels live buried in the interstitial system of rivers for a period of up to five years (Hochwald, 1997).This post-parasitic phase is generally considered the most vulnerable of all life stages. Based on information from related species, it is assumed that unionids constantly need stable physicochemical conditions in the stream substratum over a relatively long period of time (Hastie et al., 2000; McRae et al., 2004; Geist, 2010). For example, sites with natural *M.margaritifera* recruitment were found to be dominated by coarse, well–sorted substrata and a low fraction of fines, providing well-oxygenated water, nutrients and organic matter to the interstitial zone. Increased fine sediment loads, e.g. caused by agricultural land-use, can reduce hyporheic exchange rates by clogging the interstitial macropores (Schaelchli, 1992), resulting in reduced oxygen supply. Due to their similar life cycle and co-occurrence in the same habitat in several German streams (Björk, 1962), it is often assumed that functional *U.crassus* populations have similar requirements concerning substratum quality as *M.margaritifera* (Zettler et al., 1994; Engel, 1990, Buddensiek, 1993). However, the validity of these assumptions has not yet been tested, and information on physicochemical conditions in the interstitial system in functional *U.crassus* streams is still scarce. Although there is still a lack of peer-reviewed information on key habitat parameters of *U.crassus* (Koehler, 2006), various conservation efforts targeting habitat restoration for this species have been initiated (Perez-Quintero, 2007).

In order to improve conservation strategies, profound knowledge about species-specific habitat requirements with regard to substratum quality is a main prerequisite. The objective of this study was to identify habitat characteristics in a *Unio crassus* stream with recent recovery of *U. crassus.* In particular, we characterized the physicochemical conditions in the free-flowing water and in the hyporheic zone in areas with recent increase of mussel population densities and recruitment of *U.crassus.* Furthermore, we also analyzed areas where the species is currently absent. The study comprises analyses of sediment deposition and physicochemical variables such as redox potential, flow velocity, water depth, pH, specific conductance, ammonia, nitrite, nitrate and phosphate, which were previously assumed to be important key factors for the recruitment of juvenile *U.crassus* (Buddensiek et al., 1993; Engel, 1990; Hochwald, 1997).

3.3 Material and Methods

3.3.1 Study area and design

The study area is located in the Bavarian tertiary hills in the north-east of Munich, Germany (Figure 3.1). The study stream Sallingbach belongs to the upper catchment of the Danube and has a total length of 8.8 km and a width of 0.5–2 m. Substratum texture is dominated by fine gravel and sand. The mean discharge in the downstream area of the stream is $0.082\,\mathrm{m}^3\,\mathrm{s}^{-1}$.

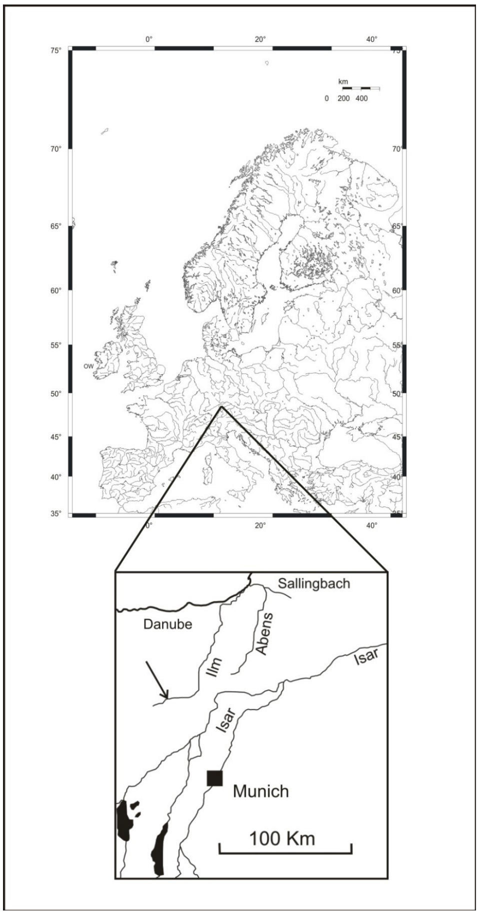

Figure 3.1: Map of the study area, arrow indicating position of the reference stretch in the River Ilm

The region is highly impacted by agricultural land-use. In the Ilm, a stream similar to the Sallingbach regarding its geomorphological and hydrological characteristics, a reference study stretch was chosen for validating the results of physicochemical parameters from the Sallingbach in a comparable environment. Here, a self-sustaining functional *U.crassus* population had been discovered by the Bavarian Mussel Coordination in 2010 (Gum, pers. comm.). The stream hosts a dense host-fish population of European minnow (*Phoxinus phoxinus*).

Due to the occurrence of *Unio crassus* and several other endangered aquatic species, the river Sallingbach was target to a statewide restoration program conducted from 1988 to 2001 (ABSP, 1991). Distribution, population size and recruitment of juvenile mussels of *U.crassus* were thoroughly analyzed in several surveys from 1988 to 2009 (Hochwald, 1988; Ansteeg, 1994; Ansteeg, 1999; Colling, 2007; Ansteeg, 2010). The monitoring data show that *U.crassus* has a core distribution area in the downstream area, where highest mussel densities and natural recruitment of juvenile mussels were found. The survey in 2009 (Ansteeg, 2010) showed that mussel densities had increased in this area for the first time since 1989. Findings of spent shells and data from over 20 years ago (Seitz, 1988; Colling, 2007) document that also upstream stretches represented the natural habitat of *U.crassus* in the past. In order to representatively cover all areas of the Sallingbach, 8 stretches were defined for analyses of physicochemical parameters. Four stretches are currently colonized with *U.crassus* and recent juvenile recruitment was observed in 2009 (distance between stretch 1 and 4: 1.7 km). In addition, four stretches were determined upstream, where *U.crassus* currently is absent (stretch number 5 and 6), and where *U.crassus* occurrence is documented (stretch 7 and 8) (total distance between stretch 5 and 8: 3.5 km). Depending on the occurrence of *U.crassus*, the stretches are referred to as colonized (C) and as non-colonized (NC) throughout the text. The reference stretch in the Ilm is classified as colonized, due to the presence of *U.crassus* in different age classes. Within each stretch, four transects were analyzed as shown in Figure 3.2. In each transect, two measuring spots in the middle and two at either side of the stream were defined ($n = 6$). After the physicochemical measurements in a transect were completed, the substratum was screened for the presence of mussels to validate the results of the survey in 2009 (Ansteeg, 2010). For this purpose, the substratum was removed to check for buried mussels. In order to avoid that investigations at one time point change habitat conditions and consequently introduce a bias to the data, sampling in the following month was carried out in a previously assigned transect, moving from downstream to upstream transects (t1-t4) within sampling stretches. Measurements at the reference stretch located in the stream Ilm were carried out once in October 2010. In each sampling period all measurements were conducted within the same day.

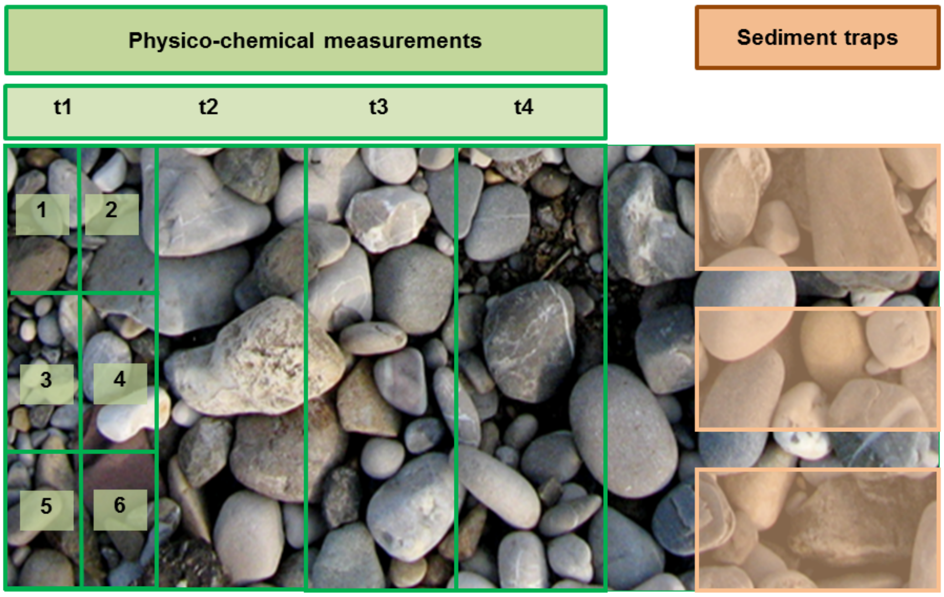

Figure 3.2: Sampling design at each study stretch (n = 8). Physicochemical parameters in the free-flowing water and in the interstitial zone were measured monthly from August until November within 1 m transects (t1-t4) at six sampling spots (n = 6, sampling spots shown for first month). Sediment traps (n = 3 per stretch) were placed upstream in order to avoid additional sediment deposition during sampling.

3.3.2 Sediment deposition

Sediment net deposition in the Sallingbach was investigated using sediment traps. The traps consisted of plastic boxes that contained a volume of 5 l (33 (length) x 19 (width) x 11 cm (height)) and had been filled with clean gravel with a standardized particle size between 16–32 mm in diameter. Prior to the physicochemical analysis in the stream, a total of 24 sediment traps (3 traps per study stretch) was deployed in the substratum such that the top of the traps was flush with the riverbed. The 3 sediment traps at each of the 8 stretches were horizontally aligned and evenly distributed across the streambed. After 28 days, the traps were removed and the deposited material was collected. The sediment traps with washed gravel were reinserted for further sediment collection in the following month. Thus, a total of

96 sediment samples were collected during August–November 2010. The deposited grain size fractions were separated at the laboratory by wet sieving (AS 200 digit, Retsch, Haan, Germany). Mesh width was 20 mm, 6.3 mm, 2.0 mm and 0.85 mm, respectively. The fractions retained on each sieve were dried at 80 ° C and weighed. The largest fraction was excluded from further analysis due to the restricted sample volumes (Sinowski and Auerswald, 1999).

3.3.3 Physicochemical Parameters

Physicochemical parameters were analyzed in 28 day intervals over a period of 4 months from August to November 2010. Flow velocity was measured with a handheld flow meter (HFA, Höntzsch, Waiblingen, Germany) and water depth was measured with a pocket rule (exactness ± 0.5 cm). Redox potentials (Eh) were analyzed according to Geist and Auerswald (2007). At each measuring spot within a transect, Eh was first measured in the free-flowing water, and then at depths into the substratum of 5 and 10 cm. Values above 300 mV imply oxic conditions, whereas values below indicate anoxia (Schlesinger, 1991). Water samples from both the interstitial system ($n = 192$ per parameter) and from the open water body ($n = 96$ per parameter) in the Sallingbach were drawn for the determination of ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphate phosphorous (NH_4–N, NO_2–N, NO_3–N, dissolved PO_4–P), pH and specific conductance. Samples of interstitial water at a substratum depth of 5 cm were collected with a fixed PVC tube attached to a flexible plastic hose in combination with a 100 ml syringe (Braun, Melsungen, Germany), which was used for creating a vacuum. Water samples were also drawn from the reference stream ($n = 3$ for free-flowing water and $n = 6$ for intersititial water, respectively). For each sample, 30 ml of free-flowing or interstitial water were extracted and transferred to 50 ml falcon tubes (Roth, Karlsruhe, Germany). Water samples were stored on ice until analysed. Prior to each analysis, water samples were filtered using paper filters (MN 615 0.25, diameter: 125 mm, Macherey-Nagel, Düren, Germany) in order to remove coarse particles. Concentrations of chemical variables were determined photometrically (Photometer: Photolab S12, WTW, Weilheim, Germany; testkits: Spectroquant, Merck KGaA, Darmstadt, Germany) according to German standard norm protocols (DIN standards). Specific conductance and pH were measured using handheld 315i conductivity and 315i pH-meters (WTW, Weilheim, Germany) in the free-flowing water and in the interstitial water from 5 cm substratum depth.

3.3.4 Statistical Analysis

Whilst the main focus of the study was an analysis of mean values, variation and range of physicochemical data from the sites where *U.crassus* is abundant and recruiting, we also compared C and NC stretches in the Sallingbach using pairwise Student's t-test or the Mann-Whitney U-test. Discriminant analysis was used to identify discriminating parameters between C and NC stretches of the Sallingbach. In a stepwise approach, seven parameters were incorporated in the model (NH_4–N, pH and specific conductance in 5 cm substratum depth, pH and specific conductance in free-flowing water, deposition of particles > 6.3 mm, water depth). All tests were implemented in PASW 18 (IBM Statistics 20, SPSS Inc., NY, USA).

3.4 Results

At C stretches in the Sallingbach, the mean nitrate nitrogen concentration in the free-flowing water was $6.39 \pm 1.98\,\mathrm{mg\,L^{-1}}$ NO_3– N (mean and SD), whereas NO_3– N concentrations in the interstitial were significantly lower ($4.13 \pm 2.65\,\mathrm{mg\,L^{-1}}$ NO_3–N, Mann-Whitney-U-test, $p < 0.05$). At the colonized stretch in the reference, a mean concentration of $5.00 \pm 1.06\,\mathrm{mg\,L^{-1}}$ NO_3– N in the free-flowing water was measured compared to $2.61 \pm 1.33\,\mathrm{mg\,L^{-1}}$ NO_3–N in 5 cm substratum.
With $7.05 \pm 3.07\,\mathrm{mg\,L^{-1}}$ NO_3–N in free-flowing water, concentrations at NC stretches of the Sallingbach were also similar to concentrations at C stretches, whereas interstitial nconcentrations at NC stretches ($5.62 \pm 3.46\,\mathrm{mg\,L^{-1}}$ NO_3–N) were significantly different from C stretches (Mann-Whitney-U-test, $p < 0.05$). Mean NH_4–N concentrations averaged $0.09 \pm 0.16\ \mathrm{mg\,L^{-1}}$ in the free-flowing water at C stretches in the Sallingbach (Table 3.1), whereas maximum values reached $3.12\,\mathrm{mg\,L^{-1}}$ NH4–N. Mean NH_4–N concentrations in the substratum at C sites were higher ($0.39 \pm 0.56\,\mathrm{mg\,L^{-1}}$ NH4–N).

Table 3.1: Arithmetic mean values and ranges of chemical parameters phosphate phosphorous (PO_4–P), nitrite nitrogen (NO_2–N), nitrate nitrogen (NO_3–N), and ammoniumnitrogen (NH_4–N) in free-flowing water (FW) and at 5 cm substratum depth (substratum) at colonized (C) and non-colonized (NC) stretches of the Sallingbach ($n = 36$ for FW, $n = 96$ for substratum) and the reference stream Ilm ($n = 3$ for FW, $n = 6$ for substratum).

Parameter	Study group	FW (mean [range])	Substratum (mean [range])
PO_4–P ($mg\,L^{-1}$)	C	0.06 [0.01 – 0.83]	0.08 [0.01 – 0.82]
	NC	0.11 [0.01 – 0.98]	0.07 [0.01 – 0.90]
	Ilm (C)	0.02	0.03
NO_2-N ($mg\,L^{-1}$)	C	0.04 [0.03 – 0.05]	0.09 [0.02 – 0.49]
	NC	0.04 [0.03 – 0.07]	0.05 [0.01 – 0.24]
	Ilm (C)	0.04	0.01
NO_3-N ($mg\,L^{-1}$)	C	6.39 [3.62 – 15.68]	4.13 [0.59 – 12.58]
	NC	7.05 [1.61 – 13.67]	5.62 [0.75 – 15.29]
	Ilm (C)	5	2.6
NH_4–N ($mg\,L^{-1}$)	C	0.09 [0.01 – 0.32]	0.39 [0.01 – 3.12]
	NC	0.15 [0.02 – 1.41]	0.22 [0.03 – 1.44]
	Ilm (C)	0.09	0.37

Measured NH_4–Ñ substratum values at NC stretches ($0.22 \pm 0.21\,mg\,L^{-1}$ NH4–N) were significantly lower than at C stretches of both the study stream and the reference stream (Mann-Whitney-U-test; $p < 0.05$). Nitrite nitrogen concentrations generally ranged between 0.03 and 0.07 $mg\,L^{-1}$ NO_2–N in the free-flowing water of all study stretches, whereas higher variations were found in the substratum. Highest nitrite nitrogen concentrations reaching a maximum of 0.49 $mg\,L^{-1}$ NO_2–N were measured at C stretches of the Sallingbach. Arithmetic mean values of the dissolved PO_4–P concentration in the free-flowing water were between 0.02 and 0.11 $mg\,L^{-1}$ PO_4–P (Table 3.1); high values between 0.8 and 1.0 $mg\,L^{-1}$ PO_4–P were measured both in the free-flowing water and in the interstitial water at C and at NC stretches.

Redox depth profiles exhibited significant differences between the free-flowing water and the substratum (Mann-Whitney U-tests, $p < 0.001$). Mean values in the free-flowing water of the Sallingbach were 450 ± 23 mV (C) and 445 ± 35 mV (NC), respectively. In 5 cm substratum depth, mean values in the Sallingbach were close to 300 mV (C: 305 ± 93 mV, NC: 317 ± 88 mV) (Figure 3.3).

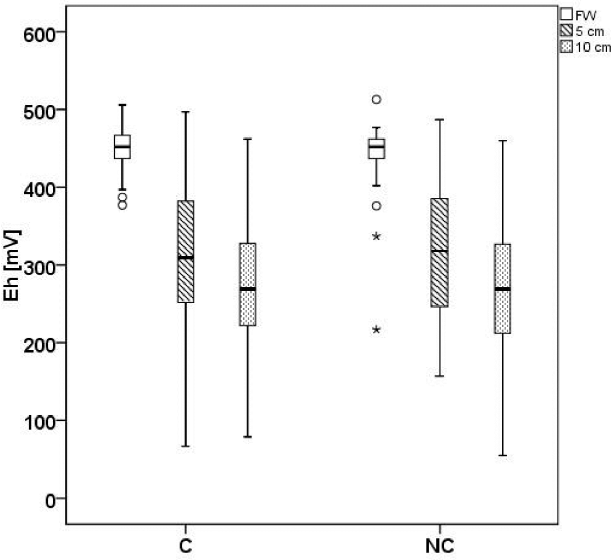

Figure 3.3: Depth profiles of redox potentials in the free-flowing water and in 5 and 10 cm substratum depth at stretches with *Unio crassus* presence (= colonized; C; n = 102, 102, 102) or absence (= non-colonized; NC; n = 96, 96, 96) in the Sallingbach (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile; circles: outliers beyond 1.5 times of the interquartile range (IQR), asterisks: extreme outliers (more than 3 times the IQR)

In 10 cm substratum depth, means were constantly below 300 mV. In the reference stream, depth profiles were similar: high delta values were found between the free-flowing water (446 ± 33 mV) and the interstitial zone (295 ± 46 mV in 5 cm substratum depth and 292 ± 57 mV in 10 cm). Redox potentials showed high microscale variability between study stretches and even between sampling spots at single study transects with ranges up to 350 mV in 10 cm substratum depth.

Specific conductance in the free-flowing water deviated from substratum values at both study streams. C stretches of the Sallingbach exhibited lower values in the free-flowing water (773 ± 125 $\mu\mathrm{S\,cm^{-1}}$ compared to 810 ± 144 $\mu\mathrm{S\,cm^{-1}}$ in the substratum) whereas at NC stretches, lower values were found in the substratum (810 ± 96 $\mu\mathrm{S\,cm^{-1}}$ compared to 753 ± 68 $\mu\mathrm{S\,cm^{-1}}$). In the Sallingbach values in the free-flowing water differed significantly between C and NC stretches (Student's t-test, p = 0.001). Variations in pH were small, ranging from 7.8 to 8.0 (free-flowing water) and from 7.5 to 8.0 (5 cm substratum depth) in the Sallingbach. In the reference, pH in the free-flowing water was 7.6, whereas pH in the substratum was 7.7. Sediment deposition in the Sallingbach was dominated by fine sediments with grain sizes < 0.85 mm. Fine sediment deposition was significantly higher at colonized stretches with a mean of 19.4 ± 10.2 $\mathrm{kg\,m^{-2}\,month^{-1}}$ than at NC stretches with 13.3 ± 6.6 $\mathrm{kg\,m^{-2}\,month^{-1}}$, respectively (Mann-Whitney U-test, $p = 0.003$) (Figure 3.4).

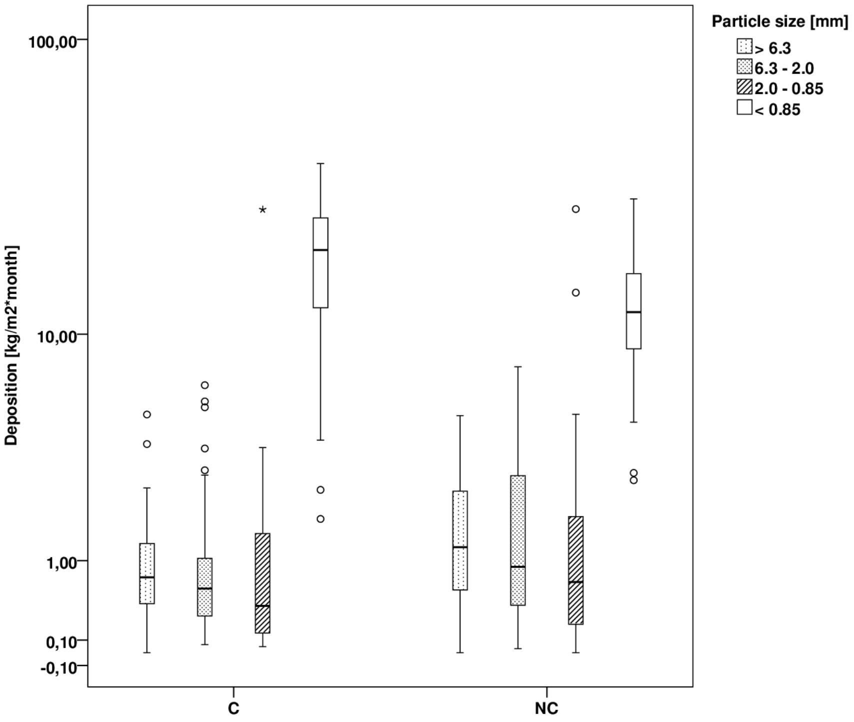

Figure 3.4: Deposition of particle size fractions > 6.3 mm, 6.3-2.0 mm, 2.0-0.85, and < 0.85 mm ($n = 90$, 93, 87, 91 respectively) at stretches with *U.crassus* presence (= colonized; C) or absence (= non-colonized; NC) in the Sallingbach (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile; Circles: outliers beyond 1.5 times of the interquartile range (IQR), asterisks: extreme outliers (more than 3 times the IQR) in $\mathrm{kg\,m^{-2}\,month^{-1}}$.

For larger particles, mean deposition rates were lower and did not exceed 2.0 $\mathrm{kg\,m^{-2}\,month^{-1}}$. Fine sediment deposition increased in downstream direction reaching its maximum at downstream stretches 1 and 2 (mean: 28.2 $\mathrm{kg\,m^{-2}\,month^{-1}}$) (Figure 3.5), where mussel density was highest. Maximum values were detected at study stretch 1 with 38.7 kg m^{-2} month^{-1} and the minimum was observed at stretch 2 (1.7 kg m^{-2} month^{-1}).

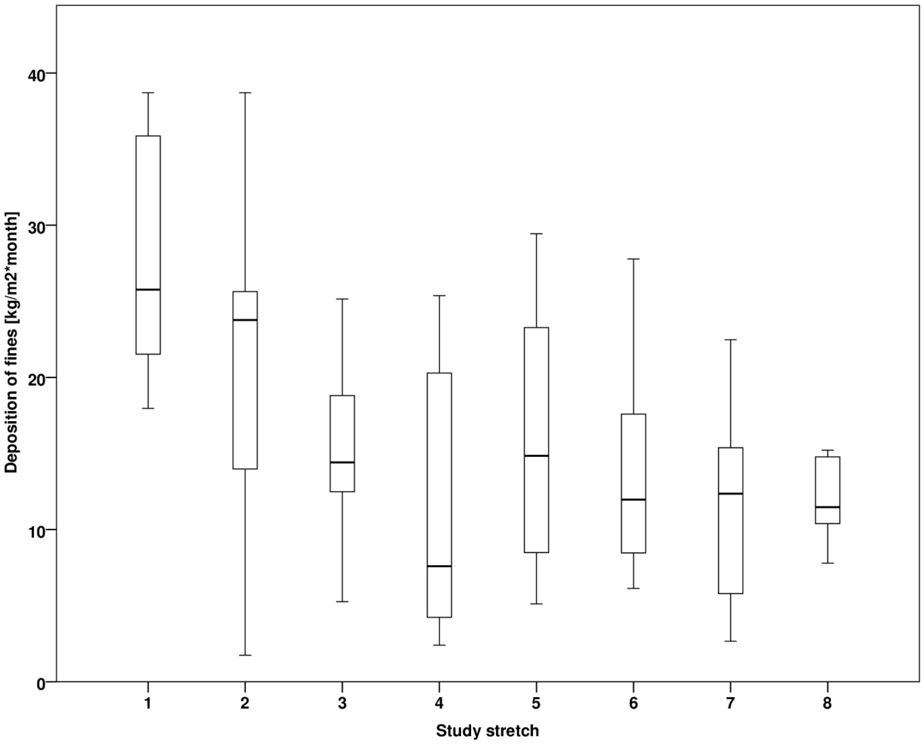

Figure 3.5: Net fine sediment deposition (particle size < 0.85 mm) at 8 sampling stretches ($n = 12$ per study stretch) in the Sallingbach. Sampling stretches are numbered in ascending order in an upstream direction (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile). The stretches 1–4 are colonized with *U.crassus*, whereas at 5–8 the species is absent.

Water depth in the Sallingbach increased in a downstream direction and from NC to C stretches with means of 13 and 22 cm respectively, whereas flow velocity decreased from upstream to downstream (mean: 0.27 m s^{-1} at NC stretches and 0.18 m s^{-1} at C stretches). The hypothesis that lower flow velocity may influence sedimentation rates of fines at the downstream stretches was not supported by regression analysis (data not shown). In the multivariate discriminant analysis, physicochemical parameters were used to characterize C and NC stretches of the Sallingbach. The discriminant analysis correctly assigned 91.7 % of the cases. The most important parameters for the discrimination between C and NC stretches were interstitial NH_4–N concentration (Canonical Coefficient: 0.676),Specific Con-

Table 3.2: Standardized canonical coefficients of physicochemical parameters incorporated as variables in a discrimant analysis for the discrimination of C ($n = 4$) and NC ($n = 4$) stretches in the Sallingbach.

Variable	**Standardized Canonical Discriminant Function Coefficients**
NH_4–N ($mg\,L^{-1}$) in 5 cm substratum depth	0.676
Specific Conductance in free-flowing water	-0.637
Specific Conductance in 5 cm substratum depth	0.492
pH in free-flowing water	0.46
pH in 5 cm substratum depth	0.666
Deposition of particles < 6.3 mm	-0.324
Water depth	0.516

ductance in the free-flowing water (- 0.637) and pH in the substratum (0.666) (Table 3.2).

3.5 Discussion

The aim of this study was to characterize the habitat of the endangered unionid species *Unio crassus* by determining physicochemical key parameters of the free-flowing water and the interstitial zone in a small upland stream.

3.5.1 Chemical water quality

In the Sallingbach, recovery of the *Unio crassus* population in C stretches (Ansteeg, 2010) occurred under elevated NO_3–N mean concentration regimes of 4.1 mg L^{-1} in the substratum and 6.4 mg L^{-1} in the free-flowing water. Furthermore, nitratenitrogen concentrations in the reference stream hosting a self-sustaining *U.crassus* population also averaged values > 2.5 mg L^{-1} NO_3–N both in the free-flowing water and in the hyporheic zone. This finding is in contradiction to other studies, where impaired vitality and reproduction of unionid mussel populations was linked to elevated nitrate nitrogen concentrations in streams (Hochwald and Bauer, 1990; Zettler et al., 1994; Hochwald, 1997; Hus et al., 2006; Douda, 2010). Hochwald (1997) reported that successful reproduction of *U.crassus* populations is reduced at 3.6 mg L^{-1} NO_3–N and completely ceases at concentrations higher than 5.0 mg L^{-1} NO_3–N. Consequently, nitrate nitrogen concentrations below 2.2 mg L^{-1} NO_3–N are commonly accepted as a threshold value for streams hosting functional populations of *U.crassus* (Hochwald, 2001; Koehler, 2006; Zettler and Jueg, 2007). In contrast to these observations in the field and in line with the results described herein, laboratory experiments also indicate an absence of direct negative effects of nitrate on juvenile mussels. Acute nitrate nitrogen toxicity tests (96 h) with juvenile *U.crassus* showed extraordinarily high LC50 values of > 1200 mg L^{-1} NO_3–N, placing the species amongst the least sensitive groups of freshwater animals (Douda, 2010). It is therefore assumed that elevated nitrate nitrogen concentrations might rather act as an indirect indicator for contamination with other nitrogen compounds such as nitrite and ammonia, which are released during nitrogen transformation pathways (Douda, 2010; Patzner and Mueller, 2001; Mueller et al., 2012).

Previous nitrate (NO_3^-) measurements in the Sallingbach show that this mussel population was exposed to elevated concentrations during the last 20 years. According to Hochwald (1990), nitrate concentrations averaged a mean of around 35 $\mathrm{mg\,L^{-1}\,NO_3{}^-}$ in 1990 ($\approx$ 7.7 $\mathrm{mg\,L^{-1}\,NO_3}$–N, n = 4) and Brandner (1997) measured a mean nitrate nitrogen concentration of 6.3 ± 2.6 $\mathrm{mg\,L^{-1}\,NO_3}$–N (n = 15) in 1997. Unpublished data of the regional watershed authorities show that there is no significant seasonal variation in the nitrate nitrogen concentration, which ranged around $6.9 \pm 0.9\,\mathrm{mg\,L^{-1}\,NO_3}$–N (n = 32) from 2007 to 2010. Therefore, the measured elevated nitrate nitrogen concentration in the Sallingbach as well as in the reference stream did not prevent juvenile recruitment, and thus cannot be used as an indicator for a functional population in this study.

Mean concentrations of nitrite and ammonium-nitrogen in the substratum, which may be formed from nitrate through respiratory denitrification and in nitrate reduction processes to ammonium (Burgin and Hamilton, 2007), were in general higher at C than at NC stretches. Ammonia is considered to be of minor toxicity to unionid mussels in its ionic form $NH_4{}^+$, which is mainly prevalent at a pH below 7 (Augspurger et al., 2003). PH values in the substratum ranging from 7.5 up to 8.0 at C and NC stretches did not significantly differ from those measured in the free-flowing water. Based on our data, it is yet not possible to determine the actual toxicity of ammonia depending on pH and temperature. Therefore, further analyses should focus on determining the causal mechanisms that explain the influence of nitrogen compounds on the chemical water quality in the interstitial depending on physical parameters such as temperature and pH.

Mean phosphate phosphorous concentrations in the free-flowing water as well as in the substratum are in line with other PO_4–P values reported from functional *U.crassus* streams (Buddensiek et al., 1993). Like Engel (1990) and Fleischauer–Roessing (1990), we did not find a correlation between the occurrence of young Unio and the concentration of phosphate phosphorous within the sediment water.

3.5.2 Physical stream bed characteristics

Both C and NC stretches in the Sallingbach are characterized by high fine sediment deposition, reaching a maximum of 38.7 $\mathrm{kg\,m^{-2}\,month^{-1}}$ in study stretch 1. The increased levels of fines seem to have a strong effect on the exchange rates between the free-flowing water and the interstitial system, which is indicated by the pronounced difference of measured redox potentials between the free-flowing water and the interstitial zone. Since increased fine sediment loads and low interstitial redox potentials were also recorded at stretches with highest mussel densities in the study stream (Ansteeg, 2010), differences in the occurrence and distribution of the *U.crassus*population at C and NC stretches within the Sallingbach cannot be explained by these habitat factors. Although other studies point out the general importance of substratum quality for the recruitment of juvenile mussels of the Unionoidea occurring in Central Europe (Buddensiek et al., 1993; Engel, 1990; Fleischauer-Roessing, 1990; Geist and Auerswald, 2007), we observed relatively low mean interstitial exchanges at river stretches with juvenile *U.crassus* recruitment. High Eh values at several measuring spots however indicate the existence of interstitial sections with better oxygen supply, which are not evident when only comparing mean values. Measured specific conductance values are in line with results from other studies on functional *U.crassus* streams (Hus et al., 2006), though they varied widely between stream stretches Elevated specific conductance at non-colonized stretches may reflect runoffs from agricultural surfaces as extensive land use is mainly found in the area of colonized stretches in the Sallingbach. The lower water levels in NC stretches of the Sallingbach may expose mussels in this area to an elevated risk of draughts. Yet, it seems unlikely that draughts caused the population decline in NC reaches, as water authorities did not observe a drying out of the stream in the last decade. The multivariate analysis for the discrimination between C and NC stretches proposed that the most important discriminant parameters were ammonium nitrogen concentration in the interstitial water, specific conductance and pH, where higher values were measured at C stretches, respectively. The variables that were found for the group assignment were chemical water compounds neglecting physical parameters which are often found to discriminate between functional and nonfunctional habitats in other studies on freshwater mussel ecology (Geist and Auerswald, 2007; Hastie et al., 2000; Buddensiek et al., 1993). Concluding from our results, we propose that functional *U.crassus* populations have a higher tolerance to poor substratum quality on a population level compared to other native endangered stream dwelling unionoid species such as *Margaritifera margaritifera* than previously thought, because i) it has a higher dispersal ability, resulting from a prolonged spawning period lasting up to 4 months, from multiple breeding events per season (Hochwald, 1997), as well as from species-specific specialities such as the spurting behavior (Vicentini, 2005). These behavioural traits may increase the chances of a successful host fish infestation which ii) is also supported by a higher number of suitable fish species, which occupy different habitats, such that *U.crassus* is able to spread glochidia over a wider range of habitats within a stream (Douda, et al., 2012; Eroes et al., 2003; Pander and Geist, 2010; Taeubert et al., 2012). This will in turn increase the probability of finding high quality substrates within the stream for the post-parasitic phase (Waechtler et al., 2001). iii) The post-parasitic phase of *U.crassus* is relatively short and can be completed after two years (Hochwald, 1997). Therefore, microsites of high physicochemical stream bed quality and substratum stability are only required during a significantly shorter period of time compared to juvenile freshwater pearl mussels, which are known to live buried within the substratum for at least five years.

3.5.3 Implications for conservation

The presented results suggest that previously assumed analogies on habitat requirements of different unionoids such as high exchange rates between the free-flowing water and the interstitial system have to be questioned critically, even if the species have a comparable life cycle. At least for the stream investigated herein, fine sediment deposition and nitrogen load could not mechanistically predict recruitment of *U.crassus*. However, both factors can also be indicative of other pollution problems and should thus not be generally overlooked. The results of this study also suggest that other factors such as predation or host fish availability (Strayer et al., 2004; Douda et al., 2012; Taeubert et al., 2012) as well as other chemical (e.g. pesticides, TOC, heavy metals) and hydrological factors (e.g. changes of discharge regimes) not investigated herein need to be considered for an effective conservation of the species.

4 Fish species composition and host fish density in streams of the thick-shelled river mussel (*Unio crassus*)–implications for conservation

A similar version of this chapter was published: Stoeckl K, Taeubert, JE, Geist J. 2014. Fish species composition and host fish density in streams of the thick- shelled river mussel (*Unio crassus*)– implications for conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 25: 276–287.

4.1 Abstract

The recruitment and dispersal of freshwater mussels of the Unionidae is strongly linked to their host fishes on which the larvae develop into juvenile mussels. Host limitation has been discussed to be one of the potential causes for the decline of the formerly widespread thick-shelled river mussel (*Unio crassus*), although quantitative data analyzing the importance of this factor are still missing. In this study, the status of host fish populations was assessed in a total of 9 *U.crassus* streams within the Danube drainage system situated in southern Germany. In particular, links between recruitment patterns of *U.crassus* populations, fish species composition and quantitative data of host fish densities in functional and non-functional streams were established. Fish species composition and richness ranged between three and nine species with no difference in presence data between functional and non-functional streams. Despite this high variability, functional *U.crassus* streams with high proportions of juvenile mussels had significantly higher (mean: 40 Ind. $100\,\mathrm{m}^{-2}$) densities of primary hosts (*Phoxinus phoxinus*, *Squalius cephalus*, *Gasterosteus aculeatus*) than non-functional streams (mean: 8 Ind. $100\,\mathrm{m}^{-2}$). In contrast, no relationship was found between the numbers and densities of juvenile mussels and densities of poor hosts as well as of non-hosts. The results of this study indicate that the recruitment status of *U.crassus* strongly depends on the density of primary host fish species. Host limitation is likely to play a major role for a lack of juvenile recruitment and consequently for the decline of central European *U.crassus* populations. Therefore, future conservation for this endangered mussel species should more thoroughly incorporate management of host fish species and

consider both the requirements of mussels and their hosts in habitat restoration.

4.2 Introduction

Freshwater mussels are considered one of the most threatened biota worldwide (Lydeard et al., 2004; Bogan, 2008). Due to dramatic declines of a high number of populations during the last century, they have become priority species in aquatic conservation (Geist, 2010). Among the European unionoid species, the thick-shelled river mussel (*Unio crassus*) was one of the most widespread and highly abundant freshwater bivalve species until the beginning of the 20^{th} century (Israel, 1913; Zwanziger, 1920; Jaeckel, 1962). *U.crassus* formerly occurred in high densities up to 700 $\mathrm{Ind.\,m^{-2}}$ (Tudorancea and Gruia, 1968) and inhabited a wide range of different habitats, which is attributed to its high environmental plasticity (Hochwald, 1997). Today, the majority of the remaining populations lack juvenile recruitment and are in a non-equilibrium state in which extinction exceeds recolonization rates, similar to observations in other freshwater bivalves (Vaughn and Taylor, 2000). At present, *U.crassus* is considered endangered in several European countries. For instance, a population loss of up to 90 % within the last decades was estimated for Germany (Engel and Waechtler, 1989; Zettler and Jueg, 2007). The species is listed in annexes II and IV of the EC Habitats Directive (Council of the European Communities, 1992; Bouchet et al., 1999). The Habitats Directive aims to protect species and habitats of European interest, following particular criteria given in the directive. For example, species on Annex II require designation of Special Areas of Conservation, whereas Annex IV lists species which are in need of strict protection. In addition, *U.crassus* is listed as endangered in the IUCN Red List of threatened species (Van Damme, 2011).

Unionoids have a complex life cycle involving an obligate phase on suitable host fish. It has long been known that the dispersal and population status of unionoid mussels is highly dependent on the availability of appropriate host fish during the reproductive period (Bauer et al., 1991; Watters, 1992; Bogan, 1993; Vaughn and Taylor, 1999; Oesterling et al., 2008). For instance, Kat and Davis (1984) as well as Watters (1995) found that extirpation of mussels from several rivers was linked to the disappearance of their host fishes. In addition, mussel species have been documented to reinvade streams after reintroduction of their hosts (Smith, 1985). Host fish limitation has also been proposed to be the main factor for declines in mussel species with a broad habitat tolerance (Vaughn and Pyron, 1995), whereas no such limitation seems to occur in highly specialized species such as *Margaritifera margaritifera* (Geist et al., 2006). Previous studies on host fish suitability for *U.crassus* development identified pronounced differences in metamorphosis success among different host fish species as well as highly different fish communities in functional *U.crassus* streams (Taeubert et al., 2012a, b). However, these studies did not consider comparisons with populations that currently have insufficient recruitment. The disruption of the host-parasite relationship may have played a crucial role in the current decline of *U.crassus.* Results of a host availability analysis in current and former *U.crassus* streams in the Czech Republic suggests that the evaluation of host limitation without precise data on host compatibility or fish abundance is ineffective for identifying the risk of host limitation (Douda et al., 2012). To date, information on the links between densities of host fishes with mussel recruitment is still lacking.

The core objective of this study was to compare the status of host fish populations in functional and non-functional *U.crassus* streams in order to deduce management implications for fish stock conservation; in particular, we tested the hypothesis that the quality and quantity of fish hosts in natural streams is directly linked to the density and the recruitment patterns of *U.crassus* populations.

4.3 Material and Methods

4.3.1 Sampling area and mussel populations

Fish community structure and host fish density was quantitatively assessed at 18 sites in a total of nine streams in Upper and Lower Bavaria, Germany (Figure 4.1).

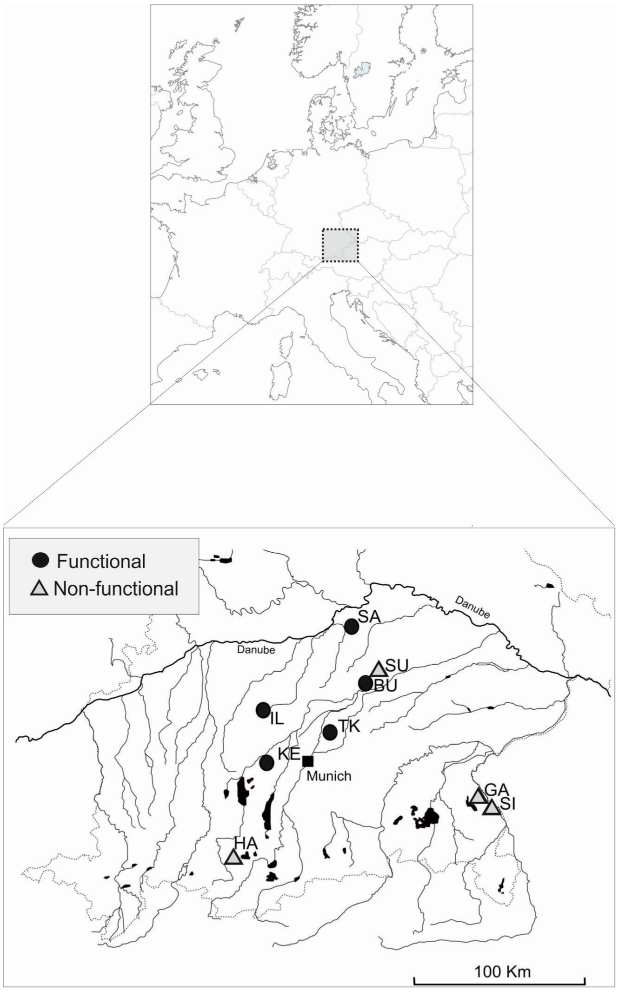

Figure 4.1: Location of the study streams, subdivided into functional (circles) and non-functional (triangles) populations. Codes of the study streams are given in Table 4.1

All investigated streams belong to the upper Danube drainage and are characterized by calcareous water; their mean widths range from one to six meters and the total length varies between 1.5 and 17.0 km (Table 4.1). The selection of the study streams and sites was based on the available information on the distribution and recruitment status of the *U.crassus* populations obtained from recent mussel surveys (for references concerning the relevant mussel surveys, see Table 4.1).

The streams were grouped into two categories according to current presence, density and juvenile recruitment of *U.crassus* (data see Table 4.1) : 1) five streams with recent recruitment and a proportion of juveniles of $> 20\,\%$ as well as mussel densities of $>$ three individuals m^2 were classified as *functional* ('F'); 2) four streams which had low numbers of juvenile mussels $\leq 20\,\%$ or no vital *U.crassus* population (recent extinctions, existing mussel population further downstream = 'E') were categorized as *non-functional* ('NF'). Recruitment of juvenile mussels in the F streams occurred naturally except for the "Sallingbach": here, European minnows (*Phoxinus phoxinus*, Linnaeus, 1758) that had been infested with glochidia were stocked every two years between 1989 and 2001.

4.3.2 Assessment of fish communities and densities

Fish populations were sampled with a 1.5 kW portable electrofishing backpack unit (FEG 1500, Efko, Leutkirch, Germany), wading from downstream to upstream direction with a single anode following the German standard (VDFF, 2000).
In order to assess the fish community in the area of potential natural infection with glochidia and to account for the structural diversity of streams, sampling was performed in two 200 m stretches per stream directly adjacent to the sites were mussels were present. In 'E' streams, electrofishing was carried out in areas where spent shells documented the former occurrence of *U.crassus*.
For assessment during the relevant time of glochidial release, electrofishing was carried out during the reproductive season of*U.crassus* between April and June 2012. To allow a quantitative analysis of the fish assemblage, prior to the sampling, each single study stretch was blocked with stop nets at the upstream and downstream ends to prevent fish leaving or entering the sampling area during the investigation. Fishing was conducted in three subsequent removal runs per study stretch. The stunned fish were collected with a dip net and kept in plastic tanks with permanent oxygen supply. After sampling, all fish were released into the same stream stretch they were taken from. Species richness and host fish density

Table 4.1: Location and classification of the studied *U.crassus* streams in the upper Danube drainage. Streams are categorized as functional (F) and non-functional (NF). [1] Streams with extinct populations are marked with E (extinct). Information on the *U.crassus* populations are based on [a] Ansteeg, 2012a; [b] Nagel, 2009; [c] Ansteeg, 2012b; [d] Ansteeg, 2010; [e] Stoll, 2004; [f] Stoeckl and Hamp, 2011, [g] Stoeckl (unpublished results from 2013), [h] Mueller (unpublished results from 2012), [i] Haas (pers. Comm. from 2012). *For the stream GT, the populated length is given in brackets.

Code	Stream	Subdrainage	Mean width	Total length	*U.crassus* population size	Max. Mussel density	Juveniles	Functionality categories
SU	Sueßbach[h]	Pfettrach	1.5	5.5	0	0	0	NF (E)[1]
HA	Haselbach[i]	Staffelseeach	2.0	4.0	0	0	0	NF (E)[1]
GT	Goetzinger Ache[a]	Salzach	6.0	17.5 (4.5)*	500	0.03	10	NF
SI	Schinderbach[ag]	Salzach	1.5	4.5	800	n.A.	20	NF
BU	Bucher Graben[c]	Pfettrach	1.5	8.5	4.000	6.0	39	F
SA	Sallingbach[dg]	Abens	2.0	8.8	2.600	1.6	31	F
KE	Krebsenbach[eg]	Amper	1.5	4.0	10.000	7.5	44	F
IL	Ilm[g]	Abens	1.0	2.0	500	4.0	50	F
TK	Theresienkanal[f]	Isar	1.5	1.5	1.500	4.9	70	F

was calculated for each stream, with all data referring to the cumulative catch.

4.3.3 Classification of host fish

According to published literature (Bednarzcuk, 1986; Engel, 1990; Hochwald, 1997; Maaß, 1987, Douda et al., 2012; Taeubert et al., 2012a, b), several fish species are suitable hosts for the glochidia of *U.crassus*.
As evident from different transformation rates, the fish species differ significantly in their suitability. The classification of the fish hosts in this study was mainly based on Taeubert et al. (2012a, b) who analyzed suitability of fish hosts originating from the upper Danube drainage system. Depending on their suitability, the caught fish species were assigned to four groups: A) “primary hosts”: species with highest transformation rates in the study region, i.e. referred to as “good hosts” in Taeubert et al. (2012a, b): chub (*Squalius cephalus* Linnaeus, 1758), European minnow (*Phoxinus phoxinus* Linnaeus, 1758) and stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) for the species found in this study; B) “poor hosts”: fish species that revealed low transformation rates, i.e. referred to as “hosts” in Douda et al. (2012); C) “non-hosts” includes fish species that are unsuitable for hosting *U. crassus* according to Taeubert et al. (2012a, b) and Douda et al. (2012); D) “unknown”: fish species whose suitability is currently unknown and has not been tested. A full categorization of all fish species caught in this study is shown in Table 2.

4.3.4 Statistical analyses

Differences in mean host fish densities as well as differences in the proportion of juveniles between F and NF streams were tested using the non-parametric Mann-Whitney-U test. Possible links between density of hosts and proportion as well as density of juvenile mussels were investigated using linear regression analyses considering the different categories of host suitability. Densities of juvenile mussels were calculated from the estimated proportion of juveniles within the populations and the populated area. All statistical analyses were performed in R, version 2.12.0 (R Foundation for Statistical Computing, 2010).

4.4 Results

4.4.1 Fish species richness and composition

A total of 3013 fish representing 20 different species were caught at 18 study sites (Table 4.2). Fish species richness per stream was highly variable both in functional and non-functional *U.crassus* streams and ranged between three and nine species. Maximum species diversities of nine different fish species were found in one F and in two NF streams. Likewise, species composition differed widely among the study streams. *S.cephalus*, a primary host of *U.crassus*, was found most frequently, occurring in 89 % of study streams. In contrast, the primary host *P.phoxinus* was predominantly recorded in functional streams (Table 4.2). Interestingly, *P. phoxinus* was not detected in the Sallingbach, where the fish species had been stocked over a period of more than 10 years. *G.aculeatus*, the third primary host, was only present in 3 study streams. The occurrence of poor host fish species (*L.leuciscus*, *S.trutta f.*, *A.bipunctatus*, *P.fluviatilis*) ranged between 33 and 44 % in the study streams and was also not associated with the functionality status of the mussel population. European Eel (*Anguilla anguilla*), which is considered to be a predator for juvenile Unionoid mussels (Geist et al., 2006), was found in 56 % of all study streams and occurred in functional and non-functional streams. Other non-native fishes, *Oncorhynchus mykiss* and *Pseudorasbora parva*, were recorded in one stream. *P.parva* reached high densities and co-occurred with *P.phoxinus* in one functional stream (IL).

4.4.2 Host fish density

Similar to species richness, total fish density was highly variable among streams and ranged between 6.2 (F, SA) and 212.3 (F, IL) $\mathrm{Ind.\,100\,m^{-2}}$. F streams with high recruitment rates had higher densities of primary hosts (41.6 ± 37.5 $\mathrm{Ind.\,100\,m^{-2}}$) compared to NF streams (8.4 ± 5.4 $\mathrm{Ind.\,100\,m^{-2}}$) (Figure 4.2), although this difference was only significant if data from the F stream SA (where stocking with primary hosts was carried out) were excluded from the analysis (Mann-Whitney U-test, $p < 0.05$).

Table 4.2: Fish species distributions and fish densities in a total of nine *U.crassus* streams with a different population status (F = functional. NF = non-functional). Classification for the suitability of fish species as hosts for U. crassus is based on Taeubert et al., 2012a,b; Bednarczuk, 1986; Engel, 1990; Hochwald, 1997 and Douda et al., 2012. Note that in the Sallingbach (SA**) artificially infected host fish species were stocked regularly.

		Study streams											
		F					**NF**						
Host status	**Species**	**TK**	**IL**	**SA****	**KE**	**BU**	**SI**	**GT**	**SU**	**HA**	**Mean density F [range]**	**Mean density NF [range]**	**Mean density**
		Ind. / 100 m²											
Primary (A)	***Squalius cephalus*** *(Linnaeus, 1758)*	42.3		1.0	37.8	5.6	13.1	9.9	3.8	0.6	**17.3** [0.0 – 42.3]	6.9 [0.6 - 13.1]	14.3
	Phoxinus phoxinus *(Linnaeus, 1758)*	2.5	101.3			16.8			4.3		**24.1** [0.0 – 101.3]	1.1 [0.0 - 4.3]	31.2
	Gasterosteus aculeatus *(Linnaeus, 1758)*	0.3		0.1					2.0		**0.1** [0.0 – 0.3]	0.5 [0.0 - 0.2]	0.8
Poor (B)	***Leuciscus leuciscus*** *(Linnaeus, 1758)*				0.2		13.3	3.4			**0.0** [0.0 - 0.2]	4.2 [0.0 - 13.3]	5.6
	Salmo trutta f. *(Linnaeus, 1758)*				7.3	0.4			1.3	17.1	**1.5** [0.0 - 7.3]	4.6 [0.0 - 17.1]	6.5
	Alburnoides bipunctatus *(Bloch, 1782)*				1.3		0.3	6.9			**0.3** [0.0 - 1.3]	1.8 [0.0 - 6.9]	2.8
	Perca fluviatilis *(Linnaeus, 1758)*	1.8			21.3		2.3	0.1			**4.6** [0.0 - 21.3]	0.6 [0.0 - 2.3]	6.3
	Pseudorasbora parva *(Temminck & Schlegel)*		83.5								**16.7** [0.0 - 83.5]	0.0 [0.0]	83.5
Non-host (C)	***Barbus barbus*** *(Linnaeus, 1758)*						1.1	2.1			**0.0** [0.0]	0.8 [0.0 - 2.1]	1.6
	Alburnus alburnus *(Linnaeus, 1758)*						0.5				**0.0** [0.0]	0.1 [0.0 - 0.5]	0.5
	Tinca tinca *(Linnaeus, 1758)*				0.3			1.7			**0.1** [0.0 - 0.3]	0.4 [0.0 - 1.7]	1.0
	Rutilus rutilus *(Linnaeus, 1758)*	0.5			0.2		5.4	0.3			**0.1** [0.0 - 0.5]	1.4 [0.0 - 5.4]	1.6
	Abramis brama *(Linnaeus, 1758)*						0.1				**0.0** [0.0]	0.0 [0.0 - 0.1]	0.1
	Anguilla anguilla *(Linnaeus, 1758)*			0.3	0.7		3.0	0.1		3.3	**0.2** [0.0 - 0.7]	1.6 [0.0-3.3]	1.4
	Barbatula barbatula *(Linnaeus, 1758)*		27.5			33.4			12.5		**12.2** [0.0 - 33.4]	3.1 [0.0 - 12.5]	24.5
	Silurus glanis *(Linnaeus, 1758)*							0.1			**0.0** [0.0]	0.0 [0.0 - 0.1]	0.1
	Gobio gobio *(Linnaeus, 1758)*			4.8	0.7	0.4			4.8		**1.2** [0.0 - 4.8]	1.2 [0.0 - 4.8]	2.6
	Oncorhynchus mykiss *(Walbaum, 1972)*								2.0		**0.0** [0.0]	0.5 [0.0 - 2.0]	2.0
Unknown (D)	***Esox lucius*** *(Linnaeus, 1758)*			0.4							**0.1** [0.0 - 0.4]	0.0 [0.0]	0.4
	Lota lota *(Linnaeus, 1758)*			0.3							**0.1** [0.0 - 0.3]	0.0 [0.0]	0.3
	Number of species (n)	5	3	6	9	5	9	9	7	3	5.6	7.0	6.2
	Number of A + B species (n)	4	2	2	5	3	5	5	4	2	3.2	4.0	3.6
	Density category A Ind. / 100 m²	**45.1**	**101.3**	**1.1**	**37.8**	**22.4**	**13.1**	**9.9**	**10.1**	**0.6**	41.6	8.4	26.8
	Density category B Ind. / 100 m²	1.8	83.5	0.0	30.1	0.4	17.0	12.5	1.3	17.1	23.2	7.7	18.2
	Density category C Ind. /100 m²	0.5	27.5	5.1	1.9	33.8	9.0	2.2	19.3	3.3	13.8	8.5	11.4
	Ratio of (A+B)/C	93.8	6.7	0.2	35.7	0.7	3.3	10.2	0.6	5.4	27.5	4.4	17.1

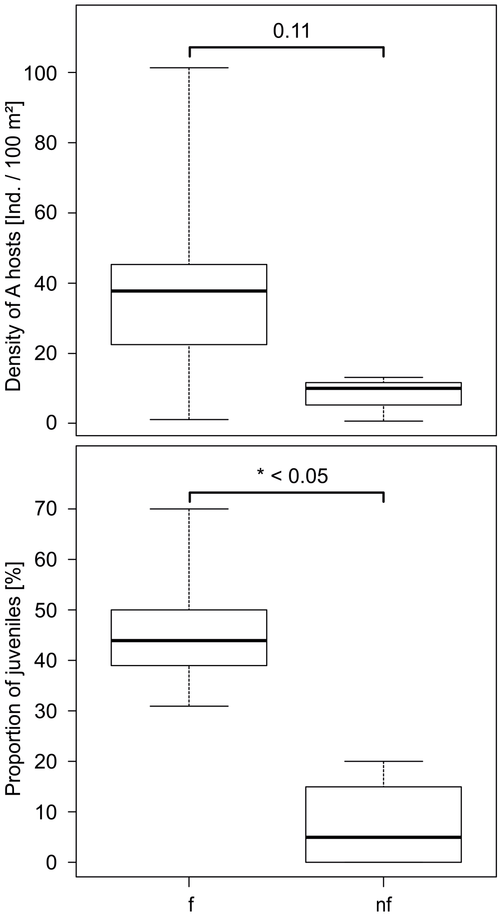

Figure 4.2: Proportion of juvenile mussels (%) and density of primary host fish (Ind. $100\,\mathrm{m}^{-2}$) in functional ‘F’ (n = 5) and non-functional (n = 4) streams. Significant differences are marked with * (Mann-Whitney U-Test; $p < 0.05$). Note that the density of A host fish significantly differs between F and NF streams ($p < 0.05$) if data of SA are excluded from the analysis (In SA, *P.phoxinus* had been artificially stocked, but is now absent). Boxes are 0.75 and 0.25 percentiles and medians. Whiskers indicate the maximum range of data points

As evident from the high standard deviation in F streams, no distinct threshold value of primary hosts could be determined. The minimum density of primary hosts was found in the NF stream HA (0.6 Ind. $100\,\mathrm{m}^{-2}$), whereas maximum density of 101.3 Ind. $100\,\mathrm{m}^{-2}$ was recorded in the F stream IL (see Table 4.2). Along with total fish densities, densities of primary hosts were only 1.1 Ind. $100\,\mathrm{m}^{-2}$ in the F stream SA, despite of the stocking with high numbers of *P. phoxinus.* Densities of the primary hosts *S. cephalus* and *P.phoxinus* in F streams averaged 17.3 and 24.1 Ind. $100\,\mathrm{m}^{-2}$ respectively, compared to 6.9 and 1.1 Ind. $100\,\mathrm{m}^{-2}$ in NF streams. *G.aculeatus* was only present at low abundances in three study streams, ranging between 0.1–2.0 Ind. $100\,\mathrm{m}^{-2}$. In line with the results for primary hosts, mean densities of poor host fish (category B) were also higher in F streams, averaging 27.5 Ind. $100\,\mathrm{m}^{-2}$, while the mean density in NF sites was 7.7 Ind. $100\,\mathrm{m}^{-2}$, although this relationship was not significant (Mann-Whitney U; $p > 0.05$). Density of non-hosts (category C) was not significantly different between F (mean: 13.8 Ind. $100\,\mathrm{m}^{-2}$ and NF streams (mean: 8.5 Ind. $100\,\mathrm{m}^{-2}$) (Mann-Whitney U; $p > 0.05$).

4.4.3 Relationship between host fish density and juvenile recruitment

Regression analyses revealed a significant positive relationship between the density of primary hosts (category A) and the proportion of juvenile *U.crassus* within a population ($y = 0.892x + 0.673; R^2 = 0.45$; $p < 0.05$) (Figure 4.3).

The strength of this relationship strongly decreased if only poor hosts ($y = 0.285x+9.825; R^2 = 0.066, p = 0.51$) or unsuitable fishes (referred to as ´no hosts´; ($y = 0.035x + 10.359; R^2 = 0.005; p = 0.86$) were considered. Using total fish density, an intermediate result was obtained ($y = 1.033x + 26.115; R^2 = 0.161; p = 0.28$). Considering absolute juvenile mussel densities instead of the proportion of juvenile mussels of the total population, no significant relationship was obtained (data not shown) since the total number of mussels likely depends on other factors such as the carrying capacity of the streams. Also, mussel population size and maximum density were not related to any of the host fish density parameters, as revealed by regression analyses ($p > 0.05$).

4.5 Discussion

The conservation status of freshwater mussel species has recently been linked to a limited availability and dispersal of appropriate host fish (Strayer, 2008; Schwalb et al., 2011), although the frequency and severity of host limitations as well as the conditions, under which host availability controls Unionid abundances, is still unknown for most mussel species (Strayer, 2008). Population losses of the formerly

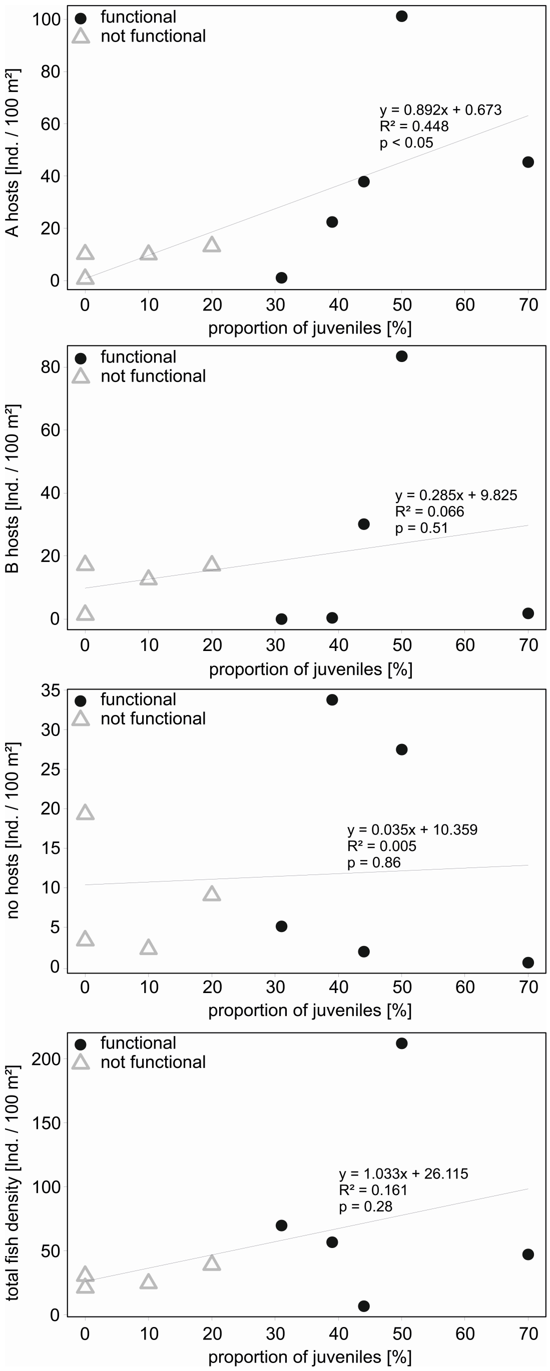

Figure 4.3: Relationships between primary (category A), poor (B) and no-host fish (C) as well as the total host fish density (Ind. $100\,\mathrm{m}^{-2}$) and the proportion of juvenile mussels (%) in *U.crassus* streams. Note that the relationship between A host fish and the proportion of juvenile mussels (%) is stronger if data of SA are excluded from the analysis (In SA, *P.phoxinus* had been artificially stocked, but is now absent). To facilitate comparisons between different categories of host suitability, regression lines are shown in all graphs, irrespective of the relative strength of the relationship

widespread European freshwater mussel *Unio crassus* have been assumed to result from an impaired status of the host fish assemblage (Douda et al., 2012), although detailed information on the relationship between juvenile recruitment of *U.crassus* and host fish density is still lacking.

To our knowledge, this is the first study which links quantitative data on fish species assemblage and densities with the current recruitment status of endangered *U.crassus*. The results of this study suggest that i) the functionality status of an *U.crassus* population is independent from fish species richness, but ii) high recruitment rates and the proportion of juvenile mussels are closely linked to a high density of primary host fish species. Consequently, the availability and abundance of primary host fish is one of the most important factors for the recruitment of the thick-shelled river mussel, at least in the upper Danube drainage. The finding of host limitation being the most important bottleneck for *U.crassus* is also supported by investigations into its habitat requirements during the post-parasitic phase where the species appears to be more tolerant to high fine sediment loads and low redox potentials (Denic et al., in press) compared to the freshwater pearl mussel (Geist and Auerswald, 2007).
In line with previous studies on host fish availability for *U.crassus* under natural conditions (Taeubert et al., 2012a), high numbers of co-occurring fish species were found. Since fish species richness did not differ between F and NF streams and since it was not associated with a high proportion of juvenile mussels, the consideration of presence-absence data appears insufficient in assessments of host limitations and habitat suitability for *U.crassus*. The occurrence data of *Phoxinus phoxinus* and *Squalius cephalus*, which are considered the most important host for *U.crassus* in the Danube drainage (Taeubert et al., 2012a), could not be linked to the distribution and functionality status of the mussel populations. Both in functional and non-functional *U.crassus* streams, these host fish species were detected. In particular, *S.cephalus* was found in eight out of nine study streams. *S.cephalus* is a very common and widespread species throughout Europe (Caffrey et al., 2008) with a conservation status of least concern (Freyhof and Kottelat, 2008). The species is known to populate and successfully reproduce in a high variety of different habitats, including artificial streams (Arlinghaus and Wolter,

2003). In contrast, *Scardinius erythrophthalmus* which is also described to be a good host for *U.crassus* in the Danube drainage (Taeubert et al., 2012a) was not detected in any of the nine study streams. The low discriminative power of fish species data in separating F and NF streams in this study is in line with results from previous studies that were unable to predict mussel distribution data based strictly on host fish distribution data (Bauer et al., 1991; Strayer et al., 1994). Douda et al. (2012) examined the fish species assemblages in current and extirpated *U.crassus* populations in the Czech Republic and found that the mussel population status could not be explained by host limitation if only fish distribution data were analyzed.

In contrast to the findings in *U.crassus*, high fish species richness can be indicative of adverse habitat conditions in the highly specialized freshwater pearl mussel (Geist et al., 2006). Pristine *Margaritifera margaritifera* streams are typically oligotrophic habitats with a low number of highly specialized fish species, such as *Salmo trutta f.*, the main host of *M.margaritifera* in central Europe. Eutrophication and habitat destruction can result in increased species numbers due to the occurrence of generalists. In contrast, *U.crassus* has been described to have a much broader environmental plasticity and natural distribution range (Hochwald, 1997), naturally encountering a greater diversity of fish communities. Therefore, fish species assemblages are expected to be highly variable in functional *U.crassus* streams, depending on the type of stream habitat.

An important finding of this study is the positive correlation between the density of primary hosts and the proportion of juvenile mussels, indicating that host fish density effects play a crucial role for the functionality of an *U.crassus* population. Whilst regression and correlation analyses must not be interpreted in a causal way, the results from this study are in line with findings from North America, where Mulcrone (2004) found that abundance of several mussel species was also positively correlated with abundance of individual host species. The weaker relationship between poor hosts and the proportion of juvenile mussels indicates that they are less important for determining recruitment success. Also in previous studies, no functional *U.crassus* population which is exclusively dependent on poor hosts was identified (Taeubert et al. 2012a). The absence of a negative relation between

unsuitable hosts (´no hosts´) and the proportion of juvenile mussels, in spite of up to 34 Ind. $100\,\mathrm{m}^{-2}$ in F populations, indicates that competition for glochidia between suitable and unsuitable hosts does not seem to be relevant under natural conditions. The high variability of host communities and the different suitability of various fishes as hosts for *U.crassus* make the determination of one critical threshold value as a minimum requirement for a functional population very difficult, particularly since the survival rates of other phases in the life cycle are currently unknown and probably also highly variable among the diverse habitats. Taeubert et al. (2012a) found densities of primary host fishes in functional *U.crassus* populations in a range of 1–12 Ind. $100\,\mathrm{m}^{-2}$ in single electrofishing runs. Based on three electrofishing runs of this study, the mean value of > 40 Ind. $100\,\mathrm{m}^{-2}$ and the range of 22–101 Ind. $100\,\mathrm{m}^{-2}$ primary hosts (excluding the stocked SA) can provide a rough guideline for orientation, but should be used with caution due to different catch efficiencies depending on gear and stream types.
The observed host limitation in *U.crassus* was unexpected, particularly since the species has a distinct spurting behavior to attract fish hosts when glochidia are being released (Vicentini, 2005). Haag and Warren (1998) and Haag (2012) proposed that host limitation is less likely in bivalves that have specific strategies for active host attraction, such as mantle lures in some North American species. Obviously, the spurting behavior, the broad spectrum of host fishes and the multiple spawning events are not sufficient to ensure sustainable recruitment of *U.crassus* where there are low densities of primary hosts.

Low abundances or the complete absence of primary host fishes may have multiple causes that are highly different between streams. For example, Douda et al. (2012) states that several host species of *U.crassus* such as the primary host *P.phoxinus* are in decline because of water pollution, habitat modification and other anthropogenic pressures, including excessive stocking of Salmonid species for angling. As for the non-functional streams SU, HA as well as in the functional stream SA in this study, fish species assemblages are known to be altered by stocking of predatory fish species (Schaefer, Haas, pers. Comm.), which may be indicative for the low densities of primary hosts in these streams. Furthermore, although *S.cephalus*, the most important host for *U.crassus* populations in the

Upper Danube drainage, is considered a generalist fish species and often occurs in highly altered habitats, habitat fragmentation may permanently limit fish migration and isolate the mussel population from its host fishes.

Given the wide distribution of the primary hosts within streams of the Danube drainage, correlations between distribution data of host fish species and mussel occurrence may be weak, because mussel populations are controlled by additional factors that do not affect all of their host fishes similarly, e.g. substratum quality and availability of nutrients. The geographic ranges of mussel species may be therefore smaller than those of their hosts, as already proposed in Gordon and Layzer (1993) and Bauer et al. (1991).

4.6 Implications for conservation

Our results underscore the importance of considering the host fish community within conservation and restoration programs for thick-shelled river mussel streams. Host limitation may be a widely distributed, yet an underestimated problem in *U.crassus* streams, because current host fish mapping for this species is mostly performed on a qualitative level only. To date, assessments for *U.crassus* in the scope of the Habitats Directive monitoring programs do not necessarily require a profound analysis of the host fish community, leading to highly different ways of host fish assessment practices in European member states. For example, according to the national guideline for monitoring the host fish community of *U.crassus* streams in Germany, it is considered sufficient for host fish assessments to rely on the opinion of experts instead of conducting electrofishing. Thus, we recommend to standardize host fish community analyses and incorporate a systematic approach into monitoring programs for *U.crassus* and other endangered mussel species in order to effectively detect bottlenecks.

Conservation strategies for *U.crassus* populations require an integrative management approach aiming at sustainably stabilizing the complex relationship between this endangered mussel species and its hosts. First, public awareness of the importance of host fish species for *U.crassus* streams needs to be increased in order to implement suitable fisheries management practices. For example, the active removal of the primary host species *Squalius cephalus* from streams by electrofishing used to be a common practice in fisheries management in order to reduce competition with more highly valuable Salmonid species such as Salmo trutta. In addition, excessive stocking of predators for angling is described to be one of the major threats for the host species *P. phoxinus* (Freyhof and Kottelat, 2008). Practices that have adverse effects on the host fish community should therefore no longer be supported.

The finding that none of the stocked host fish in the functional river Sallingbach were detected in the current study shows that stocking of infected host fish may help to bridge inadequate host fish populations and to promote the recovery of the mussel population as a short-term conservation action. However, for the effective implementation of sustainable conservation strategies that are functional on the long-run, a high priority should be given to improve habitat conditions for primary host fish species in *U.crassus* streams. For example, the strong substratum dependency of some host fish species such as *P.phoxinus* (Bless, 1992) requires the improvement of catchment management practices in order to reduce fine sediment discharge to streams, which negatively affect the quality of stream beds. Furthermore, water temperature plays an important role in development and metamorphosis of *U.crassus* (Taeubert et al., 2013). Thus, effects that substantially impact water temperature, e.g. water withdrawals or discharges of heated wastewater, should also be managed on the catchment level. With regard to the wide host range of *U.crassus* and the highly different habitat requirements of the host fish species, restoration programs should also put effort in increasing structural diversity within streams, e.g. by the creation of microhabitats that differ in current speeds and water depths as well as in their substratum quality.

5 General discussion

The studies presented in this thesis provide novel information on the ecological requirements of the endangered thick shelled river mussel *Unio crassus* as a basis to plan and implement adequate conservation strategies for this species. The results show that current assumptions and knowledge of the habitat requirements of *U.crassus* need to be updated. As presented here, the species can populate streams that vary considerably in substrate and flow regime and it is is not restricted to clear headwater streams. Furthermore, it is more tolerant to low substrate and water quality than previously postulated. Host fish limitation, which had received little attention in conservation for this species so far, was identified as an important threat for *U.crassus*. Based on the new findings, the indicator as well as the ecosystem function of freshwater mussels in highly degraded stream systems is critically questioned (Chapter 5.2.)

5.1 The importance of species-level analysis in freshwater mussel ecology – aspects to consider for the definition of the fundamental niche of *U.crassus*

While most published studies dealing with European freshwater mussels focus on the prominent flagship species *Margaritifera margaritifera*, information on less popular species such as *U.crassus* was still incomplete. Denic (2014) noted that because of the limited availability of scientific studies for some species, current knowledge may thus often be generalized. Since *U.crassus* and *M.margaritifera* are both stream-dwelling mussels co-occurring in some streams and show comparably high population declines, it was frequently concluded that both species are riverine specialists and have highly specific and – at least to some extent – similarly high habitat requirements.

Despite the population losses and the high conservation status, the results of this thesis raise doubt as to wether *U.crassus* can be considered a habitat specialist. Colonization of different microhabitats that vary in current, substrate and depth (Chapter 2) as well as successful recruitment in areas with high nutrients and fine sediments (Chapter 3) strongly suggest that the species has a significantly broader fundamental ecological niche in terms of abiotic habitat conditions and may be regarded as a generalist in this respect. The large natural distribution area (see Introduction, Chapter 1), the occurrence in both uplands and lowlands as well as in streams with variable sizes also justify this classification on a macrohabitat level.

Strayer (2008) and Haag (2012) state that traditional habitat variables fail in many cases in predicting mussel occurrence and aggregations in streams. The reason for this is that most freshwater mussels – except for specialists such as *M.margaritifera* (Geist and Auerswald, 2007) – do not show pronounced microhabitat specialization based on variables such as substrate, current or water depth (e.g. Strayer, 1981, Strayer and Ralley, 1993). This observation suggests that many freshwater mussel species have a broad fundamental niche with respect to abiotic habitat variables. The realized niche is in general much narrower and may be significantly determined by the host fish distribution and availability.

A commonly observed specialization in riverine mussel species is the preference for lentic microhabitats. Haag (2012) distinguishes between lentic habitat specialists, riverine habitat generalists and riverine lentic specialists. The latter occur mainly in lentic environments and are rarely found in the main channel. As shown in Chapter 2 of this thesis, areas characterized by low shear stress values had significantly higher mussel densities and other authors (Zajac and Zajac, 2011) found *U.crassus* predominantly in near-bank areas. Although not having a narrow niche width, *U.crassus* thus may be assigned into the riverine lentic specialists group, at least in the streams studied herein.

While the concept of substrate stability as primary factor determining mussel occurrence has been widely accepted by freshwater mussel researchers in North America (Haag, 2012), it is a relatively new emerging theme in the scientific discussion in Europe. The findings in this thesis (Chapter 2) corroborate the results of North American studies, that measures of substrate stability, such as shear stress, are excellent predictors of mussel occurrence in small brooks as well as in large rivers (Layzer and Madison, 1995, Gangloff and Feminella, 2007, Allen and Vaughn, 2010). Since mussels are long-lived and sedentary animals, stable substrates appear to be a crucial habitat characteristic. Strayer (2008) proposes that substrate stability depends on the local hydraulic conditions, which may

also explain the missing consistency in microhabitat associations across varying stream types (Haag, 2012). I therefore propose to shift from traditional habitat descriptions or at least include measures of complex hydraulic variables describing substrate stability into future assessments of mussel habitats in Europe.
In conclusion, the results of this thesis illustrate the importance of species-specific analysis of habitat requirements to be able to define the ecological niche of a mussel species.

5.2 Is *Unio crassus* an indicator species?

Indicator species are used in ecosystem monitoring and management to assess the state of the overall species community as well as the physicochemical state of their habitat (Simberloff, 1998). Following the definition of Hellawell (1986), presence of a biological indicator species means that certain predefined parameters such as nutrients or dissolved oxygen are within the tolerance limits of the species. The Saprobic System, mainly used in Central Europe to assess water quality, is based on macroinvertebrate indicator species and it assigns streams to different zones of pollution according to the benthic community. In this system, *U.crassus* is listed as an indicator species being characteristic for clean and well-oxygenated streams with low organic pollution. Since the species is able to persist and successfully reproduce under elevated nutrient levels and in various stream types as shown in this thesis, and occurrence of functional populations in degraded streams was also observed elsewhere (Bodis et al., 2015, Lewin, 2014), I critically scrutinize this current classification. In comparison, the distribution of the freshwater pearl mussel is restricted to pristine headwater streams, and a lack of juvenile recruitment indicates the degradation of substrates. Therefore, this riverine mussel species clearly fulfills the criteria of an indicator species (Geist, 2010). In contrast, *U.crassus* has a significantly broader habitat spectrum and it does not seem to be indicative for pristine, unpolluted waters. Juvenile mussels of this species can even be observed in streams, where the streambed has been concreted (pers.observ.).

However, the occurrence and the reproduction of this species in streams may be indicative for other habitat characteristics, such as long-term substrate stability (see Chapter 2), the grade of physical habitat degradation, appropriate nutrient supply or an absence of stochastic events such as drought or floods. Furthermore, it may indicate the status of the co-occurring fish community, which in turn signficiantly determines the realized niche of the mussel species.

One might contentiously question the need for habitat restoration and improvement of water quality for the persistence of certain freshwater mussel species since they seem to be tolerant to adverse conditions. First of all, the results presented herein indeed give some hope for the persistence of the species itself, because populations probably can be sustained in degraded systems that likely do not reach a natural state anymore. However, there is some evidence that the functional role of freshwater mussels is diminished or lost in disturbed systems. While nutrient heterogenity mediated by mussels increased biodiversity of the co-occurring macroinvertebrate communites (Spooner et al., 2013), the relevance of nutrients provisioned by mussels was nearly eliminated in systems with an increased nutrient status. Thus, the ecological function of freshwater mussels may become lost without restoration and improvement of water quality.

5.3 The importance of host fish limitation for *U.crassus*

As a result of the obligate parasitic life stage, distribution and persistence of unionid freshwater mussel populations are critically dependent on the availability of suitable host fish. On a large scale, the geographic distribution of the hosts defines the potential habitat of mussel species (Haag, 2012). On the local level, suitable host fish species must be available in space and time – i.e. during the reproductive season and close to the populated area within a stream–so that glochidial larvae are able to infest their hosts. The case study of this thesis (Chapter 4) clearly revealed the significant role of host fish limitation in *U.crassus* population declines. The finding of a positive correlation between the density of primary hosts and the proportion of juvenile mussels indicates that host fish density is a crucial factor for the functionality of *U.crassus* populations.

Limited host fish availability may not fully explain the Pan-European decline of the species within the last decades. Excessive water pollution from untreated sewage and industrial byproducts (e.g. Johnson et al., 2001) as well as channelization and fragmentation of streams (Dynesius et al., 1994) in the 19^{th} and 20^{th} century in Europe had with no doubt detrimental and long-term effects on the aquatic fauna as a whole. Extensive habitat destruction of streams throughout in Europe probably resulted in many small and isolated mussel populations that can be observed today. Although a threshold value for a minimum viable population size has not been determined yet for any mussel species (Haag, 2012), it is likely that a considerable number of populations became extinct because of too few individuals left, being necessary for population recovery (see Allee-effect, e.g. Rosenzweig, 1995), after habitat destruction.

Nevertheless, it is proposed that the importance of sufficient host fish densities for *U.crassus* populations had been underestimated so far. This assumption is in line with Douda et al. (2012), who suggest that local extirpations of the mussel species in the Czech Republic are linked to an impaired status of the associated fish community as well as to a low density of primary host fish species. Taeubert et al. (2012 a,b) as well as Douda et al.(2012) showed that *U.crassus* uses a wide array of different host fishes that vary in their habitat requirements and in their sensitivity to anthropogenic disturbances. Suitability and availability of host fish species also differs within the natural distribution area of *U.crassus* (Douda et al., 2012; Lamand et al., 2016). The risk of host fish shortages is likely higher in streams where the mussel species is dependent on sensitive host fishes or where only few host fishes co-occur. Small headwater streams feature a fish community that is naturally composed of low numbers of fish species, mostly specialized to riverine conditions. In this type of habitat, primary hosts of *U.crassus* are mainly European minnow *Phoxinus phoxinus*, as well as bullead *Cottus gobio*, two species with declining populations (Kottelat and Freyhof, 2007).

Both fish species inhabit cold, well-oxygenated streams and require clean gravel to complete their life-cycle (Mills and Mann, 1983;) They are sensitive to water pollution, habitat destruction and stocking of predators such as *Salmo trutta f.* for angling (Kottelat and Freyhof, 2007). Mussel population declines in those stream types may be thus determined by the habitat requirements of the hosts rather than by the mussels themselves. This hypothesis is supported by the finding that *U.crassus* is able to inhabit and successfully recruit in highly degraded streams with high densities of less specialized host fish species. In medium – sized to large streams, *U.crassus* uses generalist fish species as primary hosts, such as *Squalius cephalus*, *Scardinius erythrophthalmus*, *Perca fluviatilis* and *Gasterosteus aculeatus*. Interestingly, the decline of *U.crassus* in those habitat types - particularly in large rivers - is considerably less well-documented than in the headwater streams (but see Bodis et al., 2015), probably because of the required high monitoring effort. Regardless of the habitat type, the case study of this thesis clearly shows that assessment and management of the co-occurring fish community need to be incorporated into conservation strategies for *U.crassus*.

5.4 Can we protect what is left? A systematic approach for the conservation of *U.crassus*

The case studies in this thesis notably broadened and updated the knowledge about the ecology of the thick-shelled river mussel, which is necessary to implement effective conservation strategies (Geist, 2010). At the same time, identification of a potential threat that previously had scarcely been considered (Chapter 4) indicates that generalizations or uncritical acceptance of the multiple cause hypothesis of population declines (Haag, 2012) may lead to unfocussed, broad conservation actions that do not address specific causal factors. Since budget is limited for conservation actions and restoring all the remaining mussel habitats thus seems unlikely, a systematic strategy for the protection of populations and their habitats is necessary. I suggest to apply a step-wise approach for the conservation of *U.crassus* adapted from the approach from Geist (2015) for freshwater biodiversity conservation. This approach includes the following seven elements (modified from Geist (2015): 1) Decision on conservation objectives 2) Determination of status quo 3) Identification of problems 4) Setting priorities and making decisions on conservation action 5) Conservation action 6) Evaluation and adaptive management 7) Exchange and share information. Publication of results from scientifc research, accompanying the conservation strategy, should be done during the entire process at each of the seven steps.

1) Decision on conservation objectives: In a first step, the conservation objectives

for *U.crassus* need to be determined. As with all recovery plans for other species, the overall goal of the recovery actions should be to declassify and finally delist the species in its natural distribution area. The objectives thus are to ensure the long-term viability of *U.crassus* in Europe by stabilizing and protecting priority populations. Specific recovery criteria which are a necessary prerequisite to be able to downlist the species in the future should be discussed and determined in accordance with experts and authorities on a European level as well as on a local level. Since properties of a viable population have not yet been fully investigated, results of future research on population dynamics need to be incorporated into the list of recovery criteria.

2) Determination of status quo: Increased efforts in monitoring programs in the last years contributed to a significant improvement in the data set of the present status of the populations. However, for comparable census population estimates, it is necessary to consider new scientific results on the species and consequently update and standardize monitoring guidelines, which has currently been done insufficiently. For example, Zieritz et al. (2014) showed that *U.crassus* displays increased vertical and horizontal movements in summer compared to autumn/winter and the visible proportion of mussels differed by up to 69 % between sites. In the German monitoring protocol however, excavation of mussels is not necessarily required in surveys. Thus, population sizes may be considerably under – or overestimated using the current methodology. Furthermore, Nagel et al. (2016) observed growth cessation in adult individuals of *U.crassus*, indicating that the currently used method for age determination by counting growth lines may underestimate the age of older mussels.

Most habitat surveys in the past years focused on wadeable, small to medium sized streams, probably because those stream types are easy to access and the survey is less time consuming. In order to monitor the species in all its habitat types and to fill critical data gaps, the application of various survey techniques such as SCUBA Diving (e.g. in deep and/or turbid streams), or snorkeling is necessary. Similar to the European Standard for monitoring freshwater pearl mussels and their habitats that is currently being developed (CEN-Standard), I propose to establish a standard for other freshwater mussels species of European interest such as *U.crassus*, that provides standardized guidelines on monitoring and habitat assessments, including population census estimations and survey techniques.

3) Identification of problems: this is a crucial step since it will decide on which conservation measures are required for the persistence of the mussel populations. For example, in contrast to the conservation strategy for *M.margaritifera*, where most populations do not have natural recruitment of juvenile mussels, captive breeding programs for *U.crassus* appear to have less high priority, because there are considerable numbers of populations with juvenile mussels. As shown in this thesis (Chapter 3), juvenile recruitment in this species even occurs in degraded streams with high nutrient levels.
As species-specific conservation projects require to consider the complete life cycle of a species, other problems that were not identified in the cases studies of this thesis may exist on an individual or population level. Information on nutrient requirements of both juvenile and adult mussels, life stage specific sensitivities to waterborne contaminants or on the genetic constitution of populations will help to identify other threats and to deduce appropriate conservation measures.

4) Setting priorities and making decisions on conservation action: prioritization in species conservation should be based on a systematic, objective and scientific approach that considers, amongst others, the threat, viability and genetic distinctness of a population as well as socioeconomic aspects (Geist, 2015). Currently, most conservation projects for *U.crassus* focus on small headwater streams, probably primarily because the stream and catchment area, i.e. the management area, is smaller and thus the financial effort for restoring a suitable habitat seems to be lower. However, I want to point out that those stream types carry a high risk of local extinctions by natural stochastic events, such as drought or floods. The probability of some events may even increase in some areas in the near future, resulting from the effects of climate change (e.g. Lehner et al., 2006). Since headwater streams are only one type of habitat that the species is able to populate, I suggest to considering the probability of local extinctions in the prioritization process to develop a sustainable conservation strategy. Larger streams appear to be important habitats for the persistence of *U.crassus* populations, because a) the probability of host fish shortage is lower b) the probability of local extinction

by natural stochastic events is lower c) Populations in larger streams may act as source for (re-)colonization of smaller streams.

Information on the genetic structure of *U.crassus* populations has not yet been used for prioritization, since development of genetic markers for *U.crassus* being necessary for population genetics is still in its early stages (Sell et al., 2013).Yet, the risk of genetic drift and the loss of genetic variability is particularly high in small populations, which leads to inbreeding and rapid extinctions (Geist, 2010). At the same time, genetic diversity in large populations can also be low, as shown for the freshwater pearl mussel (Geist, 2005), as a result of founder effects or population bottlenecks in the past. In order to sustain evolutionary lineages of *U.crassus* that are able to adapt future changes in the environment, I recommend integrating genetic analyses of populations in selecting priority populations for conservation of this species.

Furthermore, *U.crassus* populations in biodiversity hot spots should have high priority in conservation, because limited budget spent on restoration of those areas will have effects on the overall community and biodiversity. Sophisticated tools such as GAP analysis (e.g. Sowa et al., 2007) may help to identify those priority areas. Overall, I suggest to establish a new prioritization protocol for selecting priority populations by including missing information on *U.crassus*, such as genetic diversity.

5) Conservation action: In a first step, compilation of a working group that assists the implemenation of the recovery actions for *U.crassus* appears necessary. This team should be composed of species experts and represenatives from authorities that should meet periodically to assess the implementation of the recovery objectives and actions. Frequently, conservation projects dedicated to *U.crassus* work independently on a regional scale. In order address the overall objective of conservation for *U.crassus*, information exchange between project managers should be improved and the establishment of an overarching coordination is necessary. The next step is to discuss and define appriopriate conservation action tools, including habitat restoration, development of emergency strategies for severe conditions (e.g. major droughts), research on population biology and ecology as well as development of initiatives to increase public awareness. Since host fish limitation

was identified as an important threat for *U.crassus*, protection and management of the co-occurring fish community should be considered a central element in conservation projects for this species. Depending on the local situation, conservation action may need to include habitat restoration - e.g. by increasing structural heterogeneity, by removing fish migration barriers or by restoring substrate suitable for gravel spawning species (see Pander et al., 2015) – reintroduction of fish infested with glochidia as well as management of stocking activities. The latter requires a close cooperation with stakeholders, such as fishery clubs and local fisheries authorities. Since the host fish species of *Unio crassus* do not have a high economic importance, their conservation is not a central element in fisheries yet. Some species have even been actively removed from streams to reduce competition with the more highly valuable fish species, such as *Salmo trutta f.* Excessive stocking of predators such as Salmonid species is also seen as a threat for some host fishes, e.g. for *Phoxinus phoxinus* (Kottelat and Freyhof, 2007). Effective conservation management for *U.crassus* is thus crucially dependent on an increased public awareness of the importance of the relationships between mussels and fishes as well as on a collaborative involvement of the stakeholders.

6) Evaluation and adaptive management: As proposed by Geist (2015) for freshwater restoration projects, evaluation of the conservation actions should be considered an inherent part in future conservation projects for freshwater mussels species. In order to avoid poor results from restoration actions, it is necessary to shift from conservation mainly based on intuition, to a more evidence-based conservation approach. Using a BACI (before-after-control impact, e.g. Stewart-Oaten et al., 1986) monitoring framework, pre- and post-assessment should be performed to validate conservation action success. Ideally, this information should be incorporated into a database and thus, be available for future projects and for the implementation of an adaptive management process.

7) Exchange and share information: Publication of both success and failure of conservation action is essential in order to avoid repeating mistakes and reaching the overall goal, i.e. delisting the mussel species, very slowly or too slow. Exchange of information between different stakeholders, including scientists, practitioners,

public officials and the public, should take place e.g. by publishing articles in scientific as well as in practice-oriented journals, as well as by holding local and international meetings and conferences. A good example of successful communication between all involved stakeholders is the establishment of a freshwater mussel conservation office in the state of Bavaria, Germany. This office is a central point of contact for freshwater mussel conservation in Bavaria, acting as an interface between research and practice. Various communication tools are used to share knowledge information, including conferences, newsletters and local meetings.

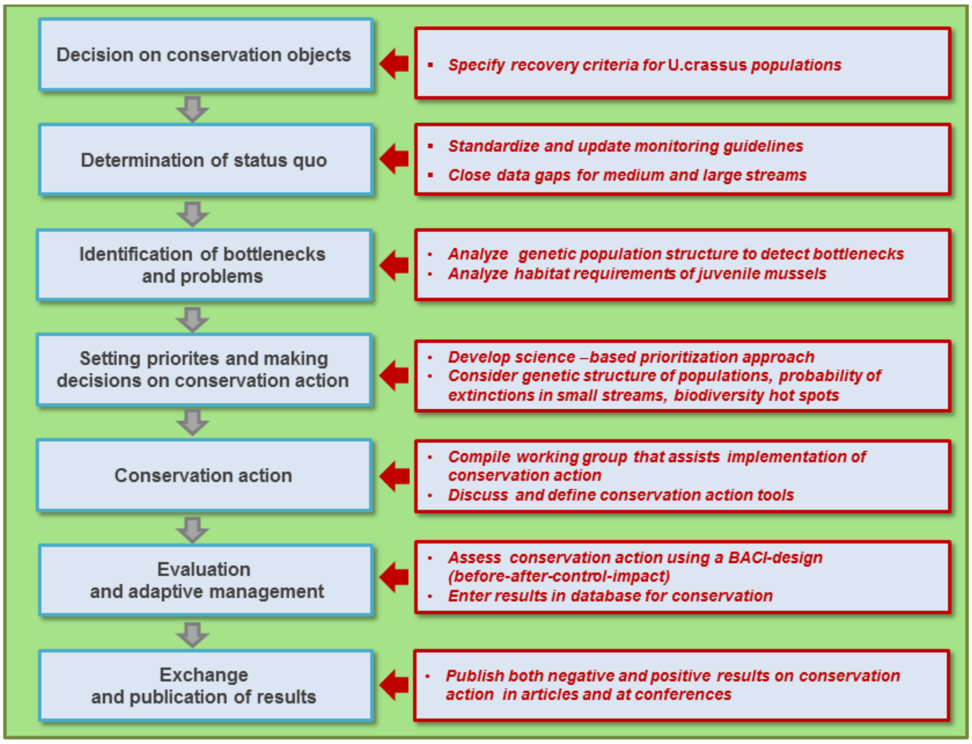

Figure 5.1: Systematic conservation approach for *Unio crassus*, based on the stepwise approach as proposed in Geist (2015)

5.5 Outlook

In conclusion, this study aimed at defining the ecological niche of a freshwater mussel species by analyzing abiotic and biotic key variables that play a crucial role during distinct phases of the life cycle. In terms of the study species *Unio crassus*, further research, accompanying the implementation of the conservation strategy, is suggested to focus on biological and ecological aspects that had not been investigated herein. For example, identification of suitable habitats and detection of unknown populations could be performed on a larger geographical scale by applying ecological niche modelling approaches. Furthermore, information on nutrient requirements of juvenile mussels and potential effects of waterborne contaminants potentially explaining population declines is still missing. Finally, development and application of molecular tools to analyse populations on a genetic level are necessary to be able to prioritize existing populations. The approach presented herein may be used as an example for defining the habitat requirements and identifying bottlenecks of other endagered unionoid mussel species in Europe, for most of which only little information is available at present.

6 Publication list

The following papers were included in this thesis:

Stoeckl K, Geist J. 2016. Hydrological and Substrate Requirements of the thick-Shelled River mussel *Unio crassus* (Philipsson 1788). Aquatic Conservation: Marine and Freshwater Ecosystems.
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Denic M*, Stoeckl K*, Gum B and Geist J. 2014. Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation. Hydrobiologia 735: 111-122. *Equal contribution authors

Stoeckl K, Taeubert JE, Geist J. 2014. Fish species composition and host fish density in streams of the thick- shelled river mussel (*Unio crassus*)–implications for conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 25: 276–287.

Co-authorships (not included in this thesis):

Richter A, Stoeckl K, Denic M, Geist J (accepted). Association between the occurrence of the Thick-shelled River Mussel (Unio crassus) and macroinvertebrate, microbial, and diatom communities. Freshwater Science.

Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, Bespalaya Y, Bódis E, Burlakova L, van Damme D, Douda K, Froufe E, Georgiev D, Gumpinger C, Karatayev A, Kebapçi Ü, Killeen I, Lajtner J, Larsen BM, Lauceri R, Legakis A, Lois S, Lundberg S, Moorkens E, Motte G, Nagel KO, Ondina P, Outeiro A, Pau-novic M, Prié V, von Proschwitz T, Riccardi N, Rudzīte M, Rudzītis M, Scheder C, Seddon M, Şereflişan H, Simić V, Sokolova S, Stoeckl K, Taskinen J, Teixeira A, Thielen F, Trichkova T, Varandas S, Vicentini H, Zajac K, Zajac T, Zogaris S. 2016. Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biological Reviews. doi: 10.1111/brv.12244.

Oral contributions related to the PhD thesis:

Stoeckl K, Geist J. 2015. New information on the habitat requirements of Unio crassus as a basis to optimize supportive breeding. 2nd International seminar on the rearing of unionoid mussels, November 24-26 2015, Clervaux, Luxemburg.

Stoeckl K, Geist J. 2015. Mussel monitoring in the context of the European Habitats Directive: new information on the ecological requirements of *U. crassus*. 2nd International Meeting on Biology and Conservation of Freshwater Bivalves, October 4-8 2015, Buffalo, NY, USA.

Stoeckl K, Geist J. 2015. Freshwater mussels conservation in Southern Germany: status, threats and conservation strategies. Institute Seminar at the US Geological Survey Institute, October 13, La Crosse, Wisconsin, USA.

Stoeckl K, Geist J. 2015. FFH-Monitoring von Großmuscheln: neue Erkenntnisse zur Autökologie der Bachmuschel *Unio crassus*. Jahrestagung der Deutschen Gesellschaft für Limnologie und der deutschsprachigen Sektionen der SIL, September 21-25 2015, University Duisburg-Essen, Germany.

Stoeckl K, Geist J. 2015. Habitat requirements of the thick-shelled river mussel *Unio crassus*: an integrative approach. 4th Biennial Symposium of the International Society of River Science (ISRS), August 23-28 2015, University of Wisconsin, La Crosse, Wisconsin, USA.

Stoeckl K, Geist J. 2013. Look before you leap: new information on habitat and host fish requirements of Unio crassus implies new conservation management practices. Symposium of the Freshwater Mollusk Conservation Society (FMCS). Species recovery and restoration–from concept to implementation, 10-14 March 2013. Lake Guntersville, Alabama, USA.

Stoeckl K, Geist J. 2012. New aspects and implications for the conservation of the thick-shelled river mussel (*Unio crassus*) International Meeting on Biology and Conservation of Freshwater Bivalves, 4-7 September 2012, Braganca, Portugal.

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