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Biotic interactions between native freshwater unionids and aquatic invasive species in relation to conservation management

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For Aribert

In grateful and loving memory

What is a scientist after all?
It is a curious man looking through a keyhole,
the keyhole of nature,
trying to know what's going on.

Jacques-Yves Cousteau, French undersea explorer,
researcher, photographer and documentary host
(1910-1997)

Preface

The objectives of this dissertation were to assess the functional interactions of two aquatic invasive species and their impacts on native freshwater unionids, to evaluate the current conservation management of two highly endangered native unionids species, and to derive further strategies for systematic conservation and invasion management. This work consists of three scientific manuscripts, which represent the three main chapters (Chapters 3-5) of this thesis.

After an introductory chapter providing fundamental background information on invasion biology and ecology and the importance of systematic conservation strategies, and a second chapter summarizing the methodology used in this thesis, the three main chapters follow.

In the first of these chapters (Chapter 3), the actual distribution and habitat characteristics of a non-native unionid in Bavaria, the Chinese pond mussel (*Sinanodonta woodiana*), and its potential impact on native bivalves are highlighted. The second chapter (Chapter 4) uses an experimental approach to compare the predatory effects of a native and the most common invasive crayfish, the signal crayfish (*Pacifastacus leniusculus*) on native and non-native freshwater unionids. The third chapter (Chapter 5) presents an approach to spatially prioritize conservation areas for two highly threatened native freshwater bivalve species, the freshwater pearl mussel (*Margaritifera margaritifera*) and the thick-shelled river mussel (*Unio crassus*). Each of these main chapters has been published as an independent research paper in a slightly modified form (according to different journal requirements).

The thesis concludes with a general discussion of the main findings, the distribution of aquatic invasive bivalves, the known impacts of invasive aquatic species on native bivalves, management recommendations and a future outlook.

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Abbreviations

A

AD	anterior-dorsal
AIC	Akaike information criterion
AK	water body: Aischgraben / Kleine Weisach
AUC	area under the receiver operating characteristic curve
AV	anterior-ventral

B

BI	water body: backwater Inn
BNatSchG	Bundesnaturschutzgesetz, German federal nature conservation act
BR	biosphere reserves; German area protection category designated by § 25 BNatSchG
BW	water body: Burlafinger Weiher

C

CL	carapace length
cond	electric conductivity

E

e.g.	*exempli gratia,* Latin for ‘for example’
Eh	redox potential
ENM	ecological niche model
EU	European Union

F

FW	open water

G

GH	water Body: Großer Hirschbergweiher
GLM	generalized linear model

H

HW	water body: Hirtenweiher

I

i.a.	*inter alia,* Latin for 'among others'
IAS	invasive alien species
IN	substratum depth of 10 cm

L

LPA	landscape protection area; German area protection category designated by § 26 BNatSchG

M

macroph	macrophytes
MB	water body: Moorbach
mc	60% depth of the water column

N

NASA	National Aeronautics and Space Administration
NATP	national park; German area protection category designated by § 24 BNatSchG
NP	nature park; German area protection category designated by § 27 BNatSchG
NR	nature reserve; German area protection category designated by § 23 BNatSchG

O

O_2	dissolved oxygen

P

PD	posterior-dorsal
pers. obs.	personal observation
PM	water body: pond near Muenchsmuenster
PR	penetration resistance
PV	posterior-ventral
PVC	polyvinyl chloride

R

RS	water body: Rothsee

S

SAC	special area of conservation; area protection category designated by the European Union Habitats Directive (92/43/EEC) and by §§ 31-36 BNatSchG
SD	standard deviation
SDM	species distribution model
su	2 cm below the surface

T

T	water temperature
Turb	turbidity

V

v	flow velocity

W

WO	water body: Woernitz

Summary

Biological invasions are a major and growing threat to biodiversity worldwide. Invasive non-native species can displace native species and alter entire ecosystems. Especially in freshwater systems, which are already altered by other factors such as degradation, climate change, overexploitation and pollution, the introduction of non-native species can cause significant ecological and economic damage. In particular, invading species can pose a major threat to freshwater bivalves, one of the most threatened groups of animals. Effective protected areas, which are often more resilient to invasions, can therefore be seen as strategic refugia for threatened native unionids. This thesis aims to examine the functional interaction between two less studied invasive aquatic species and native freshwater bivalves, and to identify conservation gaps for two highly threatened native freshwater unionids for conservation prioritization.

In the first study, the actual distribution of the invasive Chinese pond mussel *Sinanodonta woodiana* in Bavaria, Germany, was investigated and its ecological niche was defined using a systematic monitoring of water systems in which this species was only randomly observed or anecdotally reported. In a second study, predation impact of the invasive signal crayfish and of the native noble crayfish on three native and the invasive *S. woodiana* was compared in laboratory experiments. Repeating these experiments with the same crayfish specimens, a potential learning effects was also tested. In the third study, ecological niche models were conducted for two highly endangered native freshwater unionids. In a following gap analysis, the predicted suitable habitats were overlaid with actual conservation areas to reveal conservation gaps.

The results showed that *S. woodiana* is already widespread in Bavarian water systems, where it often co-exists with other native and invasive bivalve species, indicating a high invasion potential. A broad ecological niche for this species was confirmed by the wide range of habitat variables recorded. Predation by invasive crayfish was significantly higher than by native crayfish in the experiment, with native unionids being the most affected compared to invasive *S. woodiana*. A learning effect was observed for both crayfish species. Gap analysis based ecological niche models revealed that a higher percentage of suitable habitat for *M. margaritifera* are already protected, albeit in lower protection categories, while the overall protection status of suitable habitats for *U. crassus* was found to be low.

The already widespread distribution of *S. woodiana* shows that the current management of invasive species is inadequate. The increased predation by the invasive crayfish, and the fact that the Chinese pond mussel is less affected by this, poses a serious threat to native unionids, especially in water systems where both species have invaded in parallel. The methodology used has proved successful in filling the existing knowledge gaps on the interactions of the two studied invasive species with native bivalves and should therefore be extended. Conservation management of native unionids should be adapted and extended to include management of invasive species in order to achieve sufficient protective effects.

Zusammenfassung

Biologische Invasionen sind eine große und wachsende Bedrohung für die Biodiversität weltweit. Invasive nichtheimische Arten können einheimische Arten verdrängen und ganze Ökosysteme verändern. Insbesondere in Süßwasserökosystemen, die bereits durch andere Faktoren wie Degradation, Klimawandel, Übernutzung und Verschmutzung verändert sind, kann die Einführung nichtheimischer Arten erhebliche ökologische und wirtschaftliche Schäden verursachen. Vor allem für Süßwassermuscheln, eine der am stärksten bedrohten Tiergruppen, können invasive Arten eine große Gefahr darstellen. Wirksame Schutzgebiete, die oft widerstandsfähiger gegen Invasionen sind, können daher als strategische Refugien für bedrohte einheimische Unioniden angesehen werden. Ziel dieser Arbeit ist es, die funktionelle Interaktion zwischen zwei weniger untersuchten invasiven aquatischen Arten und einheimischen Süßwassermuscheln zu untersuchen und Schutzlücken für zwei stark bedrohte einheimische Süßwassermuscheln zu identifizieren, um Prioritäten für deren Schutz zu setzen.

In der ersten Studie wurde die tatsächliche Verbreitung der invasiven Chinesischen Teichmuschel *Sinanodonta woodiana* in Bayern untersucht und ihre ökologische Nische durch ein systematisches Monitoring von Gewässersystemen definiert, in denen die Art nur zufällig beobachtet oder anekdotisch berichtet wurde. In einer zweiten Studie wurde die Prädationswirkung des invasiven Signalkrebses und des heimischen Edelkrebses auf drei heimische und die invasive *S. woodiana* in Laborexperimenten verglichen. Durch Wiederholung dieser Experimente mit denselben Krebsexemplaren wurde auch ein möglicher Lerneffekt getestet. In der dritten Studie wurden ökologische Nischenmodelle für zwei stark gefährdete einheimische Süßwasserunioniden durchgeführt. In einer anschließenden Lückenanalyse wurden die vorhergesagten geeigneten Lebensräume mit den tatsächlichen Schutzgebieten überlagert, um Schutzlücken aufzudecken.

Die Ergebnisse zeigten, dass *S. woodiana* in bayerischen Gewässern, wo sie häufig mit anderen einheimischen und invasiven Muschelarten koexistiert, bereits weit verbreitet ist, was auf ein hohes Invasionspotenzial hinweist. Eine breite ökologische Nische für diese Art wurde durch die große Bandbreite der erfassten Lebensraumvariablen bestätigt. Die Prädation durch invasive Krebse war in den Experimenten deutlich höher als durch einheimische Krebse, wobei einheimische Unioniden im Vergleich zu den invasiven *S. woodiana* am meisten betroffen waren. Ein Lerneffekt wurde bei beiden Krebsarten beobachtet. Die Gap Analysis auf Basis ökologischer Nischenmodelle ergab, dass

ein höherer Prozentsatz geeigneter Lebensräume für *M. margaritifera* bereits geschützt ist, wenn auch in niedrigeren Schutzkategorien, während der Schutzstatus geeigneter Lebensräume für *U. crassus* insgesamt niedrig ist.

Die bereits weite Verbreitung von *S. woodiana* zeigt, dass das aktuelle Management von invasiven Arten nicht ausreichend ist. Die stärkere Prädation durch invasive Flusskrebse sowie die Tatsache, dass die Chinesische Teichmuschel davon weniger betroffen ist, stellt besonders in Gewässern, in die beide Arten einwandern, eine ernsthafte Gefahr für heimischen Muscheln dar. Die angewandte Methodik hat sich bewährt, um die vorhandenen Wissenslücken zu den Interaktionen der zwei untersuchten Arten mit den heimischen Muscheln zu schließen und sollte deshalb ausgeweitet werden. Auch sollte das Schutzmanagement der heimischen Muschelfauna angepasst und um ein Management invasiver Arten erweitert werden, um eine ausreichende Schutzwirkung zu erzielen.

Contents

1 General introduction

1.1 Biological invasions

Biological invasions are an increasing threat for biodiversity worldwide and the spread of invasive alien species (IAS) can lead to enormous ecological and economic damages (e.g., Cox 2005; Millennium Ecosystem Assessment 2005).

The term ‘invasive alien species’ is often used to describe those species that are intentionally or accidentally introduced by humans into habitats outside their historical range and, which additionally have great impacts on the new habitat (Davis and Thompson 2000). In contrast, some authors recommend to use the term ‘invasive’ only for species with high capability to reproduce and to spread excluding ecological or economic impacts of such species (e.g., Richardson et al. 2000). Nevertheless, with the implementation of the regulation on the prevention and management of the introduction and spread of invasive alien species, the European Parliament as well as the Council of the European Union included both ecological as well as economic impacts in the definition of ‘invasive species’ (European Parliament 2014). The term IAS used throughout this thesis, follows the definition of the European Parliament.

Over billions of years, the Earth's ecosystems have been formed and natural barriers such as i.a. mountains, rivers or desserts have been created resulting in formation of species that have adapted to the specific conditions of their habitat. With the industrial revolution, humankind has over and over softened these barriers and moved some species, sometimes deliberately, but often unintentionally, into areas that were previously inaccessible for them. The many movements of people but also of goods offer an ideal opportunity for such translocations. Thus, the main introducing pathways and vectors of

IAS result predominantly from these global travels and trades (Kolar and Lodge 2000). However, these pathways must be distinguished between deliberate and unintentional introduction. Many species were deliberately brought to new habitats to meet a human demand, e.g., for recreational fishing or aquaculture (e.g., rainbow trout *Oncorhynchus mykiss* Walbaum, 1792), as bait (e.g., freshwater shrimp *Gammarus pulex* Linnaeus, 1758) or as biological control (e.g., grass carp *Ctenopharyngodon idella* Valenciennes, 1844) (Hulme 2007).

As another example for a deliberate introduction, American crayfish such as the signal crayfish (*Pacifastacus leniusculus* Dana, 1852) were brought to Europe after the spread of the oomycete, crayfish plague (*Aphanomyces astaci* Schikora, 1906), which was also brought to Europe unintentionally with other American crayfish species carrying this pathogen (e.g., Martin-Torrijos et al. 2021), leading to an enormous die-off of many native crayfish populations of the noble crayfish (*Astacus astacus* Linnaeus, 1758) and the stone crayfish (*Austropotamobius torrentium* Schrank, 1803). Since most of the non-native crayfish are immune to the crayfish plague, introductions of such crayfish should ensure a further crayfish production. Another example is the earlier mentioned *C. idella*. In the 1960th, this fish species originating from Eastern Asia was introduced to Europe for its high ability to consume large quantities of aquatic macrophytes, and thus, for an ideal aquatic plant management to improve fish production in, e.g., fishponds (e.g., Pípalová 2006). Even today, this fish species is still stocked as a by-product in Bavarian aquaculture (pers. obs.).

In contrast to these deliberate imports, many of the non-native species that were brought to new areas were introduced unintentionally or escaped from farms into the wild. Besides the earlier mentioned *A. astaci*, the zebra mussel (*Dreissena polymorpha* Pallas, 1771) is another example for an unintentional introduction of a non-native species that is now widely distributed. In the 19th century, this mussel was brought within the ballast water or attached to the hull of container ships from their natural range in the estuary of the black sea to Central Europe (Bij de Vaate et al. 2002; Kinzelbach 1992; Thienemann 1950). In the same way, this species started its further dispersal more upstream along the big waterways and canals and not least with recreational water sport equipment like vessels into unconnected water systems such as alpine lakes (Pollux et al. 2010).

In addition to these examples, even some rodents were brought from America to farms in Europe and Asia as a fur resource, but without the intention to release them into the wild. In the case of the American mink (*Neogale vison* Schreber, 1777) or the muskrat (*Ondatra zibethicus* Linnaeus, 1766), this failed when some individuals escaped from the fur farms (Hulme 2007). Both species got established and spread throughout a wide range with some remarkable threats for native species. As an example, the mainly herbivore *O. zibethicus* can destroy whole populations of the highly endangered native thick-shelled river mussel (*Unio crassus* Philipsson, 1788) during winter, when herbal food is scarce (Stoeckl et al. 2020). As a newly threat, this can thwart existing conservation efforts.

Many other examples of terrestrial but also of aquatic invaders can be found in the literature and the number is further increasing. In fact, the number of non-native species in freshwater ecosystems is still exponentially increasing (Keller et al. 2009). However, as there is often a long time lag between the introduction of a species and its establishment and dispersal (Kowarik 1995), it can be assumed that many new invasions will be discovered in the near future (Crooks 2005; Jeschke and Strayer 2005).

1.2 Biotic interactions between non-native and native species

Once arrived in the new habitats, some non-native species will adapt to the local conditions, start reproducing without direct human intervention and thus become established (Keller et al. 2011). While many of the introduced species will not succeed, only a fraction will further spread and become invasive (Kolar and Lodge 2001). Even if the proportion depends on the taxonomy of the species and the regions they were introduced, some authors use the ‘tens rule’ assuming that approximately 10% of all introduced species will become established and only approximately 10% of those species will become invasive (Williamson 1996). However, when occupying the new habitats that often are already inhabited by native species, the invading species start to interact with them. Native species, however, are often perfectly adapted to their habitat, thus the invasion of non-adapted non-native species should not pose a noticeable threat to them.

Over the past approximately 150 years, many different hypotheses have been proposed trying to explain the success of invasive species. Jeschke and Heger (2018) have collected more than 1100 studies and sorted them into 12 major hypotheses, such as the earlier mentioned “tens rule”, the “biotic resistance” or the “invasional meltdown” hypothesis, to name just three of them. However, one of the most common hypothesis is the “enemy release” hypothesis (Enders et al. 2018), which states that in the new habitat invasive species often do not have to face their natural enemies leading to a reduction in enemy pressure and an increased performance at the same time (e.g., Maron and Vilà 2001; Torchin and Mitchell 2004). This enemy release hypothesis seems to be especially true for freshwater systems, since Prior et al. (2014) have shown that native enemy effects were stronger in freshwaters than in terrestrial systems.

In addition, when habitats are already altered, which is often the case in freshwater systems, and highly specialized native species have to struggle with these changes, non-native generalist species can have a significant advantage, which meets another hypothesis, the “disturbance” hypothesis (Nordheimer and Jeschke 2018). Due to higher tolerance to non-optimal habitat conditions, the often generalist invasive species are more easily able to adapt to their new environment. In contrast, the often highly-specialized native species have to struggle with changing conditions and since these species often have a small geographic range they are more vulnerable to get locally extinct (Groom et al. 2006). Thus, factors such as climate change (Walther et al. 2009), aquatic pollution (Crooks et al. 2010) or even land-use change (Scott and Helfman 2001) can be strong drivers for invasive species in aquatic systems. Especially, land-use intensification can alter aquatic habitats, which leads to a reduction in species richness further reducing biotic resistance to invasions (Havel et al. 2015).

However, a small movement of scientists and academics has formed that criticize the actual field of invasion science, although most of the criticisms they propagate are already rebutted (Richardson and Ricciardi 2013). Some authors, predominantly from the invasive species denialism movement, postulate, e.g., that invasive species also have positive effects, since an invasion will increase the biodiversity, which consequently leads to an increase in ecosystem services. As the fact of an increase in biodiversity can be true for α-diversity, a simultaneous reduction in β-diversity or even in γ-diversity is often disregarded. Even though, in case that an invasive species is rare in its natural range, the global biodiversity can benefit from the invasion into a new habitat. But at the same time, when endemic species get extinct as a reason of this invasion, at least β-diversity as well as γ-diversity will be reduced (Lean 2021).

Besides the contribution to biodiversity, some other positive effects of invasive species such as ecological, economic and socio-cultural benefits were reported (Kapitza et al. 2019). In case that invading species can provide important ecosystem services that got lost with extinct species, the ecosystem can benefit of this replacement. Nevertheless, ecosystem processes are complex and often not all functions can be replaced. As an example, freshwater bivalves are considered keystone species (e.g., Geist 2010), which, e.g., importantly contribute to water purification by filter-feeding (Atkinson et al. 2013; Lummer et al. 2016; Vaughn 2018; Vaughn et al. 2008). If native unionids such as the native *Anodonta* species get replaced by non-native Chinese pond mussel *Sinanodota woodiana* (Lea, 1934; **Fig. 1**)*,* the ecosystem service of biofiltration can still be provided from such non-native species. However, contrary to native ones, this non-native unionid cannot be used from the highly endangered European bitterling (*Rhodeus amarus* Bloch, 1782) for reproduction, which can result in a secondary die-off of this endangered fish species (Douda et al. 2017a).

Fig. 1 Large and fast growing unionid, the Chinese pond mussel (*Sinanodonta woodiana* Lea, 1934), non-native to European water systems is considered a major potential threat to native mussels. Yet, little knowledge is available on its distribution.

Nevertheless, besides positive effects of non-native species, there is always a risk that negative impacts may predominate, as positive effects often are temporary whereas negative impacts are more lasting and often irreversible (Richardson and Ricciardi 2013). Such negative impacts can result from non-native species that can affect ecosystem functions and other (native) species by several direct and indirect mechanisms (**Fig. 2**). As non-native species starts to interact with the resident species as soon as they have invaded a new system, this may result in a strong competition for resources like food or space, but also in predation or herbivory. AIS can even have potential impacts on community structure as well as on certain ecosystem functions. Thus, invasive species are often found to restructure food webs (Vander Zanden et al. 1999) or alter processes and functions of certain ecosystems (Mack et al. 2000; Pyšek and Richardson 2010).

Whereas for some non-native species, such as *D. polymorpha* or Asian clam (*Corbicula fluminea* Müller, 1774), ecological consequences for native bivalves are well known (e.g., Ferreira-Rodríguez et al. 2018; Ożgo et al. 2020; Sousa et al. 2011; Strayer 1999; Strayer and Malcom 2007; Vaughn and Hakenkamp 2001), the risk resulting from species of the same family are less studied. In case of the non-native bivalve *S. woodiana*, it is already known that this fast-growing species has some advantages in reproduction, since it can breed earlier and several times a year with high amounts of glochidia (Labecka and Czarnoleski 2021; Labecka and Domagala 2018), which can survive longer (Benedict and Geist 2021). Additionally, it can use more fish species as hosts (Douda et al. 2012; Huber and Geist 2019), and block them for usage of native mussel species (Donrovich et al. 2017). Even though some impacts of this non-native species are already known, the invasion of this species is comparably new and its ecological range and thus its potential for further spread remains unclear in some regions of Central and Western Europe.

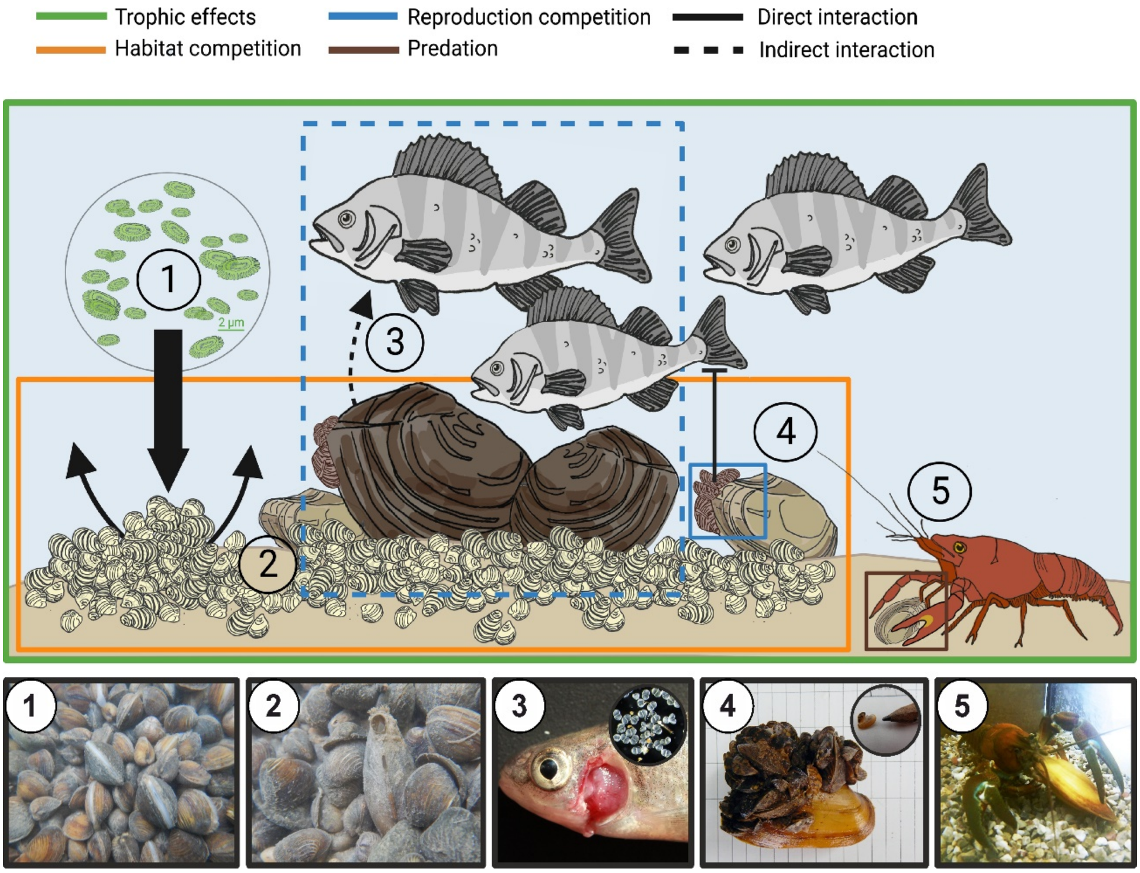

Fig. 2 Functional interactions of invasive and native aquatic fauna: 1) Trophic competition with native fauna by high filtration activity of dense assemblages of invasive bivalves such as the Asian clam *Corbicula fluminea*; 2) Habitat competition between dense aggregations of invasive bivalves and native unionids; 3) Competition for host fish, e.g., by infestation with high rates and volumes of non-native glochidia can indirectly lead to cross-resistance to native glochidia; 4) Physical fouling by invasive bivalves limits reproduction, movement, and filter activity of native unionids; 5) Predation by invasive fauna upon native mussels occurs at higher rates (adapted and changed from Geist et al. (2023))

Invasion of non-native species, however, can be highly problematic for aquatic ecosystems, affecting freshwater ecosystems more strongly, since freshwater species are spatially more restricted compared to marine species (Lopes-Lima et al. 2017). Especially in the case of native freshwater bivalves, AIS seem to be a major problem (Geist et al. 2023; Sousa et al. 2014), with negative effects resulting from functional interactions, such as competition, consumption, physical impairment, genetic alteration, pathogene transmission, invasional meltdown, habitat alterations:

Competition

If species colonize the same habitat, they start to compete with each other for available resources such as food, space or even hosts. Since native as well as non-native bivalve species are filter feeder, the often large assemblages of the non-native dreissenid and corbiculid bivalves can filter high rates of water and thus limit plankton also available for native species as this was shown from the Hudson River in New York, USA (Fig. 2.1; Strayer et al. 2011; Strayer et al. 2014; Strayer et al. 2019a; Strayer and Malcom 2014; Strayer and Smith 1996; Strayer et al. 2019b). In addition, it has been shown that *Corbicula* spp. can assimilate a wider range of food sources than native bivalves, which could be the reason for the declines of native bivalve species with a simultaneous spread of this invasive species in the U.S. (Atkinson et al. 2010; Haag et al. 2021).

In freshwater unionids, which are dependent on fish hosts at an early stage of their life cycle, competition for suitable hosts can arise when these species live in the same waterbody and have the same host preference. As already known, the non-native *S. woodiana* is a generalist using different native as well as invasive fish species as suitable hosts (Douda et al. 2012; Huber and Geist 2019), blocking them for a subsequent use by native species (Fig. 2.3; Donrovich et al. 2017).

Physical impairment

Effects of physical impairment can also negatively affect native species. The non-native bivalve *D. polymorpha*, e.g., can use strong byssus threads to attach on solid grounds. In habitats with fine sediments and an occurrence of native freshwater bivalves, the shell of these natives can get overgrown by invaded *D. polymorpha* (**Fig. 2.4**). This can lead to shell deformations but also impair the movement of or even turn over native mussels, which will result in higher mortality (Ożgo et al. 2020).

Consumption

Besides direct competition, consumption like predation or herbivory can also pose a novel threat to native species. However, in some cases predation seems to be an even greater threat than competition (Pyšek et al. 2017). Invasive freshwater crayfish, e.g., are omnivorous, with opportunistic feeding behaviour often being observed (Guan and Wiles 1998; Lewis 2002; Nyström et al. 1996). Thus, strong effects of both, predation and herbivory, can be found in invasive crayfish (Machida and Akiyama 2013; Nyström and Strand 2003; Sousa et al. 2019; zu Ermgassen and Aldridge 2011). For example, the invasive *P. leniusculus* (**Fig. 3**) appears to be a more effective grazer than native crayfish species, leading to large reductions in macrophyte biomass, cover and species richness (Nyström et al. 1996; Nyström and Strand 2003). Predation by invasive crayfish has also been identified as a potential threat to many native freshwater bivalves (**Fig. 2.5**). While such effects have already been shown to be problematic for Western European unionids (Meira et al. 2019; Sousa et al. 2019), this has so far only been suspected for freshwater pearl mussels in Central Europe (Schmidt and Vandré 2012), but has not yet been systematically investigated.

Genetic effects

Genetic effects are also suspected to have negative impacts on native species. Even though this is often not visible in the field, genetic analyses in the laboratory can provide evidence of, e.g., hybridization. In some cases, especially in marine bivalve aquaculture, hybridization has already been used to introduce desired traits (Guo 2009). However, in freshwater bivalves, potential hybridization is poorly examined up to now, but would be more expected between more closely related taxa such as *Anodonta* and *Sinanodonta* species (Sousa et al. 2014). Nevertheless, this aspect of interaction is an important point. By outbreeding or genetic swamping, the fitness of highly adapted native species can be reduced, treating the genetic integrity of wild bivalve populations (e.g., Adavoudi and Pilot 2021; Todesco et al. 2016).

Fig. 3 Invasive signal crayfish, *Pacifastacus leniusculus*, with distinctive marks (light oval patches at the joint of the fingers of the claw), is already a known factor for the decline of native crayfish in Europe, though little is known about functional interactions with mussels in Central Europe. Picture taken from Dobler and Geist (2022).

Pathogene carrier/vector

In addition to the direct mechanisms described above, invasive species can also affect native species through indirect mechanisms. Some non-native species carry diseases or parasites that could harm native species in the invaded system (Hulme 2007). For example, many non-native crayfish in Europe, such as *P. leniusculus*, can carry the oomycete *A. astaci* and act as vectors, carrying this pest to new areas where it can cause massive die-offs of native crayfish populations (Holdich et al. 2009). Another example is freshwater bivalves, which are known to carry many diseases and parasites (Cichy et al. 2016; Taskinen et al. 2021). When mussels are translocated, the introduced pathogens could infect the bivalves in the new habitat, potentially causing mass mortality (Brian et al. 2021). Although some non-native species, such as the Asian clam, *C. fluminea*, were not found to carry any parasites, the role of parasites appears to be invader-specific, as the non-native unionid *S. woodiana* was found to carry many parasites (Taskinen et al. 2021), making this species more problematic for native unionids.

Invasional meltdown

As explained above, invasive species often colonize new habitats where there is a lack of enemies of the invader. In some cases, however, other invading species may become the new enemy, as *N. vison* has been shown to be a strong predator of invasive crayfish (Yanuta et al. 2022). Conversely, in some cases, when several non-native species invade the same system, invasional meltdown effects may occur (Simberloff and Von Holle 1999). For example, the non-native *C. idella* can reduce native macrophytes, which may also facilitate the invasion of new non-native plant species (Pípalová 2006). As another example, it has been shown that several Ponto-Caspian species, such as *D. r. bugensis*, often subsequently colonize habitats that were first invaded by *D. polymorpha* (Haltiner et al. 2022; Hetherington et al. 2019).

Habitat alteration

In some cases, invasive species can also have great impact on whole ecosystems, in which this species is invading, which can modify the key rules of existence for all organisms of this ecosystem (Vitousek 1996). With its ability to assimilate more food resources than native bivalves, the Asian clam *C. fluminea* can alter the whole nutrient dynamic in an entire ecosystem (Atkinson et al. 2010). Even, the invasive *D. polymorpha* has to be shown to reduce suspended solids and phytoplankton concentrations due to high filtration activities, which can increase light transmittance and thus macrophyte reproduction (Macisaac 1996) but at the same time limit plankton available for native unionids. As one of the most prominent examples of such effects, long-term studies from the Hudson River in New York reported a parallel decrease in turbidity due to filter feeding by *D. polymorpha* and a decline in native species (Strayer et al. 2011; Strayer et al. 2014; Strayer et al. 2019a; Strayer and Malcom 2014; Strayer and Smith 1996; Strayer et al. 2019b).

As shown above, many functional interactions of non-native aquatic species may be a potential threat for native ones. This can be observed in many aquatic ecosystems, however, it is highly problematic for freshwater systems, being among the most endangered ecosystems worldwide (Dudgeon et al. 2006). With less than 1% of the earth's surface, freshwater habitats inhibit almost 10% of all species and many of these species are threatened (Strayer and Dudgeon 2010). As some prominent examples, freshwater mussels as well as crayfish are highly endangered animal groups with a high number of threatened species (Strayer and Dudgeon 2010). However, compared to other ecosystems, European aquatic ecosystems often have the greatest presence of non-native species due to human activities and connectivity with other ecosystems (Keller et al. 2011). This makes it more important to consider this issue as part of conservation management for native aquatic fauna.

1.3 Spatially explicit approaches

Freshwater bivalves are among the most endangered species worldwide and many native species are assigned high priority in aquatic conservation and restoration efforts, since freshwater bivalves are considered key faunal elements of diverse aquatic habitats (Geist 2010; Geist 2015; Lopes-Lima et al. 2018; Lopes-Lima et al. 2017; Sousa et al. 2023). However, even for this animal group introduction of non-native species was identified as one of the major threats (Ferreira-Rodríguez et al. 2019; Lopes-Lima et al. 2017). To address problems such as invasive species, systematic approaches need to be adapted, since freshwater ecosystems are often underrepresented in actual conservation management (Abell 2002; Herbert et al. 2010; Hermoso et al. 2016; Lopes-Lima et al. 2017; Nel et al. 2009; Nel et al. 2007).

With the implementation of protected areas, many negative impacts such as habitat degradation could be prevented. However, freshwater ecosystems are often not adequately protected, since catchment areas of entire rivers can be huge and may only be partially covered by protected areas (Hermoso et al. 2015). Given that stream ecosystem-level processes are longitudinally linked (Vannote et al. 1980), species that occur in the protected downstream part of an only partially protected stream can still be threatened if the upper part is unprotected. However, if a stream is fully protected, invasion of non-native species and thus a threat to native species cannot be excluded, as shown by Foxcroft et al. (2013) for invasive plant species. Even in highly isolated mountainous landscapes, in which protected areas are located, invasive species can be problematic (Alexander et al. 2016). Nevertheless, for non-native plant species, it has been shown that protected areas can be a certain barrier for colonization (Foxcroft et al. 2011; Lonsdale 1999; Pysek et al. 2003). In a study, Gallardo et al. (2017) examined current and future potential distributions of 100 of the most invasive terrestrial, freshwater and marine species in Europe and evaluated the combined threat from invasions and climate change. They found that only approximately 25% of marine and terrestrial protected areas (protected over the last 100 years) in Europe have been colonized by investigated invaders. Their prediction revealed that species richness of invaders is 11% to 18% significantly lower inside than outside of protected areas. They conclude that considering the expansion of invasive species under climate change protected areas can provide strategic refugia for native species. However, it is often not possible to predict, how susceptible a (protected) habitat is to be invaded by a certain non-native species or if a new non-native species that start to spread might be problematic for certain native species.

To optimize area protection of freshwater systems and to predict the impact of non-native species on certain native species or even the invasion potential of an already protected habitat, spatially approaches could be implemented. It has already been widely demonstrated that conservation prioritization tools, such as species distribution models (SDMs), can be useful tools for improving conservation planning (Moilanen et al. 2008; Moilanen et al. 2009). In addition, SDMs could also help to predict range shifts of invasive species due to climate change, but this has rarely been tested adequately (Jeschke and Strayer 2008).

Consequently, information on the potential niche overlap between native species of conservation concern and potentially invading non-native species is needed to assess the risk of invasion. Niche overlap could be identified by comparing ecological niche models for each of these species. However, such models are often based on spatial information on recent occurrences, and this information is often lacking for new non-native species and only available for the native ones, making this approach not yet feasible.

1.4 Thesis objectives and outline

Many different threat factors such as the introduction of non-native species, habitat loss and fragmentation, overexploitation, pollution, loss of host fishes, water abstraction and climate change have led to the decline of European native freshwater bivalve populations (Lopes-Lima et al. 2017), and the impact of human activities is constantly introducing new risks. It is therefore essential to identify new threats, such as biological invasions, at an early stage and to fully understand the impact of functional interactions of non-native species with native bivalves. In the case of non-native *S. woodiana*, this species has already been identified as a major potential threat to native bivalves (e.g., Urbańska et al. 2021). However, little is known about its actual distribution in Central Europe. In contrast, the distribution of invasive crayfish, such as *P. leniusculus*, has been well studied (Holdich 2003), while their impact on native bivalves is only suspected. In order to maintain conservation areas as strategic refugia for threatened native unionids, systematic conservation strategies should be implemented as a first step to identify conservation gaps and ultimately improve the conservation of native freshwater bivalve populations before they disappear completely. Addressing all aspects mentioned above, this thesis focused on the actual distribution of a non-native unionid, the effects of functional interactions of two non-native freshwater species with native unionids, and the conservation gap of two highly endangered native freshwater bivalves. More specifically, the main objectives of this thesis were:

- Assessment of the actual distribution and potential impacts of the non-native Chinese pond mussel (*Sinanodonta woodiana*) in Bavaria,

- Assessment of the predation risk of native and invasive freshwater crayfish on native and invasive freshwater mussels,

- Identification of conservation gaps and prioritization of protection areas for the two native freshwater mussels *Margaritifera margaritifera* and *Unio crassus.*

2 General methodology

2.1 Study design

The core study concept of this thesis was based on the three pillows field monitoring, laboratory experiments and systematic conservation and management (**Fig. 4**).

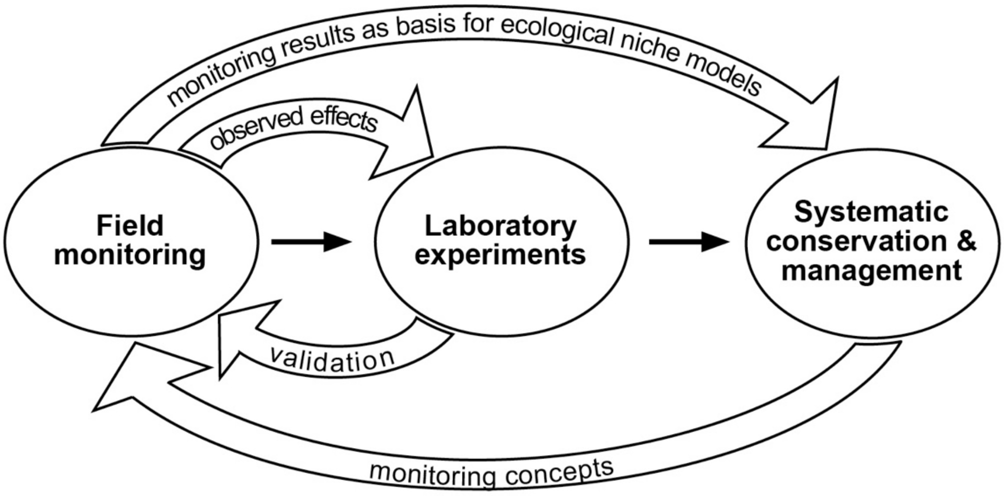

Fig. 4 Schematic illustration of the study concept this thesis was based on. Field monitoring was conducted to obtain information on actual distribution, abundance and population demography of a non-native unionid species and to potentially observe effects functional interactions with native species. Laboratory experiments were conducted to assess effects of mechanistic interactions between invasive and native species, which were subsequently validated in the field. A systematic conservation approach was used to develop ecological niche models for native bivalve species based on regional monitoring results. Such models can be used to derive new monitoring concepts.

In the first study (Chapter 3), in which the actual distribution of a non-native unionid bivalve and potential impacts on native unionid populations were assessed, a field monitoring approach was used to obtain *in-situ* information (**Fig. 4**). Since up to date there is a lack of systematic monitoring of invasive species in Germany, only randomly observed and anecdotally reported occurrences of this non-native species were available and used as a basis for the sampling design. As described in Dobler, Hoos & Geist (2022), density and biomass of native and invasive bivalve species as well as physicochemical parameters such as turbidity, water temperature, specific conductivity, or pH-value for habitat characterization were assessed.

In the second study (Chapter 4), the functional interaction between native and invasive crayfish and native and invasive unionids was investigated through defined laboratory experiments on predation effects. In controlled laboratory experiments, the intensity of damage on native and invasive unionids and a potential learning effect were compared between native and invasive crayfish species. Subsequent field monitoring served as an *in-situ* validation of the experimental results (**Fig. 4**).

The third study (Chapter 5) applied an approach that translates this knowledge into spatial conservation and management aspects of protected areas. Thus, the recent conservation concept of two highly endangered native mussel species was evaluated by comparing ecological niche models of each species with actual conservation areas. As basis for the niche models, the results of a wide-ranging field monitoring in Bavaria were used (**Fig. 4**). These three steps are discussed in more detail below.

2.2 Distribution sampling and habitat characterization

To assess the distribution and the ecological niche of a certain species, it is necessary to monitor this species in the habitats it is suspected to occur. Such sampling data can also be used for further analyses of valid field data, as this was shown in the study presented in Chapter 5, where such data formed the basis for distribution models of two highly endangered native unionids species (see Section 2.4).

In the case of newly invaded non-native freshwater bivalves, only random observations and anecdotal reports of these species are therefore available in Germany. If only the distribution range of such a species in a certain system is required, the monitoring can be simple, but if distribution overlaps and potential competition effects are expected, a sampling design with a higher resolution has to be chosen. For the study of Chapter 3, not only the distribution but also population and habitat characteristics as well as co-occurrence with native bivalve species were assessed. Including all these aspects, a monitoring design with a finer scale was therefore chosen. Sampling frames with three different sizes (0.25 m^{-2},1 m^{-2}, 9 m^{-2}) were used to define the sampling spots as well as the area that has to be searched. Sampling plots were distributed around the area of the suspected *S. woodiana* occurrence. Starting with the smallest frame, the next larger frame was chosen after 5 sports without mussels.

However, the water bodies strongly differed from each other and thus, such field monitoring had to be adapted to the specific conditions. In slow flowing streams or in fish ponds with shallow and clear water, visual monitoring by wading using an aqua-scope was applied (**Fig. 5 left**). With this approach only visible bivalves sitting on the top of the sediment can be found. However, if the turbidity is too high to find the mussels visually, a tactile approach has to be chosen. Since freshwater bivalves can also burry themselves in the sediment, all buried mussels within the certain frame were also collected by disturbing the sediment by hand up to 50 cm depth, if possible.

When the water depth exceeded one meter, it was not possible to do the monitoring by wading. In this case, a sampling via snorkeling or even scuba diving was necessary (**Fig. 5 right**) to detect mussels properly. Especially in lakes and deeper ponds, the monitoring was performed using the methodology “scientific diving” according to the existing national German regulations (DGUV-Regel 101-023 “Einsatz von Forschungstauchern”).

All found unionid specimens were collected, the individual wet body mass was weighed using a field scale (± 1 g) and the length of each individual was measured using calipers (± 0.1 mm). Before weighing the mussels, attached *D. polymorpha* were removed and weighed separately. The number of all *Corbicula* spp. specimens that were found within one sampling spot were counted and the individuals were weighed together. Due to the German law, all protected species were returned to the original spots immediately after measurements, while non-native species were kept. These biotic data were used to determine the species composition at each sampling spot as well as the biomass and density of each species.

Fig. 5 Sampling methods adapted to the sampling habitat, with a visual and tactile sampling approach in a smaller stream (left) and a scuba diving sampling in a deeper lake with high turbidity (right).

Besides the distribution and abundance of non-native and native bivalve species, abiotic habitat characteristics of all examined water systems were also assessed to determine the ecological tolerance of *S. woodiana*, except of the pond near Muenchsmuenster, as this system was drained at the time of sampling. At each sampling spot, water temperature (in °C), dissolved oxygen (in mg L^{-1}), pH-value, and electric conductivity (in µS cm^{-1}, relative to 25 °C) was measured using a handheld probe Multi 3630 IDS F (WTW, Weilheim, Germany) as well as the redox potential (in mV) using a handheld pH 3110 meter (WTW, Weilheim, Germany) together with a platinum electrode and an Ag/AgCl2-reference electrode in the open water. Additionally, turbidity (in NTU) was measured using a handheld probe Turb® 355 T (WTW, Weilheim, Germany). Flow velocity (in m s^{-1}) was measured at 60% depth of the water column and at 2 cm below the surface (see Stoeckl and Geist 2016), according common techniques in hydrological studies, using a handheld flowmeter (Flowtherm NT, Höntzsch, Waiblingen, Germany) (**Table 1**).

For interstitial and substratum characterization, penetration resistance (in kg cm^{-2}) was measured using a handheld penetrometer (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands), and, as explained above, the temperature, dissolved oxygen, pH-value, electric conductivity and the redox potential was measured in an interstitial water sample taken at a substratum depth of 10 cm (Geist and Auerswald 2007) (**Table 1**).

At diving spots, samples of interstitial water and of open water were taken from the diver at each spot and immediately delivered for further measurements.

In addition, water depth and silt layer were measured using a measuring rod (± 0.5 cm), at diving sites water depth was measured using a diving gauge (± 0.25 m). The width of the water body was measured in flowing waters using a measuring tape (± 0.05 m) for sampling sites up to 25 m wide, and using an online image viewer measuring tool for sites greater than 25 m wide (**Table 1**). Macrophyte coverage (in %) within each sampling frame was estimated.

As this study involved the handling of protected species and sampling within protected areas, all necessary permissions were obtained (reference numbers: RMF-SG55.1–8646-7–111-2 for Middle Franconia, 55.3–8646-2/856 for Swabia, ROP-SG55.1–8622.1–47-2–3 for Upper Palatinate and 41.0.03/8231 for the administrative district of Passau).

Table 1 List of variables, their units and material and method used for sampling

variables	unit	used material and method
Bivalve population assessment		
shell length	mm	Total shell length of each individual measured with calipers
shell width	mm	Total shell width of each individual measured with calipers
shell height	mm	Total shell height of each individual measured with calipers
wet bodymass	g	Wet bodymass of each individual weighed with field scales
density	Ind m^{-2}	Counting of all mussels within the defined sampling frame
Habitat characterization		
macrophytes coverage	%	Coverage estimation of macrophytes within sampling frame
penetration resistance	kg cm^{-2}	field measurements using a handheld penetrometer (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands)
silt layer thickness	cm	field measurements using a measuring rod
turbidity	NTU	field measurements using a handheld Turb® 355 T (WTW, Weilheim, Germany)
water temperature	°C	field measurements (in open water and in interstitial water samples) using a handheld probe Multi 3630 IDS F (WTW, Weilheim, Germany)
dissolved oxygen	mg L^{-1}	field measurements (in open water and in interstitial water samples) using a handheld probe Multi 3630 IDS F (WTW, Weilheim, Germany)
pH-value	pH	field measurements (in open water and in interstitial water samples) using a handheld probe Multi 3630 IDS F (WTW, Weilheim, Germany)
electric conductivity	µS cm^{-1}, relative to 25 °C	field measurements (in open water and in interstitial water samples) using a handheld probe Multi 3630 IDS F (WTW, Weilheim, Germany)
redox potential	mV	field measurements (in open water and in the interstitial/interstitial water sample) using a handheld pH 3110 meter (WTW, Weilheim, Germany) combined with a platinum electrode and an Ag/AgCl2-reference electrode
flow velocity	m s^{-1}	field measurements at 60% depth of the water column and at 2 cm below the surface using a handheld flowmeter (Flowtherm NT, Höntzsch, Waiblingen, Germany)
depth	m	field measurements using a measuring rod or a diving gauge for diving sports
width	m	field measurements at each sampling spot using a measuring tape (for spots ≤ 25 m stream width)/measuring tool of an online satellite image viewer (for sports > 25 m stream width)

2.3 Predation assessment

To assess effects of functional interactions of invasive species such as predation by crayfish systematically, it is necessary to quantify this in controlled laboratory experiments. This approach allows to delete most of disruptive factors that normally occur under natural conditions and may affect experimental results and to set the focus on the desired effect. Furthermore, such a design also allows to investigate an impact that is not yet problematic in natural systems due to the lack of co-occurrence, but may become so in the future as non-native species continue to spread and invade susceptible systems. The laboratory experiments of Chapter 4 built up the basis to quantify the damages on each mussel species for each crayfish species under artificially created conditions. Since this setting was not yet found in natural systems, but could be problematic in the future, this chosen design using laboratory experiments allowed to compare the impact of native and invasive crayfish on different native as well as invasive mussel species simultaneously. Using crayfish species that never had been in contact with freshwater bivalves for a first experiment, and reusing the same specimen after holding them combined with freshwater bivalves for 25 days allowed to compare both results considering a possible learning effect.

For these experiments, the native crayfish *A. astacus*, obtained from a regional breeder, and the invasive *P. leniusculus*, caught in the river Moosach next to the laboratory, were used. To show predation differences in light of shell conditions, two native thin-shelled pond mussels, *Anodonta anatina* and *A. cygnea*, one native thick-shelled mussel, *Unio pictorum*, as well as and non-native species, *S. woodiana*, whose shell is more thick than native pond mussel but much thinner than of *U. pictorum*, were used. All mussels were obtained from online shops.

In each of the four experimental parts (*A. astacus* without previous contact to freshwater mussels; *A. astacus* with previous contact to freshwater mussels; *P. leniusculus* without previous contact to freshwater mussels; *P. leniusculus* with previous contact to freshwater mussels), one crayfish specimen that was acclimated for 10 days and starved for three days was placed together with one specimen each of three native (*A. anatina*, *A. cygnea* and *U. pictorum*) and of one non-native (*S. woodiana*) bivalve species in one aquarium. The experiments were performed with eight replicates for each experimental part. Each aquarium (length: 40 cm; width: 25 cm; height: 25 cm) was filled with 5 cm of fine gravel (4-8 mm) and with 13 L of tap water. One PVC tube (diameter: 7 cm; length: 25 cm) was provided in each aquarium as a shelter for crayfish.

After three days, each experimental part was terminated and mortality as well as shell damage were assessed. To assess the full dimension of damages and not only estimating them, an approach using georeferenced photos of the shells was used. Before and after the certain experiment, each shell was placed on a reference grid and photographed individually. Each image was georeferenced using polynomial transformation (polynomial 3) of the software Quantum GIS v3.4.5 (QGIS Association) distributing 12 control points on the grid around the valves and using nearest neighbor as resampling method. Therefore, a custom coordinate reference system with a transverse Mercator projection was used, setting latitude and longitude origin as well as the coordinate value at *x* and *y* origin to 0 and the scale factor to 1. As ellipsoid, WGS84, and as unit, meter was used.

All damages were counted and the depth and the length of each damage was measured using the measuring function in ArcGIS v10.7.1 (ESRI 2019). For analyses, each shell was divided into four quadrants representing the four parts of a shell, posterior-dorsal, posterior-ventral, anterior-dorsal and anterior-ventral, respectively. A more detailed description of this can be found in Subsection 4.3.6.

However, if such effects are demonstrated in experiments, a validation under natural conditions should be added if possible. Thus, a subsequent field study was therefore performed as an *in-situ* validation of the experimental results. However, only a fraction of the experimental results could be examined, since a co-occurrence of native and invasive mussels with invasive crayfish was lacking.

An official permit for the experimental setup and for the handling of crayfish was obtained from the permit agency, the District Government of Upper Bavaria, under the reference number 2631.Vet_11-4-12.

2.4 Spatial approach

For conservation concepts, valid field data are necessary. However, gaining high quality and comprehensive actual information on the occurrence of several species is time and cost intensive and such data do not show the further development due to, e.g., climate change. If enough information on the occurrence of a species is available, which is not the case for non-native species, spatial approaches could help to create models on the potential distribution of a species and on possibly overlaps of different species. Such models can also be used for systematic approaches implementing conservation concepts especially for prioritization approaches.

This was addressed in Chapter 5, in which a spatially approach based on a high number of occurrence points from a big local monitoring database was used to create ecological niche models for two highly endangered native unionids, *U. crassus* and *M. margaritifera*. Ecological niche models based on habitat suitability score estimations were developed for each species using the maximum entropy algorithm in Maxent 3.3.3 k (Phillips et al. 2006).

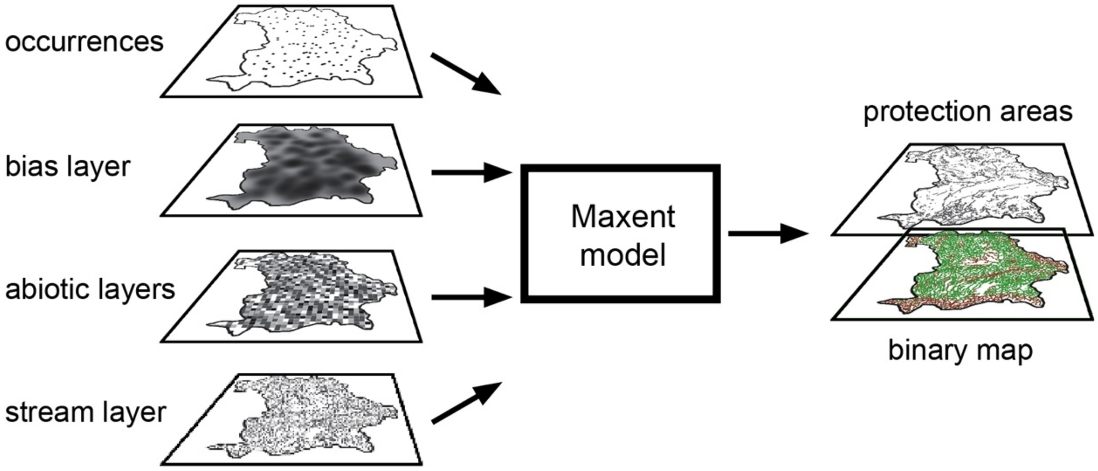

Fig. 6 Schematic illustration of the ecological niche modeling and the subsequent gap analysis (left to right). Based on different input layers (occurrence layer, a bias layer, 12 different abiotic layers and a buffered stream layer) ecological niche models were created using MAXENT. Niche models were transformed into binary maps using the 10th percentile of suitability scores, which were subsequently overlaid by maps of the actual protected areas to reveal conservation gaps.

As occurrence data, 386 data points for *M. margaritifera* and 914 data points for *U. crassus* from the Bavarian species conservation monitoring database collected between 1990 and 2016 were used. To reduce spatial autocorrelation of these data due to sampling bias, records of each species were reduced to one point within a 2-km radius (Euclidian distance) using SDMTOOLBOX 1.0b (Brown 2014) in ArcGIS 10.3.1 (ESRI, Inc. Redlands, CA, USA) following the suggestions of Kramer-Schadt et al. (2013) and Phillips et al. (2009). In addition, a bias layer of Gaussian kernel density based on the occurrence points with a bandwidth of 50 km was created and used, to control background sampling efforts that can be influenced by the sporadic distribution of a species and the higher sampling in areas with known occurrences (Elith et al. 2011).

Models were based on raster layers of twelve uncorrelated abiotic variables that were masked with a 500-m buffered line shape of Bavarian rivers: six climate variables, three landscape variables, two anthropogenic variables and one physicochemical variable (see **Table 9**).

Based on the suitability threshold that was set at the 10th percentile of suitability scores, binary maps of each of the niche models were created, with values above this threshold as suitable and values below this threshold as unsuitable parts. These binary maps were overlaid with maps of the actual conservation areas designated by §§ 23-27 and 31-36 of the German federal nature conservation act (BNatSchG) to reveal conservation gaps (see **Fig. 6**).

2.5 Statistical analyses

Statistical analyses were performed using MAXENT version 3.3.3 k (Phillips et al. 2006), R version 3.5.0 using package ‘glmmTMB’ (Brooks et al. 2017), R version 4.1.0 using package ‘stats’ (R Core Team 2020) and PRIMER version 7 with PERMANOVA+ add-on (Plymouth Marine Laboratory, Plymouth, UK; Anderson et al. 2008).

Descriptive statistics were used to characterize the mussel density of each species, the sampled *S. woodiana* habitat and shell length and wet weight of all *S. woodiana* specimens found for each sampled water body. It was also used to present the damage patterns for each bivalve species of each experimental part and the numbers of the found bivalves and crayfish at each sampling spot of the field validation. In addition, descriptive statistics was used to illustrate the percentage of suitable habitats for both bivalve species within the protected area types.

Generalized linear model (GLM) with AIC-based stepwise backward model selection were calculated to identify the decisive parameters explaining mussel occurrence. Non-parametric distance-based linear modelling (DistLM) with 999 permutation was carried out using an AIC-based BEST analysis selection to determine the proportion of explained abundance and biomass variation of native mussel species and invasive *S. woodiana* by physico-chemical parameters.

Zero-inflated generalized linear mixed models (glmmTMB) were conducted to explain length, depth and number of predation marks of native and invasive crayfish predation on three native and one non-native mussel species.

Maximum entropy algorithm was used to develop ecological niche models (ENMs) for two highly endangered native unionid species *M. margaritifera* and *U. crassus*.

3 Distribution and potential impacts of non-native Chinese pond mussels *Sinanodonta woodiana* (Lea, 1834) in Bavaria, Germany

A similar version of this chapter was published: Dobler AH, Hoos P, & Geist J. 2022. Distribution and potential impacts of non-native Chinese pond mussels *Sinanodonta woodiana* (Lea, 1834) in Bavaria, Germany. *Biological Invasions* 24: 1689-1706. DOI: 10.1007/s10530-022-02737-2

3.1 Abstract

The Chinese pond mussel, *Sinanodonta woodiana*, is non-native to European freshwater systems. Originating from Asia, it was brought to Europe in the 1970s and is now spreading across many countries. Management of the species is currently limited by a lack of information on the actual distribution and population characterisation of *S. woodiana* as well as on the co-occurrence of native mussels potentially resulting in competition.

For this paper, we examined nine water systems in the German federal state of Bavaria with randomly observed and anecdotally reported *S. woodiana* occurrences. We recorded the density and biomass of *S. woodiana* and of the co-existing mussel species as well as the habitat characteristics.

We found *S. woodiana* in eight water systems, co-existing with other native and invasive mussel species in seven of them. The distribution of *S. woodiana* was geographically widespread throughout Bavaria, indicating great invasion potential. In one fish pond, *S. woodiana* was the only mussel species occurring with the highest mean biomass (1,286.0 ± 1,067.7 g m^{-2}) and with the biggest specimen (25.0 cm and 1,616.0 g). The wide range of habitat variables matched the expectation of a wide ecological niche for the species.

This study provides evidence that *S. woodiana* has already become established in many water systems in Bavaria, with fish ponds and fisheries management practises being a likely vector. To counteract this species and to conserve native mussel species, it is important to implement effective legislation, to take measures to eradicate this invasive mussel transnationally, and to raise public awareness.

Candidate's contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by the candidate as well as by PH. The first draft of the manuscript was written by the candidate and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

3.2 Introduction

Biological invasions can have major impacts on local freshwater ecosystems (Keller et al. 2011; Malmqvist and Rundle 2002; Strayer and Dudgeon 2010). Thus, the introduction of alien species can bring about an increase of competition in several contexts (e.g., food resources, reproduction) as well as predation which could result in a decline of native species (Sousa et al. 2014; Zahner-Meike and Hanson 2001). Native freshwater mussel populations have declined globally in recent decades and most of them are now considered endangered (Lopes-Lima et al. 2018). These declines result mainly from water pollution, habitat degradation and fragmentation as well as climate change (Dudgeon et al. 2006; Lydeard et al. 2004; Regnier et al. 2009; Stoeckl et al. 2020; Strayer et al. 2004; Young and Williams 1983). In addition, the negative effects of invasive alien species (IAS) can be important. For instance, predation of invasive muskrat (*Ondatra zibethicus*) can cause considerable damages to mussel populations (Zahner-Meike and Hanson 2001) and also invasive freshwater crayfish appear to have negative impacts on native mussel populations (Meira et al. 2019; Schmidt and Vandré 2012; Sousa et al. 2019). Also, the spread of invasive freshwater bivalves can lead to increasing competition pressures (Urbańska et al. 2021). The relatively small invasive Asian *Corbicula fluminea* (Müller, 1774), which, however, occurs in high densities, and the invasive zebra mussel, *Dreissena polymorpha* (Pallas, 1771), both compete strongly with native mussels for food as a result of their high filtration rates (Cohen et al. 1984; Phelps 1994; Strayer et al. 1999). In Europe, both species are well established in many standing or slow flowing water bodies (see Karatayev et al. 2005; Van der Velde et al. 2010). Due to its ability to attach to solid objects using byssus threads, *D. polymorpha* can overgrow native bivalves, which can negatively affect the physiological condition of and also deform or at least overturn and thus kill these mussels (Ożgo et al. 2020; Sousa et al. 2011). Aside from this, the direct impact of dreissenid fouling on industrial and infrastructural facilities results in manifest technical and financial damage (Cuthbert et al. 2021; Sousa et al. 2014), which attracts widespread attention in society. In contrast, the invasion of unionids has remained relatively unnoticed and the potential effects have not yet been considered. Nevertheless, there is increasing evidence that another occurring non-native freshwater mussel, the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834), has competitive advantages over other unionids native to Europe. For example, this species can use a broader range of suitable fish as hosts, achieves higher infestation rates and develops faster than native mussel species (Douda et al. 2012;

Huber and Geist 2019). In addition, the quantity of glochidia is larger in *S. woodiana* compared to native unionids, breeding is not limited to one single action per year (Labecka and Czarnoleski 2021; Labecka and Domagala 2018) and the glochidia are also more persistent over a range of temperatures (Benedict and Geist 2021). All this leads to increased reproduction success for *S. woodiana*. Conversely, the recruitment of native unionids could be reduced if a host fish has already been infested with glochidia of *S. woodiana* as has been shown by Donrovich et al. (2017) for *Anodonta anatina*. In addition, adult specimens have been reported to tolerate a wider range of substratum conditions in comparison with native mussel species (Urbańska et al. 2021).

S. woodiana originates from the Yangtze River basin and from Taiwan (see Kondakov et al. 2018; Lopes-Lima et al. 2020) and is spreading to many countries globally (see e.g., Bespalaya et al. 2018 for Siberia; Bogan et al. 2011 for USA; Bolotov et al. 2016; Kondakov et al. 2020 for Russia; Kondakov et al. 2018 for Uzbekistan; Konecny et al. 2018 for Europe; Zieritz et al. 2018a for Malaysia and Indonesia; Zieritz et al. 2018b for Borneo; Zieritz et al. 2016 for Malaysia). In Europe, this species was first recorded in western Romania in 1979 (Sarkany-Kiss 1986) and then three years later near Arles in southern France (Adam 2010). Initially, its spread was slow and restricted to artificial heated water (see Urbańska et al. 2012) but has become faster in the last two decades (Lopes-Lima et al. 2017). Konecny et al. (2018) described the initial introduction of this species to Europe as resulting from a commercial import of Asian carp from the Yangtze River basin to hatcheries in Romania in the early 1960s, while a further spread of *S. woodiana* from the Amur River basin that were brought to hatcheries in Hungary could not be genetically verified in Europe. With its ability to adapt to cold water, this mussel species has succeeded in invading many water systems within Europe (Konecny et al. 2018). In addition, this species is sold in many outlets that provide pond products such as garden centres and construction markets as well as in aquaristics online stores, often erroneously named as “European pond mussel” (pers. obs.). This provides *S. woodiana* with an additional opportunity to spread even further.

Despite the situation that *S. woodiana* has colonised many European countries, the European Union lacks a consistent approach for classification and management. The German legal status, for example, classifies *S. woodiana* as ‘potentially invasive’ (Rabitsch and Nehring 2017) because of the lack of documentation on the actual distribution and invasiveness of *S. woodiana*. This shows the need to evaluate the actual distribution of

S. woodiana as well as its co-occurrence with native mussel species. For effective management of *S. woodiana*, systematic documentation of its habitat preferences, distribution pathways and its effects on native mussels in the event of coexistence would provide crucial information. Some studies already exist that document single populations or the spatial distribution of *S. woodiana* partly combined with an analysis of the co-occurrence and competition with native mussels, while other studies are focussed on single populations and the description of their habitat conditions. In this study, we have systematically investigated the habitat conditions and mussel community of various water bodies over a larger regional scale.

Therefore, we monitored nine water systems with randomly observed and anecdotally reported *S. woodiana* occurrences in Bavaria, Germany. Our main aims were (i) to verify the alleged occurrences of *S. woodiana* in Bavaria as well as to assess establishment of this species in these water systems; (ii) to characterise the habitat conditions where the species is found; (iii) to characterise the *S. woodiana* populations with respect to population density, mussel length and biomass and (iv) to assess the co-occurrence and potential interactions with native mussel species. We hypothesised that (i) *S. woodiana* still only occurs sporadically in the study region, (ii) the distribution of *S. woodiana* is limited to artificial or anthropogenic water bodies like fish ponds and (iii) the density and biomass of *S. woodiana* is higher than that of native mussel species.

3.3 Methods

3.3.1 Study area

The study area covers the federal state of Bavaria in Germany with its three main catchment areas of the Danube, Main and Elbe. We searched for references of known *S. woodiana* occurrences in the federal species protection database (LfU (Bayerisches Landesamt für Umwelt) 2020) as well as for hints from the public collected by the Bavarian coordination office for freshwater mussel conservation (personal communication)

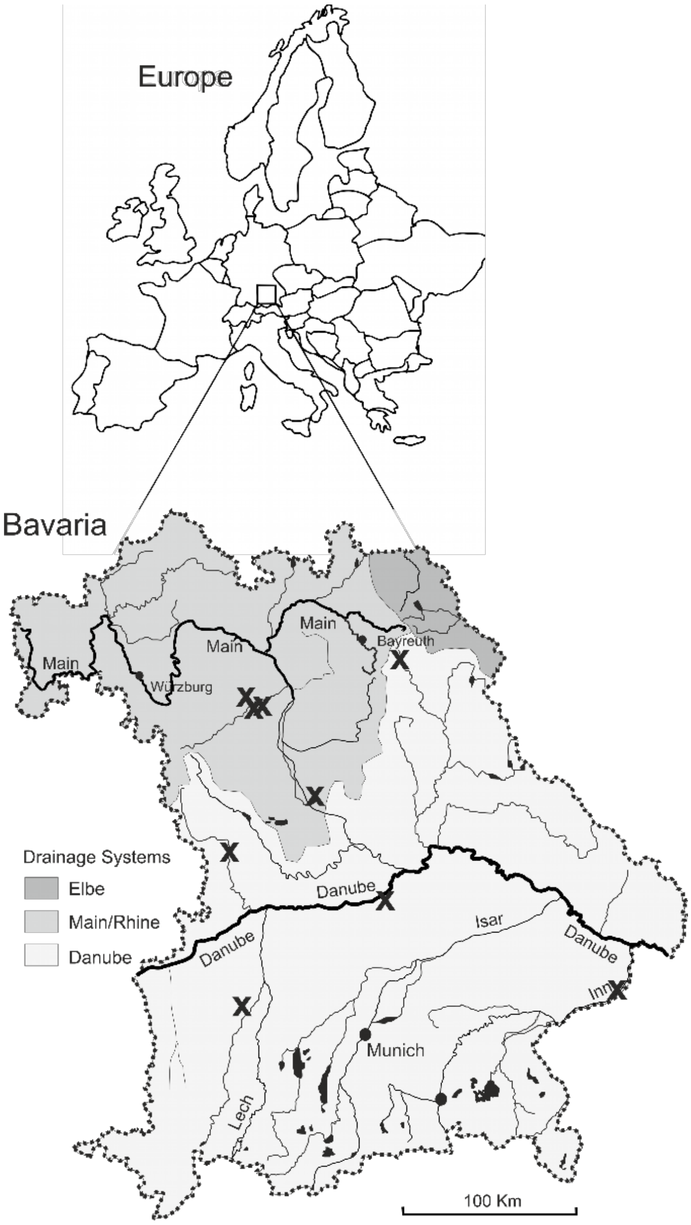

Fig. 7 Map of the study area with the three main drainage systems of Bavaria, Germany. Black crosses indicate the nine sampling locations.

over a period of four years. We systematically examined nine water bodies (**Fig. 7**) with a speculated occurrence of *S. woodiana*. All necessary permissions for this study including protected species and protected areas were obtained (reference numbers: RMF-SG55.1-8646-7-111-2 for Middle Franconia, 55.3-8646-2/856 for Swabia, ROP-SG55.1-8622.1-47-2-3 for Upper Palatinate and 41.0.03/8231 for the administrative district of Passau).

3.3.2 Data collection

Based on the different macrohabitats of each water body, we haphazardly distributed a minimum of six sampling plots around the area of the suspected *S. woodiana* occurrence by randomly placing sampling frames onto the water bottom. We used 0.5 x 0.5 m or 1 x 1 m frames respectively a twelve-metre-long and at both ends connected chain with marks all 3 meters defining the 4 corners (= 9 m^2) to define the spots. This resulted in a total of 129 quadrats of 1 m^{-2} (i.e. 0 to 50 per site) and 19 quadrats of 9 m^{-2} (i.e. 0 to 12 per site). We systematically started with the smallest frame before switching to the next largest frame after 5 spots with no mussels to ensure sufficient quantity. At sampling spots with a water depth of more than 1 m or with high turbidity, sampling was performed by scuba diving.

3.3.3 Abiotic habitat parameters

To characterise the habitats and to determine the ecological tolerance of *S. woodiana*, we measured water temperature (T, in °C), dissolved oxygen (O_2, in mg L^{-1}), pH-value, and electric conductivity (Cond., in µS cm^{-1}, relative to 25 °C) using a handheld Multi 3630 IDS F meter (WTW, Weilheim, Germany) once in the open water (FW) as well as in a sample of interstitial water taken at a substratum depth of 10 cm (IN) in the middle of the sampling spot (Geist and Auerswald 2007). We also measured the turbidity (Turb, in NTU) using a handheld Turb® 355 T (WTW, Weilheim, Germany). At spots we had to sample by scuba diving, we took samples of interstitial water as well as of open water above the ground and immediately delivered these samples to the surface for measurements.

To characterise the substrate, we measured the penetration resistance (PR, in kg cm^{-2}) using a handheld penetrometer (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) as well as the redox potential (Eh, in mV) in situ in the open water and in the interstitial water (10 cm depth) as per Geist and Auerswald (2007) using a handheld pH 3110 meter (WTW, Weilheim, Germany) together with a platinum electrode and an

$Ag/AgCl_2$-reference electrode. At scuba diving spots the interstitial redox measurement was performed within the interstitial water samples. In addition, the water depth and, if present, the thickness of the silt layer was measured using a measuring rod (± 0.5 cm). For diving spots the water depth was measured using a depth gauge (± 0.25 m).

In running waters, we measured the width of the waterbody using a measuring tape (± 0.05 m) for widths up to 25 meters and the measuring tool of a satellite image viewer (https://geoportal.bayern.de/bayernatlas) for widths over 25 meters. In accordance with common techniques in hydrological studies, we measured the flow velocities (v, in m s^{-1}) at 60% depth of the water column (mc) as well as at 2 cm below the surface (su) (see Stoeckl and Geist 2016), using a handheld flowmeter (Flowtherm NT, Höntzsch, Waiblingen, Germany). Since the pond at Muenchsmuenster was drained at the time of sampling, no abiotic water parameters could be collected.

3.3.4 Biotic parameters

We collected all mussel specimens that could be detected visibly or by touch within the defined spots. After the abiotic samples, we also disturbed the sediment by hand to a maximum of 50 cm depth if possible, e.g., in fine sediment, to find buried individuals. All collected mussels were counted and determined at the species level. The wet body mass of each unionid specimen was weighed separately using field scales (± 1 g) and its shell length was measured using callipers (± 0.1 mm). Mussels with attached dreissenids were cleaned before the measurements and the wet body mass of the attached *Dreissena* spp. specimens were weighed separately. All *Corbicula* spp. specimens found were counted and weighed together. All protected mussels were immediately returned to the sample location, whereas invasive mussels were not released.

The coverage of the macrophytes (Marcoph., in %) within the sampling frames was documented.

3.3.5 Statistical analyses

We summarised all found mussels and calculated the mussel density (in Ind m^{-2}) and biomass (in g m^{-2}) in total and for each mussel species separately for each water system. Due to the high amount of dreissenid bundles in the Rothsee consisting mainly of dead shells as well as live specimens it was not practicable to examine each single mussel specimen. Therefore, we did not include dreissenids in density and biomass calculations. We also calculated the average shell length and mean wet weight of

S. woodiana for each water body. For habitat parameters, maximum, minimum and mean values were calculated. Unless stated otherwise, arithmetic mean and standard deviation (SD) were calculated and reported as mean ± standard deviation. We computed a generalised linear model (GLM; model 1) using a “glm”-function within R (version 4.1.0; R Core Team 2020) with a binary value of plots with (1) and without (0) mussel occurrence as the response variable and with the abiotic and biotic values (O_2 FW, O_2 IN, T FW, T IN, Lf FW, Lf IN, pH FW, pH IN, v su, v mc, water depth, Macroph., Turb, PR, Eh FW, Eh IN) as predictors. We used the function “stepAIC” to select the best model and set the significance levels to $p < 0.05$.

To determine the proportion of explained variation in the abundance and biomass data of the native mussel species by physico-chemical parameters as well as the abundance or biomass of *S. woodiana*, non-parametric distance-based linear modelling (DistLM) using PRIMER (version 7) with the PERMANOVA+ add on (Plymouth Marine Laboratory, Plymouth, UK; Anderson et al. 2008) was carried out with 999 permutations. The model was based on the Bray-Curtis distance resemblance. AIC (Akaike Information Criterion) was used as the selection criterion and the procedure of selection followed a BEST analysis. To visualise the community structure and the similarity of the different spots, we plotted the DistLM in a distance-based redundancy analysis (dbRDA) for the native mussel density and biomass with bubbles representing the species contributions. We only used environmental variables with a significant contribution to the relationship with the abundance or biomass data of the native mussel species as well as abundance or biomass of *S. woodiana* as an overlay.

3.4 Results

3.4.1 Population characteristics

We sampled a total of 148 plots with a total of 300 m^{-2} (129 x 1 m^{-2} and 19 x 9 m^{-2}) in nine different water systems across Bavaria and found mussels in 55.4% of the samples. We could verify the establishment of *S. woodiana* in eight of the nine sampled water systems where it was expected to occur based on random observations and anecdotal reporting. Within 35.1% of our sampling plots we found 100 individuals of *S. woodiana*. In addition, we could find another 92 individuals outside of our plots. We found the native mussel species *A. anatina*, *A. cygnea* and *U. pictorum* in five, *U. tumidus* in one and the other invasive mussel species *C. fluminea* and *Dreissena spp.* also in one of the nine sampled water bodies. Over all samples including spots without mussels, the mean mussel density was 2.2 ± 4.4 Ind m^{-2} with a mean biomass of 35.9 ± 191.0 g m^{-2}. For *S. woodiana*, mean density was 0.45 ± 0.84 Ind m^{-2} with a mean biomass of 150.8 ± 427.2 g m^{-2}. We found the highest mussel density in the reservoir Rothsee with 5.8 ± 7.1 Ind m^{-2} and a maximum of 26 Ind m^{-2}. This reservoir also had the highest mussel diversity with a percentage contribution of *S. woodiana*, *A. anatina*, *A. cygnea*, *U. pictorum* and *C. fluminea* with 4.0%, 6.3%, 0.1%, 52.3% and 36.8% in number of individuals (see **Fig. 8a**) and with 23.2%, 18.3%, 4.7%, 49.5% and 4.2% in biomass (see **Fig. 8b**), respectively. In the fish pond Burlafinger Weiher, *S. woodiana* was the only mussel species occurring and had the highest mean biomass (1,286.0 ± 1,067.7 g m^{-2}) of all sampled water systems. We also found the largest specimen in this pond at 25.0 cm and 1,616.0 g (**Fig. 9**).

In seven of the nine sampled water systems and in 14.2% of all sampling spots, *S. woodiana* was found co-occurring with native (*A. anatina*, *A. cygnea*, *U. pictorum*, *U. tumidus*) or other invasive mussel species (*C. fluminea*, *D. bugensis* and *D. polymorpha*). The reservoir Rothsee, which is connected to the Main-Danube-channel and serves as a water compensation body, hosts most of the species mentioned above excluding *U. tumidus*.

Table 2: Population density (Ind m^{-2}) of all mussels and for each species separately for each water system separately and in total. Given values are shown with mean ± standard deviation in the first line and with the range given in brackets in the second line. An "*" indicates an invasive species.

Sampled water systems		Mussel density							
Names	Abbreviation	All	*S. woodiana**	*A. anatina*	*A. cygnea*	*U. crassus*	*U. pictorum*	*U. tumidus*	*C. fluminea**
Aischgraben/ Kleine Weisach	AK	1.5 ± 1.7 [0.0–5.0]	0.0 ± 0.0 [0.0–0.0]	0.8 ± 1.2 [0.0–3.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.8 ± 0.7 [0.0–2.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Backwater Inn	BI	1.3 ± 1.4 [0.0–4.0]	0.3 ± 0.5 [0.0–1.0]	0.3 ± 0.5 [0.0–1.0]	0.2 ± 0.4 [0.0–1.0]	0.0 ± 0.0 [0.0–0.0]	0.5 ± 0.5 [0.0–1.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Burlafinger Weiher	BW	2.0 ± 1.2 [1.0–5.0]	2.0 ± 1.2 [1.0–5.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Großer Hirschbergweiher	GH	0.3 ± 0.4 [0.0–1.1]	0.1 ± 0.1 [0.0–0.3]	0.0 ± 0.0 [0.0–0.0]	0.3 ± 0.4 [0.0–1.1]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Hirtenweiher	HW	0.4 ± 0.7 [0.0–1.9]	0.4 ± 0.6 [0.0–1.8]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.1]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Moorbach	MB	4.9 ± 4.9 [0.0–14.0]	1.4 ± 1.2 [0.0–4.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	2.3 ± 2.7 [0.0–8.0]	1.1 ± 1.7 [0.0–4.0]	0.0 ± 0.0 [0.0–0.0]
Rothsee	RS	5.8 ± 7.1 [0.0–26.0]	0.2 ± 0.5 [0.0–2.0]	0.4 ± 0.5 [0.0–1.0]	0.0 ± 0.2 [0.0–1.0]	0.0 ± 0.0 [0.0–0.0]	3.0 ± 3.3 [0.0–12.0]	0.0 ± 0.0 [0.0–0.0]	2.1 ± 4.0 [0.0–13.0]
Pond near Muenchsmuenster	PM	0.1 ± 0.3 [0.0–1.0]	0.1 ± 0.2 [0.0–1.0]	0.0 ± 0.1 [0.0–1.0]	0.0 ± 0.2 [0.0–1.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
Woernitz	WO	3.7 ± 5.1 [0.0–20.0]	1.3 ± 0.9 [0.0–3.0]	0.2 ± 0.4 [0.0–1.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]	2.2 ± 5.2 [0.0–19.0]	0.0 ± 0.0 [0.0–0.0]	0.0 ± 0.0 [0.0–0.0]
All		**2.2 ± 4.4** **[0.0–13.0]**	**0.5 ± 0.8** **[0.0–5.0]**	**0.2 ± 0.4** **[0.0–3.0]**	**0.1 ± 0.2** **[0.0–1.1]**	**0.0 ± 0.0** **[0.0–0.0]**	**1.0 ± 2.6** **[0.0–19.0]**	**0.1 ± 0.5** **[0.0–4.0]**	**0.4 ± 2.0** **[0.0–13.0]**

3.4.2 Habitat characterisation

We additionally characterised the habitat by measuring abiotic and biotic variables in 95 sampling spots. We found mussels in 76 of these sampling spots whereas in 19 spots

Table 3: Characterisation of the sampled *S. woodiana* habitats with water and sediment parameters for each water body and in total. Given values are shown with mean ± standard deviation in the first line and with the range given in brackets in the second line. Abbreviations for water systems originate from **Table 2**.

Variables	BW (N = 9)	GH (N = 6)	HW (N = 6)	BI (N = 2)	MB (N = 7)	RS (N = 6)	WO (N =12)	**All (N = 48)**
Open water								
O$_2$ FW	13.6 ± 0.7	NA	11.0 ± 0.7	9.5 ± 0.3	6.1 ± 1.5	8.7 ± 0.4	11.1 ± 2.2	**10.4 ± 2.8**
(mg L^{-1})	[12.8–14.9]		[10.3–12.3]	[9.3–9.7]	[4.5–7.9]	[8.0–9.3]	[8.0–14.2]	**[4.5–14.9]**
T FW	20.2 ± 0.6	NA	11.4 ± 0.3	10.5 ± 0.0	9.2 ± 0.6	19.1 ± 4.0	22.1 ± 2.3	**17.0 ± 5.6**
(°C)	[19.6–21.2]		[11.1–11.7]	[10.5–10.5]	[8.4–9.7]	[16.2–24.8]	[18.9–24.2]	**[8.4–24.8]**
Cond FW	270 ± 3	NA	593 ± 4	291 ± 0	589 ± 36	562 ± 9	684 ± 49	**530 ± 164**
(µS cm^{-1})	[265–274]		[587–598]	[291–291]	[543–635]	[550–568]	[614–732]	**[265–732]**
pH FW	8.8 ± 0.1	NA	8.4 ± 0.1	8.0 ± 0.0	7.5 ± 0.1	8.0 ± 0.0	8.2 ± 0.2	**8.2 ± 0.4**
	[8.7–8.9]		[8.3–8.5]	[8.0–8.1]	[7.5–7.6]	[8.0–8.1]	[8.0–8.6]	**[7.5–8.9]**
Eh FW	401 ± 9	NA	266 ± 92	247 ± 71	375 ± 51	410 ± 30	416 ± 179	**375 ± 118**
(mV)	[385–414]		[87–332]	[196–297]	[304–432]	[361–436]	[-28–670]	**[-28–670]**
Turb	7.5 ± 3.2	NA	35.7 ± 15.1	35.9 ± 35.5	21.5 ± 8.0	4.6 ± 1.7	16.1 ± 11.6	**17.3 ± 14.9**
(NTU)	[4.1–14.3]		[22.1–65.3]	[10.8–61.0]	[7.7–29.3]	[2.1–7.2]	[6.9–43.4]	**[2.1–65.3]**
v su	0.00 ± 0.00	NA	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.13	0.00 ± 0.00	0.05 ± 0.06	**0.03 ± 0.07**
(m s^{-1})	[0.00–0.00]		[0.00–0.00]	[0.00–0.00]	[0.00–0.28]	[0.00–0.00]	[0.00–0.16]	**[0.00–0.28]**
v mc	0.00 ± 0.00	NA	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.12	0.00 ± 0.00	0.06 ± 0.06	**0.03 ± 0.07**
(m s^{-1})	[0.00–0.00]		[0.00–0.00]	[0.00–0.00]	[0.00–0.29]	[0.00–0.00]	[0.00–0.19]	**[0.00–0.29]**
Depth	104.1 ± 16.8	30.7 ± 4.2	44.2 ± 19.4	89.0 ± 4.2	52.3 ± 16.8	296.7 ± 101.3	79.8 ± 23.2	**97.2 ± 88.0**
(cm)	[65.0–120.0]	[25.0–36.0]	[26.0–76.0]	[86.0–92.0]	[18.0–70.0]	[150.0–400.0]	[59.0–135.0]	**[18.0–400.0]**
Width	NA	NA	NA	29.8 ± 8.8	3.4 ± 0.5	NA	30.9 ± 13.7	**21.6 ± 16.8**
(m)				[23.5–36.0]	[2.5–4.1]		[14.8–57.9]	**[2.5–57.9]**
Substrate								
O$_2$ IN	0.6 ± 1.3	NA	0.8 ± 1.2	1.6 ± 0.1	0.5 ± 0.5	3.1 ± 1.9	1.1 ± 1.1	**1.2 ± 1.4**
(mg L^{-1})	[0.0–3.9]		[0.0–3.0]	[1.5–1.7]	[0.0–1.5]	[0.4–6.1]	[0.0–3.3]	**[0.0–6.1]**
T IN	21.6 ± 0.3	NA	12.2 ± 0.2	11.4 ± 0.7	10.0 ± 0.8	19.2 ± 4.5	21.8 ± 1.9	**17.5 ± 5.3**
(°C)	[20.9–21.9]		[11.9–12.5]	[10.9–11.9]	[9.0–11.4]	[15.6–25.3]	[19.3–24.4]	**[9.0–25.3]**
Cond IN	356 ± 81	NA	655 ± 63	394 ± 37	882 ± 227	639 ± 87	792 ± 97	**653 ± 224**
(µS cm^{-1})	[265–530]		[594–736]	[367–420]	[610–1.282]	[536–768]	[704–1.009]	**[265–1,282]**
pH IN	7.0 ± 0.2	NA	7.6 ± 0.5	7.3 ± 0.1	7.0 ± 0.4	7.2 ± 0.3	7.2 ± 0.3	**7.2 ± 0.3**
	[6.8–7.3]		[7.2–8.4]	[7.3–7.4]	[6.6–7.8]	[7.0–7.7]	[6.8–7.6]	**[6.6–8.4]**
Eh IN	296 ± 80	NA	49 ± 140	121 ± 105	24 ± 33	356 ± 83	133 ± 136	**169 ± 157**
(mV)	[192–413]		[-213–202]	[46–195]	[-5–76]	[252–450]	[-148–280]	**[-213–450]**
PR	0.00 ± 0.00	NA	0.01 ± 0.02	0.03 ± 0.01	0.19 ± 0.18	0.18 ± 0.11	0.27 ± 0.17	**0.14 ± 0.16**
(kg cm^{-2})	[0.00–0.00]		[0.00–0.05]	[0.02–0.03]	[0.01–0.55]	[0.07–0.38]	[0.00–0.51]	**[0.00–0.55]**
Silt layer	18.8 ± 10.1	1.7 ± 2.6	25.5 ± 11.3	6.5 ± 4.9	23.1 ± 35.4	NA	60.0 ± 54.8	**20.7 ± 29.6**
(cm)	[8.0–40.0]	[0.0–5.0]	[10.0–38.0]	[3.0–10.0]	[0.0–100.0]		[0.0–100.0]	**[0.0–100.0]**
Macroph.	25.0 ± 27.4	0.0 ± 0.0	0.0 ± 0.0	12.5 ± 10.6	0.0 ± 0.0	0.0 ± 0.0	4.2 ± 7.9	**6.3 ± 15.4**
(%)	[0.0–80.0]	[0.0–0.0]	[0.0–0.0]	[5.0–20.0]	[0.0–0.0]	[0.0–0.0]	[0.0–20.0]	**[0.0–80.0]**

mussels could not be detected. A significant difference of plots with and without mussels could only be detected in dissolved oxygen of the water column (ANOVA; *F* = 6.223; $p < 0.05$) with 10.2 ± 2.4 mg L^{-1} and 8.6 ± 1.0 mg L^{-1}, respectively. The other abiotic parameters were nearly identical in both groups.

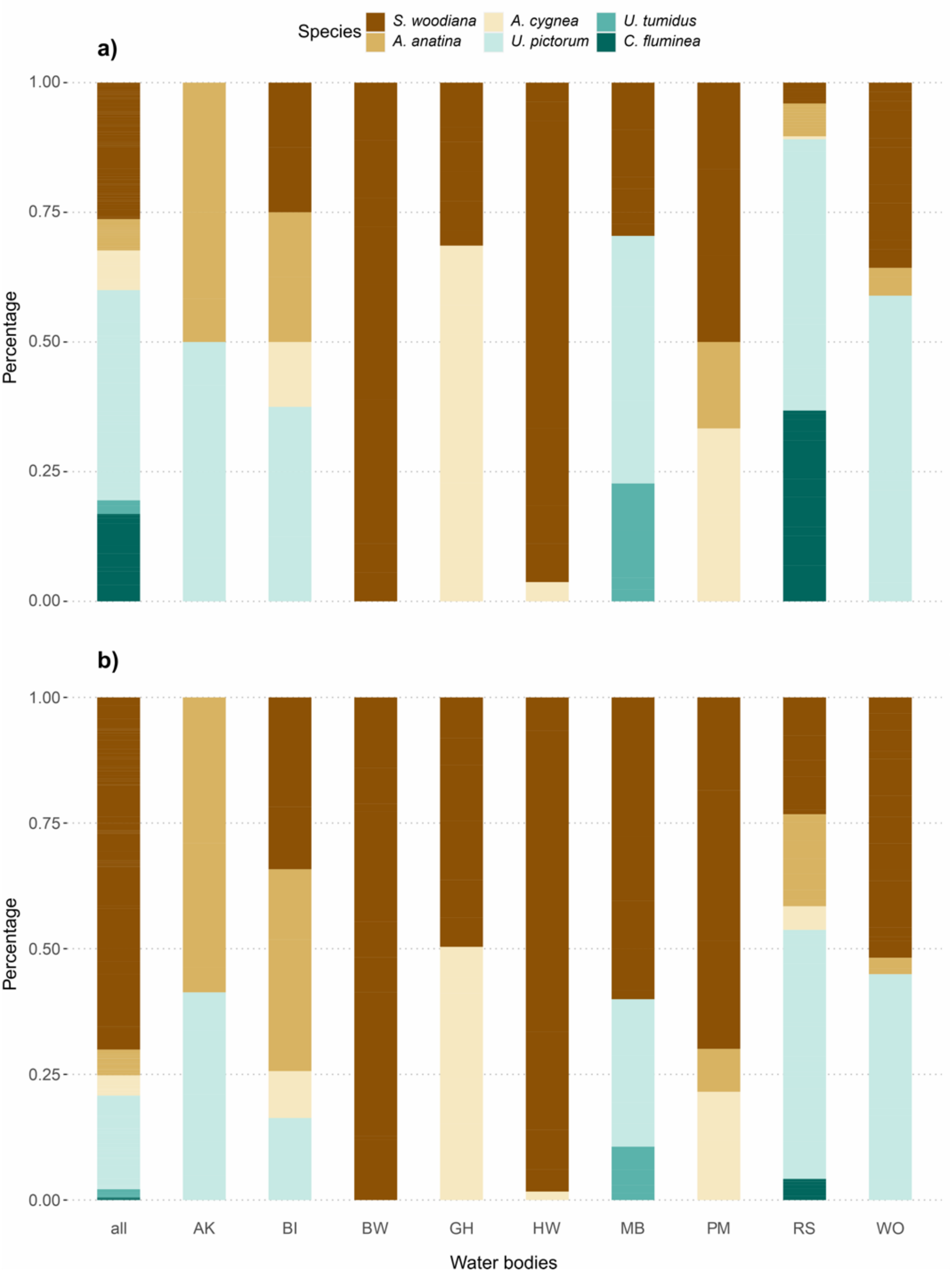

Fig. 8 Percentage contribution of a) the number of individuals and b) the biomass of all native and invasive mussel species for each sampled water body.

Within the studied water bodies, there was a great variance in structure and morphology as well as the measured physico-chemical parameters. In terms of oxygen supply, *S. woodiana* was found over a wide range of dissolved oxygen concentration with a minimum of 4.5 mg L^{-1} in Moorbach and a maximum of 14.9 mg L^{-1} in the Burlafinger Weiher (**Table 3**), even though this sampling cannot be considered representative due to seasonal and daily variation. Accordingly, the redox-potential at 10 cm depth of the interstitial showed a wide range from -213 mV in the fish pond Hirtenweiher to 450 mV in the reservoir Rothsee (**Table 3**). The latter was also the only water body with a mean above the threshold of 300 mV with values below indicating anoxic conditions (Schlesinger 1991). In addition, most of the plots had soft substratum as evident from the low means of penetration resistances (e.g., 0.00 ± 0.00 kg cm^{-2} in the Burlafinger Weiher and 0.01 ± 0.02 kg cm^{-2} in the Hirtenweiher, **Table 3**), and high mean amounts of silt (e.g., 25.5 ± 11.3 cm in the Hirtenweiher and 60.0 ± 54.8 cm in the Wörnitz, **Table 3**). *S. woodiana* was found to a maximum water depth of 4.0 meters in the Rothsee. Mean conductivity of the water bodies showed a wide range from 270 ± 3 µS cm^{-1} in the Burlafinger Weiher to 684 ± 49 µS cm^{-1} in the Wörnitz (**Table 3**).

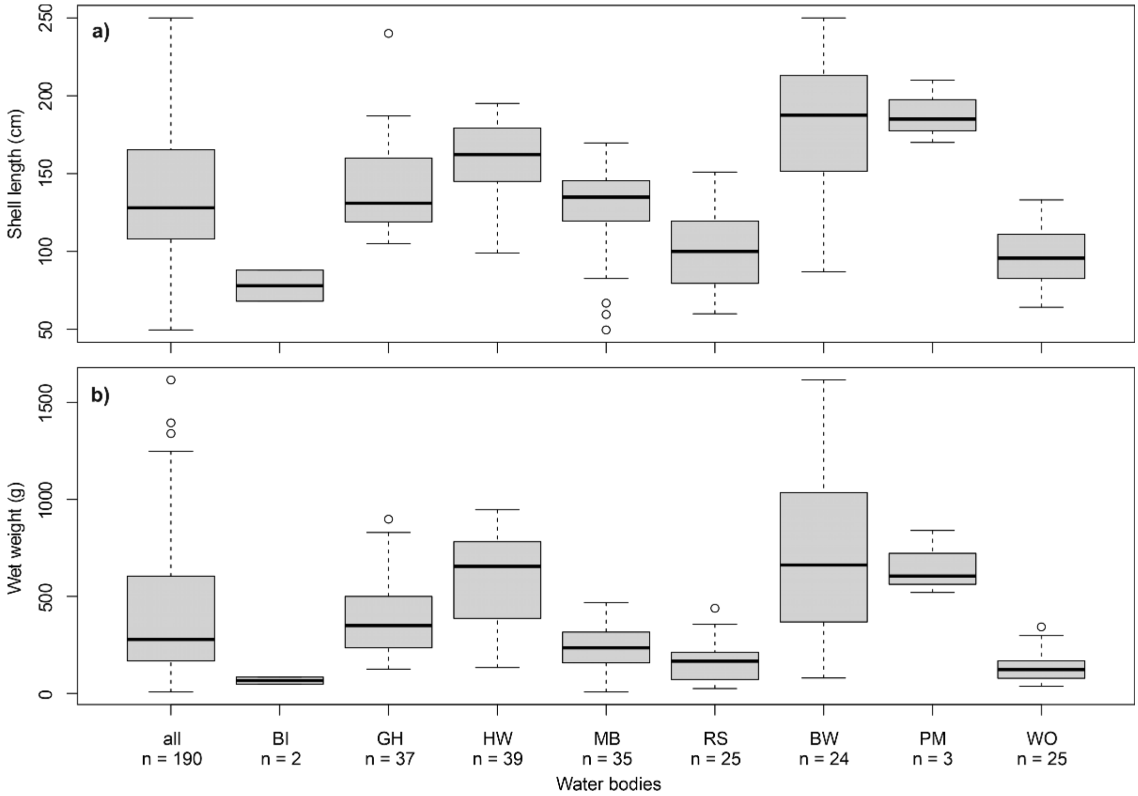

Fig. 9 Boxplots of individual a) shell lengths and b) wet weights of *S. woodiana* specimens from each sampled water body. The horizontal lines indicate median values, boxes the 25th to 75th percentiles, whiskers the lowest and highest values within 1.5 times the values observed in the percentile boxes and black dots single cases exceeding 1.5 times the values observed in the percentile boxes. Abbreviations for water systems originate from **Table 2**.

3.4.3 Co-existence of *S. woodiana* with native mussel species

Over all sampling spots, the highest number of individuals over all mussel species (20 Ind m^{-2}) were found in one spot with *S. woodiana* co-occurring with *U. pictorum* (**Fig. 10a**) whereas the highest biomass (3,312.0 g m^{-2}) was found in one spot with *S. woodiana* as the only mussel species (**Fig. 10b**). According to the DistLM, the redox of the water column (AIC = 145.9, SS-trace = 5870.2, Pseudo-F = 3.0, $p < 0.05$) and the temperature of the water column (AIC = 146.3, SS-trace = 5205.0, Pseudo-F = 2.6, $p < 0.05$) explained 15.0% and 13.3%, respectively, of the variance of native mussel abundance (**Fig. 11a**). The redox of the water column (AIC = 149.2, SS-trace = 7337.4, Pseudo-F = 3.2, $p < 0.05$) and conductivity of the interstitial water (AIC = 149.9, SS-trace = 5903.5, Pseudo-F = 2.4, $p < 0.05$) explained 15.6% and 12.6%, respectively, of the variance of the biomass of native mussels (**Fig. 11b**). Neither the abundance nor the biomass of *S. woodiana* had a statistically significant contribution to the explanation of the model. Nevertheless, based on the few data points of this study, a high biomass of native mussels was only found in locations with less than 617 g m^{-2} of *S. woodiana* (**Fig. 10**).

Table 4: Average shell length and wet weight of all *S. woodiana* specimens of each sampled water body and in total including specimens found outside of the plots. Abbreviations for water systems originate from **Table 2**.

Water bodies	n	Average length ± SD (mm)	Average weight ± SD (g)
AK	0	-	-
BI	2	78.0 ± 14.1 [68.0–88.0]	66.0 ± 25.5 [48.0–84.0]
BW	24	180.0 ± 41.3 [87.0–250.0]	721.9 ± 429.7 [79.0–1,616.0]
GH	37	143.3 ± 30.3 [105.0–240.0]	402.6 ± 206.4 [125.2–898.2]
HW	39	157.8 ± 27.8 [99.0–195.1]	581.8 ± 244.8 [133.0–947.0]
MB	35	127.6 ± 31.0 [49.6–169.7]	236.3 ± 125.3 [7.0–468.0]
RS	25	100.4 ± 25.2 [60.0–151.0]	161.4 ± 100.1 [25.0–439.0]
PM	3	188.3 ± 20.2 [170.0–210.0]	655.0 ± 165.8 [520.0–840.0]
WO	25	96.2 ± 18.8 [64.2–133.1]	138.2 ± 79.0 [36.4–343.0]
All	**190**	**135.8 ± 40.9** **[49.6–250.0]**	**383.0 ± 302.1** **[7.0–1,616.0]**

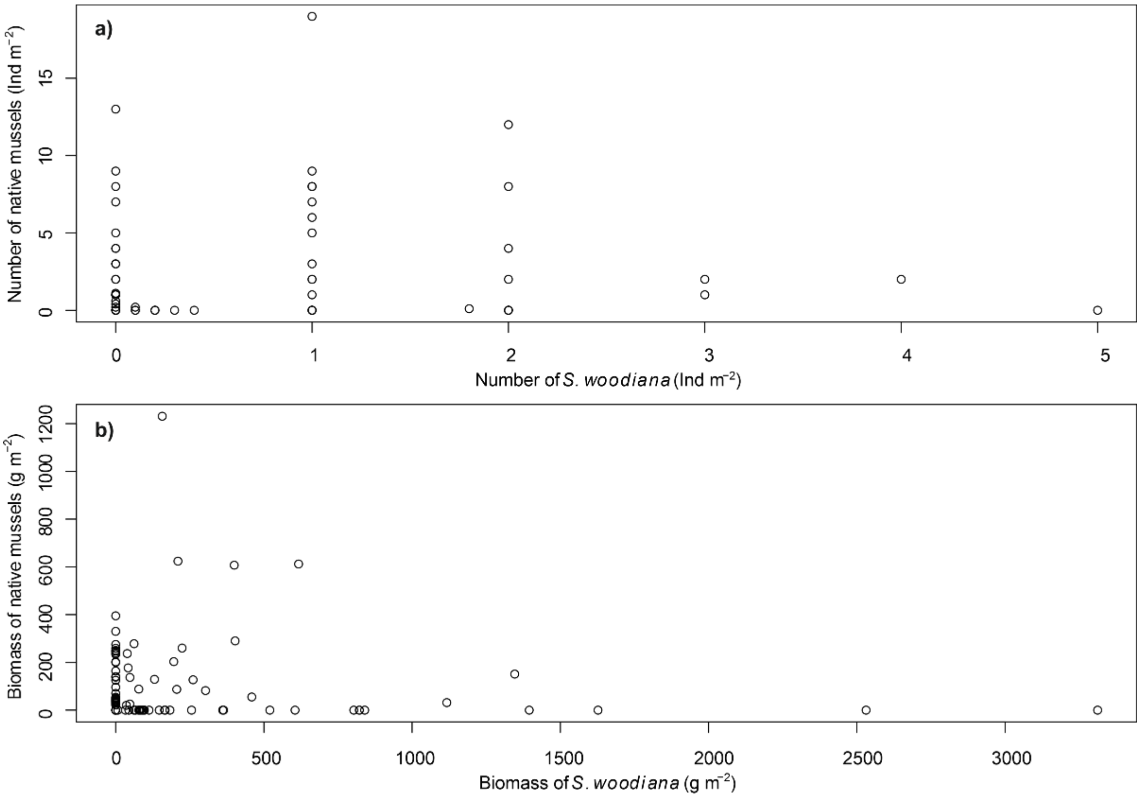

Fig. 10 Total number a) and biomass b) of *S. woodiana* and of native mussel species at each sampling spot.

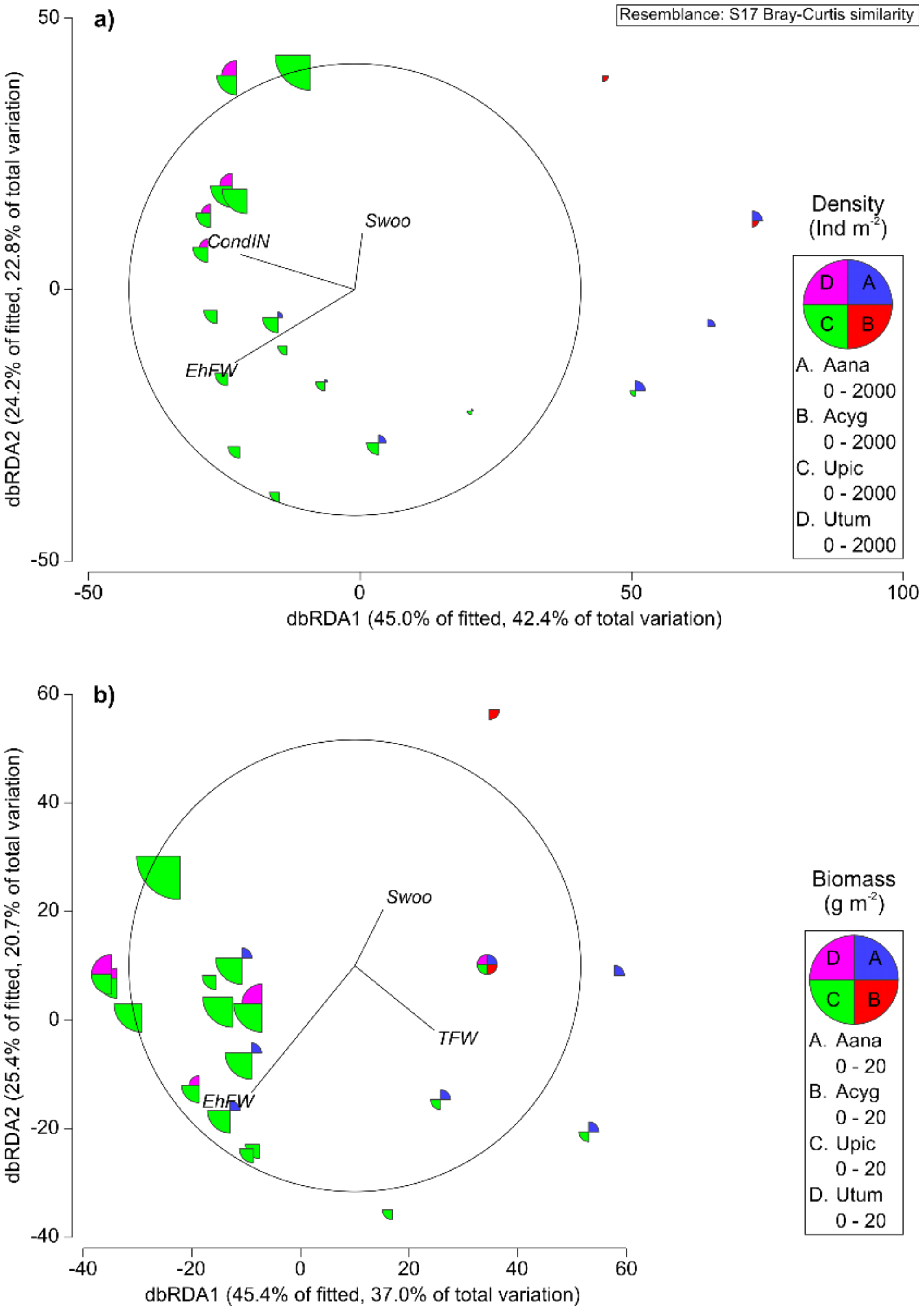

Fig. 11 Distance-based redundancy analysis (dbRDA) shows the relation of species composition in density a) and biomass b) of native mussels in relation to the physico-chemical variables as well as density or biomass of *S. woodiana*. Bubbles show the number of individuals (a) or biomass (b) per species. Physico-chemical variables shown in a vector overlay have a significant influence on the variability of the species composition and were correlated with the dbRDA plot using Pearson correlation. The length of the lines indicates the strength of correlation, with the radius of the black circle representing 100% correlation. Vectors indicating the orientation of *S. woodiana* density represent no significant influence and are shown only for illustration.

3.5 Discussion

In recent years, there have already been some individual detections of *S. woodiana* in some of the federal states of Germany, e.g., in Baden-Wuertemberg, Hessen, Saxony, Schleswig-Holstein, and Thuringia (Bahr and Wiese 2018; Bössneck and Klingelhöfer 2011; Dümpelmann 2012; Nagel and Pfeiffer 2019; Pfeiffer 2002). However, all of these studies only documented single populations and did not comprise a systematic sampling approach. In contrast, this study provides the first systematic approach to document the actual distribution of the invasive *S. woodiana* in the federal state of Bavaria in Germany by verifying punctual local information of random observations and anecdotal reporting. Alongside the distribution, we also documented the habitat characteristics as well as the density and biomass of *S. woodiana* and of the co-occurring other native and invasive mussel species.

3.5.1 Actual and potential distribution

The examined populations of this study were widespread across Bavaria. Spatial concentrations in some regions were evident, but this may be partly affected by our study approach which focused on a closer inspection of sites with speculated or expected occurrence of *S. woodiana*. Local reporting was strongly dependent on an awareness of the issue of invasive species. Nevertheless, the occurrence of *S. woodiana* was not limited to a special region or drainage system, which suggests that there may be many still undetected populations across Bavaria. It also suggests a greater than expected invasion potential of the species and a limited possibility of still being able to eradicate single hotspot populations. This is also obvious from other countries where new populations of *S. woodiana* are frequently found (e.g., Cilenti 2019; Urbańska and Andrzejewski 2019).

Aside from the absent regional limitation, we also found a broad range of suitable habitats, extending from rivers to eutrophic ponds (**Table 3**) with a great variety of habitat conditions. Our results suggest that many types of inland waters are potentially suitable for *S. woodiana,* which has already been shown by others (e.g., Benkő-Kiss et al. 2013; Douda et al. 2012; Popa 2007; Urbańska and Andrzejewski 2019; Urbańska et al. 2021). *S. woodiana* is often characterised as a thermophilic species since this mussel originates from tropical and subtropical areas (Kondakov et al. 2018) and has predominantly invaded warmer natural waters in the southern part of Europe (Lajtner and Crncan 2011) as well as artificially heated waters and fishponds in the northern part (Kraszewski and

Zdanowski 2007). Nevertheless, this species has demonstrated an adaption to colder temperatures, which facilitated its spread across Europe (Konecny et al. 2018). This is confirmed by our results. The water temperature was comparatively low in the Moorbach brook and yet we found the smallest individual of *S. woodiana* in it, indicating that this species is well established and reproducing.

3.5.2 Potential pathways

The water usage in many of the studied waters leads to the suggestion that the first spread of *S. woodiana* in Bavaria was limited to waters with artificial stocking. This may be traced back to stockings with grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844), since this fish species is often used to reduce macrophytes within fish ponds (e.g., Pípalová 2006) and is known as a suitable host for *S. woodiana* (Huber and Geist 2019). Most of the pond owners confirmed that they have stocked *C. idella* and some of them still do. Since these stocked fish are often imported from hatcheries in Poland and Hungary, it is highly likely that alongside the fish, *S. woodiana* is also introduced attached to the gills of the fish.

Nevertheless, the occurrence of *S. woodiana* is not only limited to fish ponds. In the reservoir Rothsee the main population of this mussel was mainly found in the area next to the in- and outflow of the reservoir that is connected with the Main-Danube-channel. It can be assumed that *S. woodiana* invaded this reservoir from the channel via infected fish. Since *S. woodiana* has already been found in lower parts of the Danube system (e.g., Lajtner and Crncan 2011; Paunović et al. 2006; Popa 2007), it cannot be excluded that this species has already spread throughout many places connected to the Danube drainage including the Main system. Such artificial connections of naturally separated drainage systems may work as super-spreader highways for invasive species and consequently complicate effective management.

Another pathway could be the transfer of infested fish from ponds with an occurrence of *S. woodiana* to uninhabited waters. This seems a general practice in German carp production. Besides common carp, fish farmers usually cultivate secondary species like pike (*Esox lucius*), perch (*Perca fluviatilis*), pike-perch (*Sander lucioperca*) and tench (*Tinca tinca*) which are often used for re-stocking by fishing clubs (Brämick 2019). To prevent this, pond owners and fishing clubs should be sensitised, in particular since high infestation densities of *S. woodiana* glochidia impairs fish physiology and condition (Douda et al. 2017b), and, additionally, legal regulations should be adopted to prohibit the transfer of fish from waters with *S. woodiana* populations to natural water bodies. Other possibilities whose implementation would be difficult to control could be to check each fish for glochidia, before transfer, or caging the fish in basins without *S. woodiana* for a time span long enough for glochidia to develop and drop off the fish. Due to the continuous reproduction, a simple regulation linked to timespans without *S. woodiana* glochidia production cannot be recommended (Labecka and Domagala 2018).

3.5.3 Co-existence with native mussels

Besides *S. woodiana* populations, we also documented co-occurring mussel species. In almost all sampled water bodies, *S. woodiana* co-occurs with other mussel species, although we only found this in 14.2% of the sampling spots. It is still not fully understood how the long-term effects of the invasion of *S. woodiana* will affect the native mussel populations but, nevertheless, shifts in the mussel community are already being reported (Urbańska et al. 2019). One sign of *S. woodiana* outcompeting native mussels could be a much higher ratio of fresh empty shells to live specimens of native mussels when compared with *S. woodiana*. For the water bodies, we have investigated in our study, it is not possible to explain a potential shift caused or intensified by *S. woodiana* based on this ratio. We found ponds exclusively inhabited by *S. woodiana* with not a single empty shell of native mussel species but with the largest individuals of *S. woodiana* (Burlafinger Weiher). On the other hand, such a ratio was clearly visible in some plots of the river Wörnitz. However, in this case, we had the phenomenon of significantly disturbed shell formation resulting in thinner and easily breakable shells affecting both native and invasive mussel species. Derived from this, the reason for an increased die-off of native mussels cannot only be explained by the invasion of *S. woodiana* but it might also demonstrate a higher tolerance of *S. woodiana* to some kind of unsuitable habitat conditions. Such a higher tolerance could be an aspect favouring *S. woodiana* in a potential competitive development. As our results show, a negative link between the biomass of *S. woodiana* and other mussel species could be observed. At spots with high *S. woodiana* biomass, a lower biomass of other mussels could be found and vice versa. This suggests that some kind of limitation in the carrying capacity of a water system has been reached. With a faster growth rate (Sárkány-Kiss et al. 2000), *S. woodiana* could quickly reach a high biomass which, combined with higher filtration rates, leads to an advantage competing for food sources against native mussels (Douda and Čadková 2018). In this way, the slower growing native mussels could be outcompeted as they are deprived of their nutrition. However, in the fish pond Großer Hirschbergweiher a predominant part was covered with a large layer of quite soft silt with only the slower growing and lighter *A. cygnea* occurring in these areas. The colonisation of *S. woodiana* in this pond was limited to areas with a more sandy substratum. It seems that the higher weight of *S. woodiana* prevented this mussel from gaining a foothold on this fine sediment.

However, *S. woodiana* shows many other competitive ecological advantages over native mussel species, especially in the most crucial life stage of freshwater mussels. Thus, it could be shown that the viability of glochidia of *S. woodiana* is less affected by higher temperatures than is the case for glochidia of native mussels (Benedict and Geist 2021). This could be an important aspect in times of climate change resulting in an increase in water temperatures. Combined with more frequent breeding, higher amounts of glochidia (Labecka and Czarnoleski 2021; Labecka and Domagala 2018), higher infestation rates, faster development and a broader range of suitable host fish (Douda et al. 2012; Huber and Geist 2019), this all could lead to *S. woodiana* outcompeting native mussel species. In order to clarify these processes and to document a possible shift in the mussel community, this study should be repeated after several years.

3.5.4 Implication for management

To prevent the endangered native mussel species from the negative impacts of the invasive *S. woodiana* a good management should, *inter alia*, be based on three strategies: prevention (avoiding the introduction of invasive species by national or international regulations as well as by controls at entry points), early detection and rapid eradication (detecting invasives at an early stage of invasion and rapid removal of these species completely and permanently) as well as long-term management (controlling or containing populations of invasives as well as minimising their impacts) (Robertson et al. 2020). In addition to scientific knowledge, a political regulation is imperative for effective management.

With Regulation (EU) 1143/2014, the European Commission implemented a legal basis for preventing and managing the introduction and spread of IAS in all member states. In addition to this regulation, the European Union implemented a regularly updated list of invasive alien species of concern in the Union known as the black list of invasive alien species. For listed species, the member states are urged to make every effort to prevent an introduction of to control or eradicate these species and thus, includes all the above mentioned strategies. This implies for example regulations on import, trading, transportation or releasing of IAS. Since the invasion of *S. woodiana* does not stop at borders, it is important that measures to stop further spreading as well as to eradicate this species should be taken transnationally. Furthermore, *S. woodiana* is already established in many European countries and thus, this is not just a challenge for a single country. Nevertheless, the legal regulations and thus, opportunities seem to be quite different. Thus, in Poland for example, this species has been listed as invasive since 2012, which has had significant effects on trade in this species (see Urbańska et al. 2019). This is in contrast to Germany, where *S. woodiana* is still listed as potentially invasive with the need for further investigations. For this reason, we highly recommend the inclusion of *S. woodiana* on this black list because this species fulfils all of the listed criteria of article 4 paragraph 3 of the regulation:

(criterion a) *S. woodiana* is an alien species to the territory of the European Union (see Kondakov et al. 2018; Sousa et al. 2014); (criterion b) the capability of establishing viable populations across many European countries is very well documented (e.g., Benkő-Kiss et al. 2013; Kamburska et al. 2013; Lajtner and Crncan 2011; Munjiu et al. 2020; Paunović et al. 2006; Urbańska et al. 2019). Our study provides further evidence in favour of adding Germany to the list of countries with widely established *S. woodiana* populations; (criterion c) economical damage could be caused in fish hatcheries by glochidial infestation by *S. woodiana* which can reduce the body mass or condition factor of infected fish (Douda et al. 2017b). As described above, many ecological advantages in respect of reproduction are already known (Benedict and Geist 2021; Huber and Geist 2019; Labecka and Czarnoleski 2021; Labecka and Domagala 2018) which can have significant adverse impacts on freshwater mussel biodiversity; (criterion d). The findings of our study combined with all the studies mentioned above highlight the requirement of measures at Union level to prevent a further introduction, establishment or spread (criterion e); as our study shows, the main cause for further spreading of *S. woodiana* can be traced back to fish stockings. Inclusion on the Union list would create a basis on which regulations and procedures could contribute to the implementation of measures in a more targeted and rapid manner to reduce further spreading.

Since it is practically impossible to regularly monitor all waters for *S. woodiana* and other invasive species, early detection and knowledge on the habitat preferences of the species as demonstrated in this study is essential to be able to assess the impact on native mussels. Thus, it helps to understand the processes that go hand in hand with the invasion, and it is crucial to prevent further spreading. Moreover, measures to combat those invasive species should be adopted quickly to prevent a threat for native mussel populations. In addition, public awareness should be increased concerning the identification and spread of invasive mussels. This requires considering the sale of mussels, fish stocking as well as fisheries and pond management.

4 Impacts of native and invasive crayfish on three native and one invasive freshwater mussel species

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4.1 Abstract

Freshwater mussels and crayfish provide important ecosystem functions and services. In both groups, global declines of native species are paralleled by invasions of non-native species. Knowledge on differences in predation susceptibility of native and invasive freshwater mussels exposed to native and invasive crayfish is essential for understanding their ecological interactions and for conservation management.

In this study, we compared the predation impact of the native European noble crayfish (*Astacus astacus*) and the invasive signal crayfish (*Pacifastacus leniusculus*) on three native (*Anodonta anatina*, *Anodonta cygnea* and *Unio pictorum*) and one invasive (*Sinanodonta woodiana*) mussel species in controlled laboratory experiments. We repeated the same experiments with the same crayfish specimens to investigate a potential learning effect of crayfish and assessed the *in situ*-impacts in a natural stream.

Mussel predation and damage caused by *P. leniusculus* was significantly higher than by *A. astacus*. Irrespective of the crayfish species, susceptibility was greater in native mussel species compared to invasive *S. woodiana.* Predation and damage was greatest in the thick-shelled *U. pictorum*, suggesting that other factors such as shell shape are more important in explaining susceptibility than shell thickness. A predation learning effect from previous co-exposure was evident, being most pronounced for *A. astacus*.

Even if our experimental findings could not be confirmed in the field, the experimental results suggest that crayfish may have a negative impact on mussels and this impact will likely increase with the ongoing spread of *P. leniusculus* replacing *A. astacus* populations in Europe. The co-occurrence of invasive *P. leniusculus* with the invasive *S. woodiana* could potentially exacerbate declines of endangered native mussel populations.

Candidate's contribution: The candidate primarily conceived this study with critical revision of JG. Experiments and field sampling were performed by the candidate. Photos were analysed by the candidate as well as by Alia Benedict. Manuscript was drafted, finalised and revised by the candidate with continuous input and revision by JG.

4.2 Introduction

Freshwater mussels are considered important key faunal elements of freshwater systems that need to be considered in concepts of integrative freshwater biodiversity conservation (Geist 2011). Their important ecosystem functions and services such as filtration of fine particles, bioturbation, nutrient cycling and storage are also beneficial for other species (Boeker et al. 2016; Lummer et al. 2016; Vaughn 2018; Vaughn and Hakenkamp 2001). At the same time, freshwater mussel populations are globally in decline (see e.g., Haag 2012; Haag and Williams 2014 for North Amerika; Lopes-Lima et al. 2017 for Europe). On a European scale, none of the 16 species of native unionid bivalves can be considered secure throughout their range based on IUCN threat assessments (Lopes-Lima et al. 2017). In Germany, all of the seven indigenous freshwater mussels of the order ‘Unionoida’ (Zieritz et al. 2012) are listed on the national red list of threatened species (Binot-Hafke et al. 2011) and protected by the federal species regulation. Most freshwater mussel populations are exposed to multiple threats such as habitat fragmentation and degradation, water pollution and climate change (see e.g., Dudgeon et al. 2006; Geist 2010; Lopes-Lima et al. 2017; Lydeard et al. 2004; Regnier et al. 2009; Stoeckl et al. 2020; Strayer et al. 2004; Young and Williams 1983). In addition to these globally important factors for decline, invasions by non-native species are suspected to contribute to the decline of mussel populations. Invasive mussel species, for example, tend to be more generalist in terms of the physico-chemical habitat conditions as well as the spectrum of host fishes, and can therefore adapt more easily to non-optimal conditions compared to the more sensitive native ones (Bodis et al. 2016). In addition, there are several direct negative impacts of invasive mussel species on native ones. In the case of Asian clam *Corbicula fluminea* (Müller, 1774), which often occurs in high densities, the relatively high filtration rate leads to a strong competition for food with native unionids (Cohen et al. 1984; Phelps 1994; Strayer et al. 1999). This also applies to the invasive zebra mussel, *Dreissena polymorpha* (Pallas, 1771), which has already invaded many of the lakes and slow flowing parts of rivers and streams in Europe (see Van der Velde et al. 2010) and North America (Strayer 2009). Its attachment to native unionids with its byssus threads reduces the physiological condition of the mussel (Sousa et al. 2011), and can lead to shell deformations, overturning and killing of the overgrown mussels (Ożgo et al. 2020).

In addition to competition for food, the Chinese pond mussel, *Sinanodonta woodiana* (Lea, 1834), an invasive species that was first brought from the Yangtze River basin in

China to Europe in 1979 (Sarkany-Kiss 1986), competes with native mussels during reproduction. *Sinanodonta woodiana* larvae are more persistent than larvae of native species, also tolerating warmer water temperatures (Benedict and Geist 2021). Further, *S. woodiana* has a broader range of suitable host fishes, higher infestation rates and faster development than native species (Douda et al. 2012; Huber and Geist 2019). This species can breed several times throughout the year while native unionid reproduction is limited to a shorter time span (e.g., Labecka and Czarnoleski 2021). Additionally, a cross-resistance of host fishes was detected which reduces the reproduction success for native mussel species (Donrovich et al. 2017).

Besides competition, another important effect of non-native species on native ones is predation. As with freshwater mussels, crayfish can play important roles in the functioning of aquatic food webs, and there is a similar situation with native species being prioritised in conservation and invasive species being considered a major threat. Crayfish are omnivorous (e.g., Guan and Wiles 1998; Gutiérrez-Yurrita et al. 1998; Mason 1975), can occur in high densities, and can be potentially important predators on freshwater mussels (Machida & Akiyama, 2013; Meira et al., 2019; Sousa et al., 2019). Community shifts in crayfish populations due to die-offs of native species and dominance of non-native species may exacerbate this problem. The introduction of non-indigenous crayfish species from North America to Europe led to a massive spread of the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), since the 20th century, followed by further declines of native species (Holdich 2002). Invasive crayfish tend to have a competitive advantage over native ones due to faster growth (e.g., Paglianti and Gherardi 2004), higher aggressivity (Söderbäck 1991), greater dispersal ability (Bubb et al. 2006; Wutz and Geist 2013), and due to exploitative or interference competition (e.g., Hudina et al. 2011). In addition, the North American crayfish are resistant to the crayfish plague, *Aphanomyces astaci* (Schikora, 1906), but can transmit this disease to the ones native to Europe. This typically leads, apart from some rare exceptions (Martin-Torrijos et al. 2017), to a complete extinction of local native populations within only a few weeks (e.g., Vorburger and Ribi 1999). Of nine crayfish species that currently occur in Germany, only three are indigenous, excluding *Astacus leptodactylus* (Eschscholtz, 1823) which was introduced from Eastern Europe after arrival of the crayfish plague (Holdich et al. 2009; Kouba et al. 2014). Invasive crayfish, particularly *P. leniusculus*, are now the most widespread crayfish species in Europe (Holdich et al. 2009).

Previous studies already showed evidence for crayfish predation on unionids (e.g., Machida and Akiyama 2013; Meira et al. 2019; Schmidt and Vandré 2012; Sousa et al. 2019) and on dreissenids (e.g., Glon et al. 2017; zu Ermgassen and Aldridge 2011). However, the following questions have not yet been answered: i) are invasive crayfish more problematic than native ones concerning predation on unionids ii) are crayfish able to learn using mussels as food, and iii) which mussel species and which characteristics (in particular shell thickness and shape) affect the susceptibility of mussels to be killed or damaged by crayfish?

To answer these questions, the core objective of this study was to compare the predation pressure of two crayfish species, European noble crayfish, *Astacus astacus* (Linné, 1758) and invasive *P. leniusculus* on native and non-native European freshwater bivalves, including an assessment of their ability to learn from repeated exposure to mussels. More specifically, we: i) compared the predation rates of *A. astacus* and *P. leniusculus* on three different native freshwater bivalves (*Anodonta anatina*, *Anodonta cygnea* and *Unio pictorum*) and one invasive bivalve (*Sinanodonta woodiana*); ii) assessed the ability of crayfish to learn using mussels as a source of food; and iii) assessed the shell damage and mortality resulting from co-exposure of *A. astacus* and *P. leniusculus* with three freshwater bivalves (*Anodonta anatina*, *Anodonta cygnea* and *Unio crassus*). We hypothesised that (i) mussels with more brittle and delicate shells such as *A. cygnea* (see Killeen et al. 2004) would be most prone to damage by crayfish, irrespective of the crayfish species, and that the co-exposures would result in lowest predation, damage and mortality in the most thick-shelled species *U. pictorum*, (ii) the co-exposures would result in highest damages from co-exposure with invasive *P. leniusculus*, (iii) both species of crayfish would be able to learn to use freshwater mussels as a food source as evident from greater mussel damage in repeated exposure. Impacts of crayfish on the mussels were tested in a controlled laboratory experiment and also assessed in the wild where damage patterns of three species of mussels were compared at sites with occurrence of either one of the crayfish species.

4.3 Methods

4.3.1 Animal origin and husbandry conditions

Astacus astacus were purchased from a regional breeder and directly transferred to a tank with 290 cm x 65 cm x 70 cm (length, width, height) filled with 10 cm of gravel and 600 litre of tap water (temperature: 11.2 °C ± 2.7 SD; water hardness: 18.9 °dH, electric conductivity adjusted to 20°C: 606.9 µS ± 57.0 SD; oxygen saturation: 95-100%) where crayfish were kept under natural day/night regime. The tank was closed on the top with two wooden lids connected by a durable polyvinyl chloride (PVC) foil to avoid a contamination with crayfish plague though to allow light to enter the interior. To avoid territorial struggles or cannibalism, we placed one dark brown PVC tube (7 cm x 25 cm) for each individual on the ground (see Barim-Oz 2018) and fed the crayfish every second day with fresh carrot slices. The water was changed twice a week. *P. leniusculus* were caught in the river Moosach (48°23'38''N, 11°43'25''E) next to the laboratory using crayfish traps (type "pirate", Engel Netze, Germany) with a mesh size of 11 mm x 45 mm baited with Frolic® dry dog food (Mars, Incorporated, USA) and kept under the same conditions as *A. astacus*.

Carapace length (CL) of all crayfish was measured from the apex of the rostrum to the mid-dorsal posterior edge of the carapace (± 0.1 mm) with analogue callipers (following Wutz and Geist 2013).

We obtained the three native bivalves (*A. anatina*, *A. cygnea* and *U. pictorum*) and one invasive bivalve (*S. woodiana*) from commercial aquacultures one month before the first experimental part. We kept all mussel species (a total of 130 mussels) together in the same tank under similar conditions as the crayfish and fed them with 30 ml of an algae mixture (Shellfish Diet 1800®, Nannochloropsis 3600® and tap water; 2:1:1; Varicon Aqua Solutions Ltd, Worcester, UK) every day.

4.3.2 Study design

In order to investigate differences in predation of *A. astacus* and *P. leniusculus* on three native and one invasive mussel species, we carried out co-exposure experiments under controlled laboratory conditions. We obtained the permission for these experiments from the District Government of Upper Bavaria (reference number: 2631.Vet_11-4-12).

To exclude a contamination of *A. astacus* with the crayfish plague, experiments were performed subsequently, starting with *A. astacus* followed by *P. leniusculus*. The aquaria, gravel and the air supply were disinfected with Virkon® S for a minimum of 15 minutes and thoroughly washed with tap water afterwards. Before each experiment, the crayfish were acclimated in the aquaria for ten days (see Barim-Oz 2018) with new carrot slices provided every other day. The water of the aquaria was aerated 48 hours before the crayfish were added and during the whole time of the experiment.

In each replicate of the experiments, we exposed one individual each of three native bivalve species (*A. anatina*, *A. cygnea* and *U. pictorum*) and one individual of an invasive mussel species (*S. woodiana*) of similar size classes (**Table S1**) together with one crayfish specimen in an aerated aquarium with 40 cm x 25 cm x 25 cm (length, width, height) filled with 5 cm of fine gravel (4-8 mm) and 13 litres of tap water (temperature: 16.5 °C ± 0.9 SD; water hardness: 18.9 °dH; oxygen saturation: 95-100%) for three days (**Fig. 12a**). This type of substratum was consistently used for all laboratory experiments due to previous experience of maintaining the selected mussel and crayfish species in the laboratory. Mussels were positioned in a way which mimics their natural burrowing to minimize stress and provide a realistic exposure scenario. To ensure equal access possibilities of the crayfish to each of them, they were placed in rows with equal distance of individuals in random order. We provided a PVC tube (7 cm x 25 cm) as a shelter for the crayfish in each aquarium. Before each experiment, crayfish were not fed for three days following Meira et al. (2019).

4.3.3 Co-exposure and predation

The co-exposure experiment aimed to compare the predation rates of *A. astacus* and *P. leniusculus* on the three native bivalves (*A. anatina*, *A. cygnea* and *U. pictorum*) and one invasive bivalve (*S. woodiana*). This experiment was performed with *A. astacus* and *P. leniusculus* and with eight replicates per crayfish species. We used four crayfish specimens with similar size of each male (with a carapace length of 5.09 cm ± 0.37 SD for *A. astacus* and 4.92 cm ± 0.23 SD for *P. leniusculus*) and female (with a carapace length of 4.98 cm ± 0.09 SD for *A. astacus* and 5.25 cm ± 0.27 SD for *P. leniusculus*).

4.3.4 Learning effect

Previous contact of the crayfish with mussels before the experiment could be excluded. The hatchery from which we obtained *A. astacus* does not have mussels in their facility

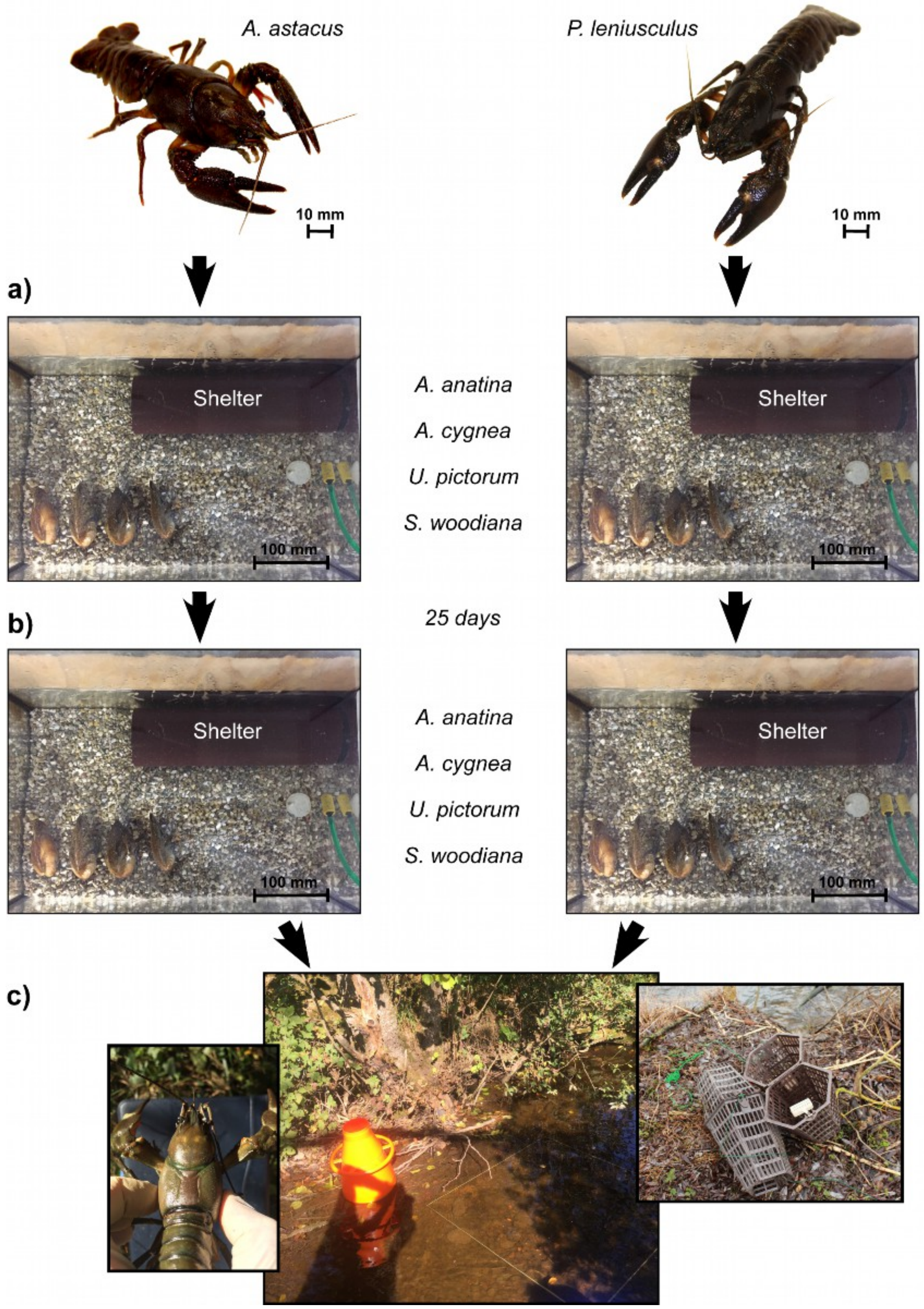

Fig. 12 Schematic illustration of the study design showing a) the co-exposure experiment placing one crayfish specimen together with one specimen each of three native (*A. anatina*, *A. cygnea*, *U. pictorum*) and one invasive (*S. woodiana*) mussel species in each aquarium with eight replicates per crayfish species (*A. astacus* and *P. leniusculus*), b) the repeated exposure experiment with the same crayfish specimens after 25 days and c) the field validation of the experimental results.

and there are no mussel populations in the stream of origin of *P. leniusculus*. This allowed testing a possible learning effect of the crayfish to use mussels as prey. Thus, the same experiment was repeated after holding the crayfish together with five mussels of each species (8-9 cm), also providing them with five dead mussels to increase attraction for 25 days (**Fig. 12b**). During this co-exposure, mussels were fed with 30 ml of an algae mixture (Shellfish Diet 1800®, Nannochloropsis 3600® and tap water; 2:1:1; Varicon Aqua Solutions Ltd, Worcester, UK) and crayfish with carrot slices every other day. We observed crayfish feeding on all of the dead mussels, but we could not detect severe damages on the living mussels. To recognize each crayfish, we individually marked the crayfish carapaces with nail polish and frequently checked the tank for freshly moulted crayfish.

Carapace length changed between the experiments because some of the crayfish moulted. In the second experiments, the crayfish had a carapace length of 5.16 cm ± 0.09 SD for female and 5.17 cm ± 0.28 SD for male *A. astacus,* and 5.25 cm ± 0.26 SD for female and 5.17 cm ± 0.34 SD for male *P. leniusculus*.

We used 8 new specimens of similar size classes of each mussel species per crayfish species (**Table S1**; **Fig. 12b**), except for four completely intact individuals of *A. cygnea* we re-used in the second experiment with *A. astacus*.

4.3.5 Field impact assessment

To assess the shell damage and mortality resulting from *in-situ* co-exposure of *A. astacus* and *P. leniusculus* with three freshwater bivalves (*A. anatina*, *A. cygnea* and *U. crassus*), we conducted field investigations (**Fig. 12c**) in the stream Mooshamer Weiherbach (47°53’25’’N, 11°31’0’’E) in October 2018. The stream belongs to the upper Danube drainage system and has a similar water chemistry as in the laboratory experiment, for details see Richter et al. (2016). Sampling frames (1 x 1 m) were placed onto the streambed every 250 m of the stream within a stretch of 4.5 km after an outflow of a pond. We collected all visible and buried mussels down to a substrate depth of 10 cm within these frames, determined their species and measured their length, width and height. We also examined all mussels carefully for predation marks. To confirm the presence of crayfish, we performed two capture events within seven days. For this purpose, we laid out crayfish fyke traps (type “pirate”, Engel Netze, Germany) with a dimension of 61 cm x 31,5 cm x 25 cm (length, width, height) and a mesh size of 11 mm x 45 mm baited with Frolic® dry dog food (Mars, Incorporated, USA) at each mussel sampling point for 24 hours. All trapped crayfish were sexed, determined to the species level and the carapace length was measured. Afterwards we released all *A. astacus* at the point they were caught. Caught *P. leniusculus* were not released.

4.3.6 Assessment of mussel damage

After each experiment, mortality and shell damage were assessed. For the assessment of mussel damage patterns, shells were positioned on a reference grid and individually photographed with a digital single-lens reflex camera (Canon EOS 650D). All images of valves with predation marks were georeferenced by polynomial transformation (polynomial 3) of the open source geographic information system software Quantum GIS v.3.4.5 (QGIS Development Team 2019) using nearest neighbour as resampling method and a custom coordinate reference system with a transverse Mercator projection, a latitude and longitude origin of 0 with a coordinate value at x and y origin of 0, a scale factor of 1, WGS84 as the ellipsoid used and the units in meter. For georeferencing, we distributed 12 control points on the reference grid around the valves. For a systematic analysis, each shell side was subdivided into four areas: posterior-dorsal (PD), posterior-ventral (PV), anterior-ventral (AV) and anterior-dorsal (AD). To distinguish between posterior and anterior, we used the axis perpendicular to the hinge and running through the umbo,

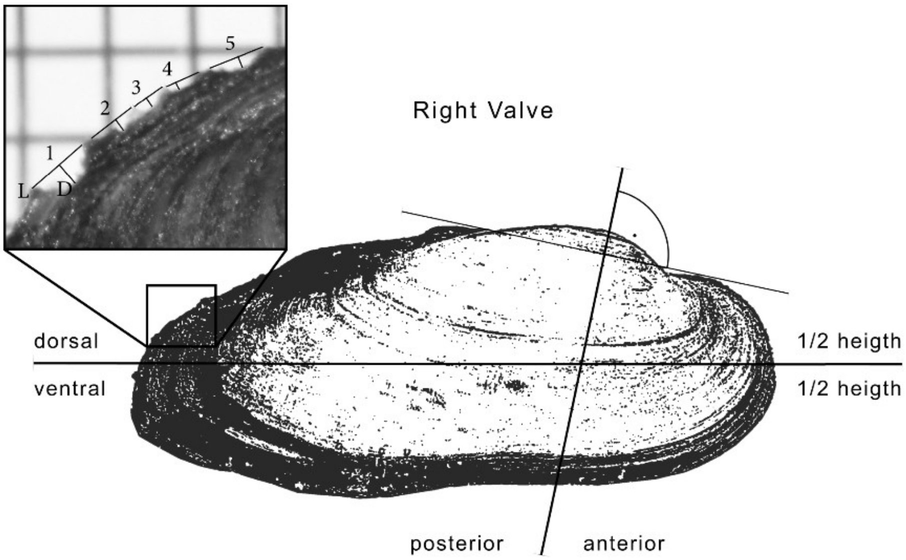

Fig. 13 Analysis of predation marks with shells divided into four sections, posterior-dorsal (PD), posterior-ventral (PV), anterior-ventral (AV) and anterior-dorsal (AD). The length (L) and the maximum depth (D) of each mark were measured. Numbers show the count of the marks.

and to distinguish between dorsal and ventral, we used the axis running from posterior to anterior in 1/2 of the height (**Fig. 13**).

We counted all marks and measured each length from the outermost, undamaged edge of one side to the outermost, undamaged edge of the other side of the mark along the natural edge of the shell and the depth of all marks from the natural edge of the shell to the deepest part of the notch (see **Fig. 13**) using ArcGIS v.10.7.1 (ESRI 2019).

4.3.7 Statistical analyses

We calculated the total damage rates (%) as the proportion of each mussel species and each quadrant of each mussel species being damaged in the pool of specimens assessed. To assess the damage patterns, we summarised the number and length of shell damages and calculated the maximum shell injury depth for each mussel used in the experiments (see **Fig. 13**).

To test the effect of the two different crayfish species and to test for the learning effect of the crayfish, we conducted three zero-inflated generalised linear mixed models using the function “glmmTMB” in the package “glmmTMB” (v. 0.2.3, Brooks et al. 2017) in R (v. 3.5.0, R Core Team 2020). The models differed in the response variable, using length of damages per quadrant in the first, maximum depth of damages per quadrant in the second, and number of damages per quadrant in the third model. In the models, the variance distribution was a Poisson regression function with a log link for the number, and a Gaussian regression function for length and depth of damages. Since we used four mussel specimens of four different species with one crayfish and eight replicates per crayfish species for the co-exposure (experiment part 1) and we repeated the co-exposure with the same crayfish individuals (experiment part 2), we included mussel species, crayfish species, experimental parts and quadrant as fixed factors. We also included interactions between crayfish species and mussel species, mussel species and quadrant as well as between crayfish species and experimental parts in each of these models. The factors tank (replicate) and mussel specimen were included as random effects to adjust for the repeated use of a tank as well as the four reused mussel specimens. Significance levels of fixed effects were assessed with Wald chi-square tests using the function “Anova” within the package “car” (v. 3.0-7, Fox and Weisberg 2019). Pairwise comparisons between treatment levels of fixed effects were calculated using the function “emmeans” in the R package “emmeans” (v. 1.4.3.01, Lenth 2019). *P*-values were adjusted using the “Bonferroni” method. We visually evaluated the residuals of the fitted models in terms of normality of errors and homogeneity of variances using diagnostic plots in the R package “DHARMa” (v. 0.2.7, Hartig 2020). For all statistical analyses, significance levels were set to $p < 0.05$.

4.4 Results

Across all mussel species, predation damages during the experiment were detected in 58.6% of all specimens, with the thick-shelled *U. pictorum* being most affected (81.3%). For all mussel species pooled, this value was lower in the first part of the experiment without previous contact (48.4%) than in the second part (68.8%). Mortality was only observed in *A. anatina*, of which five specimens died (one in the first part of the experiment with *A. astacus*, three in the second part of the experiment with *A. astacus* and one in the second part of the experiment with *P. leniusculus*). No further mortality was observed within an additional period of 7 days after the experimental parts.

4.4.1 Predation in co-exposure

In the co-exposure, each shell had an average of 10.6 ± 15.9 SD marks per specimen with a mean length of 33.4 mm ± 53.7 SD and a maximum depth of 0.5 mm ± 0.6 SD per mussel. Most of the predation marks were observed in *A. cygnea* (16.8 ± 23.8 SD) with a length of 55.5 mm ± 82.0 SD and a maximum depth of 0.6 mm ± 0.9 SD, fewest in the invasive *S. woodiana* (4.3 ± 11.8 SD) with a length of only 13.9 mm ± 40.7 SD and a maximum depth of only 0.2 mm ± 0.4 SD (**Table 5**).

Significant differences in lengths of predation marks were only observed in the factor quadrant (Model 1; $\chi^2 = 41.36$; $p < 0.001$) and for the interaction between mussel species and quadrant (Model 1; $\chi^2 = 19.3$; $p < 0.05$). Model 1 showed no preference of *A. astacus* or *P. leniusculus* for mussel species.

Considering depths of the marks, significant differences were detected for the factors mussel species (Model 2; $\chi^2 = 23.3$; $p < 0.001$), crayfish species (Model 2; $\chi^2 = 20.84$; $p < 0.001$), experimental parts (Model 2; $\chi^2 = 13.9$; $p < 0.001$) and quadrant (Model 2; $\chi^2 = 133.76$; $p < 0.001$) as well as for the interactions between crayfish species and mussel species (Model 2; $\chi^2 = 14.3$; $p < 0.01$), crayfish species and experimental parts (Model 2; $\chi^2 = 6.4$; $p < 0.05$) and mussel species and quadrant (Model 2; $\chi^2 = 39.5$; $p < 0.001$) (**Table 6**). Furthermore, pairwise comparison showed significant differences between *S. woodiana* and *A. cygnea* (Model 2; $p < 0.01$) as well as between *S. woodiana* and *U. pictorum* (Model 2; $p < 0.001$) for the depth of marks.

For *A. astacus*, the pairwise comparison only revealed significant differences in depth of the marks between *S. woodiana* and *A. anatina* (Model 2; $p < 0.05$). In the experiment with *P. leniusculus*, marks differed significantly in depth between *S. woodiana* and *A. cygnea* (Model 2; $p < 0.05$), *S. woodiana* and *U. pictorum* (Model 2; $p < 0.01$) as well as between *A. anatina* and *A. cygnea* (Model 2; $p < 0.01$).

Table 5: Percentage (%) of damaged mussels (*Anodonta anatina, Anodonta cygnea, Unio pictorum* and *Sinanodonta woodiana*), average number of predation marks per specimen and percentage of marks within the four quadrants (anterior-ventral, anterior-dorsal, posterior-ventral and posterior-dorsal) after first (part 1) and second (part 2) co-exposure.

	Mussel species	Percentage of damaged mussels	Percentage of predation marks in quadrants				Average number of predation marks
			Anterior-ventral	Anterior-dorsal	Posterior-ventral	Posterior-dorsal	
Part 1	*A. anatina*	68.8%	32.1%	4.2%	28.7%	35.1%	8.9 ± 9.1 SD
	A. cygnea	43.8%	42.0%	6.7%	22.3%	29.0%	16.8 ± 23.8 SD
	U. pictorum	62.5%	15.0%	4.5%	30.5%	50.0%	12.5 ± 13.3 SD
	S. woodiana	18.8%	68.1%	1.4%	23.2%	7.2%	4.3 ± 11.8 SD
Part 2	*A. anatina*	68.8%	26.7%	5.4%	28.8%	39.2%	15.0 ± 17.2 SD
	A. cygnea	62.5%	40.2%	2.9%	23.4%	33.6%	15.2 ± 19.6 SD
	U. pictorum	100.0%	23.9%	5.6%	31.8%	38.6%	22.2 ± 12.8 SD
	S. woodiana	43.8%	39.7%	0.0%	39.7%	20.5%	4.9 ± 6.6 SD
Change	*A. anatina*	+0.0%	-4.1%	+3.3%	-2.0%	+2.8%	+6.1
	A. cygnea	+18.8%	-1.8%	-3.8%	+1.1%	+4.6%	-1.6
	U. pictorum	+37.5%	+8.9%	+1.1%	+1.3%	-11.4%	+9.7
	S. woodiana	+25.0%	-28.4%	-1.4%	+16.6%	+13.3%	+0.6

Number of marks were significantly different in mussel species (Model 3; $\chi^2 = 23.72$; $p < 0.001$), crayfish species (Model 3; $\chi^2 = 16.36$; $p < 0.001$), experimental parts (Model 3; $\chi^2 = 8.49$; $p < 0.01$), quadrant (Model 3; $\chi^2 = 123.36$; $p < 0.001$) and in the interaction between crayfish species and experimental parts (Model 3; $\chi^2 = 12.17$; $p < 0.001$) as well as between mussel species and quadrant (Model 3; $\chi^2 = 47.12$; $p < 0.001$).

Table 6: Results of zero-inflated generalised linear mixed models investigating differences in mean length, maximum depth and mean number of damages per shell quadrant between four mussel species, two crayfish species, two experimental parts, four quadrants, as well as the interactions of crayfish species with mussel species, crayfish species with the experimental parts and mussel species with the quadrants.

Response variable	Source	χ2	d.f.	*P*
length	mussel species	-	-	n.s.
$R^2 = 0.95$	crayfish species	-	-	n.s.
	Experimental parts	-	-	n.s.
	quadrant	41.36	3	< 0.001
	crayfish species*mussel species	-	-	n.s.
	crayfish species*experimental	-	-	n.s.
	mussel species*quadrant	19.29	9	< 0.05
depth	mussel species	23.33	3	< 0.001
$R^2 = 0.58$	crayfish species	20.84	1	< 0.001
	Experimental parts	13.85	1	< 0.001
	quadrant	133.76	3	< 0.001
	crayfish species*mussel species	14.25	3	< 0.01
	crayfish species*experimental	6.35	1	< 0.05
	mussel species*quadrant	39.51	9	< 0.001
number	mussel species	23.72	3	< 0.001
$R^2 = 0.76$	crayfish species	16.36	1	< 0.001
	Experimental parts	8.49	1	< 0.01
	quadrant	123.34	3	< 0.001
	crayfish species*mussel species	-	-	n.s.
	crayfish species*experimental	12.17	1	< 0.001
	mussel species*quadrant	47.12	9	< 0.001

Pairwise comparisons showed significant differences in number of predation marks between *S. woodiana* and the other three mussel species *A. anatina* (Model 3; $p < 0.001$), *A. cygnea* (Model 3; *p* < 0,01) and *U. pictorum* (Model 3; *p* < 0.001). In *A. anatina* and *U. pictorum* most of the predation marks were found in the posterior-dorsal quadrant of the shell (35.1% respectively 50.0%), but for *A. cygnea* and *S. woodiana* in anterior-ventral (42.0% respectively 68.1%).

Astacus astacus caused a mean of 3.2 ± 7.6 SD marks per mussel with an average length of 8.5 mm ± 19.8 SD and a maximum depth of 0.2 mm ± 0.4 SD per mussel. In contrast, *P. leniusculus* caused more than five times more marks (18.1 mm ± 18.4 SD) with an average length of 58.3 mm ± 64.7 SD and a maximum depth of 0.8 mm ± 0.7 SD per mussel. For *A. astacus*, pairwise comparisons revealed significant differences in number of marks between *S. woodiana* and *A. anatina* (Model 3; *p* < 0.001) as well as between *S. woodiana* and *U. pictorum* (Model 3; *p* < 0.001); and for *P. leniusculus* between *S. woodiana* and *A. anatina* (Model 3; *p* < 0.05), between *S. woodiana* and *A. cygnea* (Model 3; *p* < 0.01) as well as between *S. woodiana* and *U. pictorum* (Model 3; *p* < 0.001).

Thus, *A. astacus* mostly damaged *A. anatina* with 6.0 ± 6.8 SD and *A. cygnea* with 4.5 ± 12.7 SD marks per specimen, whereas *P. leniusculus* mostly damaged *A. cygnea* with 29.1 ± 26.5 SD and *U. pictorum* with 22.6 ± 11.0 SD marks per specimen (**Fig. 14**; **Table S2**).

4.4.2 Learning effect

The repeated exposure revealed a greater crayfish impact on the mussels as evident from significantly deeper shell marks (Model 2; *p* < 0.001; mean: 0.7 mm ± 0.6 SD) and from a higher number of marks (Model 3; *p* < 0.001; mean: 14.3 ± 15.8 SD) compared to the first exposure. Concerning depth of the marks, pairwise comparison of mussel species were significantly different between *S. woodiana* and *A. cygnea* (Model 2; *p* < 0.01), and between *S woodiana* and *U. pictorum* (Model 2; *p* < 0.001) for the second co-exposure. Between the experimental parts, pairwise comparison revealed significant differences in the depth of marks (Model 2; *p* < 0.001) caused by *A. astacus*, but not for marks caused by *P. leniusculus*.

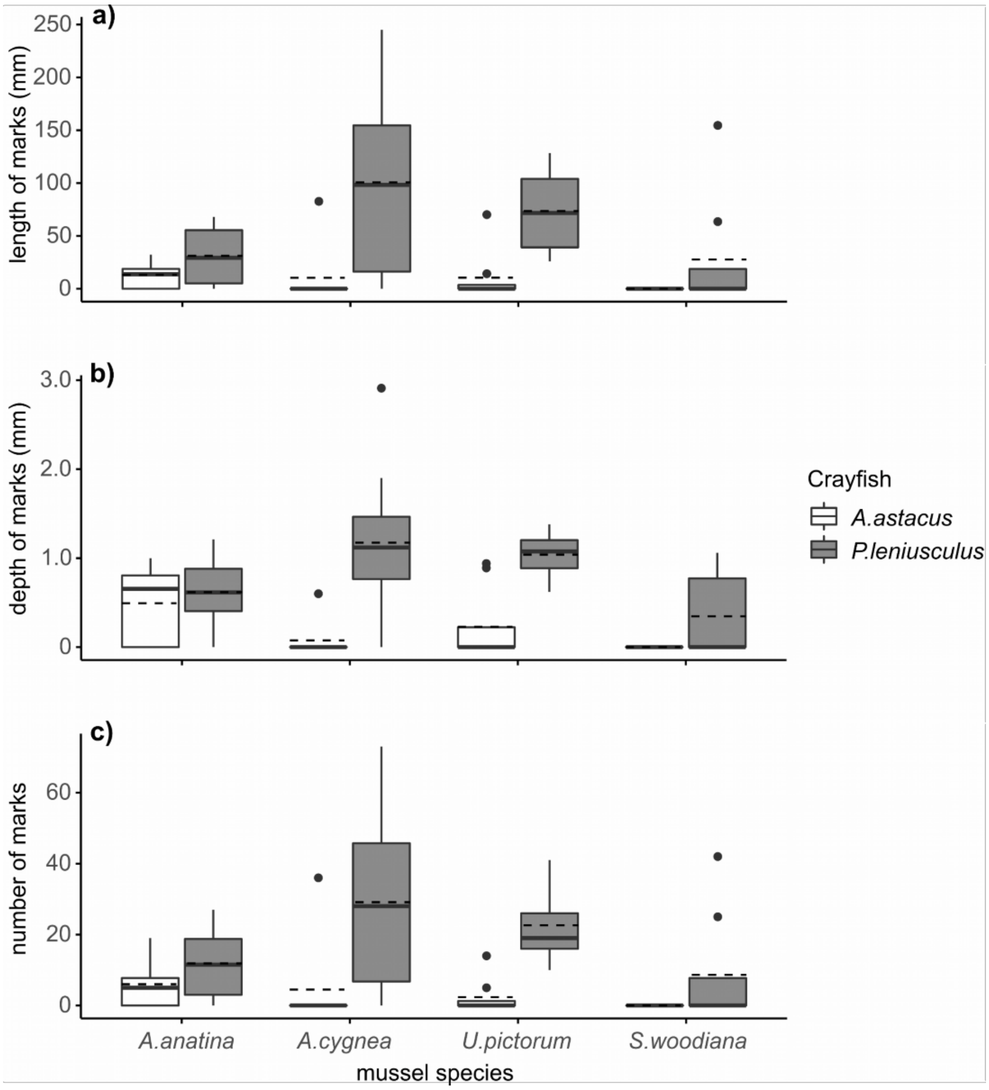

Fig. 14 Boxplots of a) the length, b) the depth and c) the number of the predation marks of each mussel species per crayfish species of the co-exposure experiment. The horizontal lines indicate median values, dashed lines the mean values, boxes the 25th to 75th percentiles, whiskers the lowest and highest values within 1.5 times the values observed in the percentile boxes and black dots single cases exceeding 1.5 times the values observed in the percentile boxes.

In line with experiment part 1, significant differences were detected in number of predation marks between *S. woodiana* and the other three mussel species *A. anatina* (Model 3; $p < 0.001$), *A. cygnea* (Model 3; $p < 0,01$) and *U. pictorum* (Model 3; $p < 0.001$). Most of the predation marks were observed in *U. pictorum* (22.2 ± 12.8 SD) with a length of 86.1 mm ± 41.1 SD and a maximum depth of 1.0 mm ± 0.3 SD, fewest in *S. woodiana* (4.9 ± 6.6 SD) with a length of 23.4 mm ± 38.4 SD and a maximum depth of 0.4 mm ± 0.5 SD **(Table 5)**.

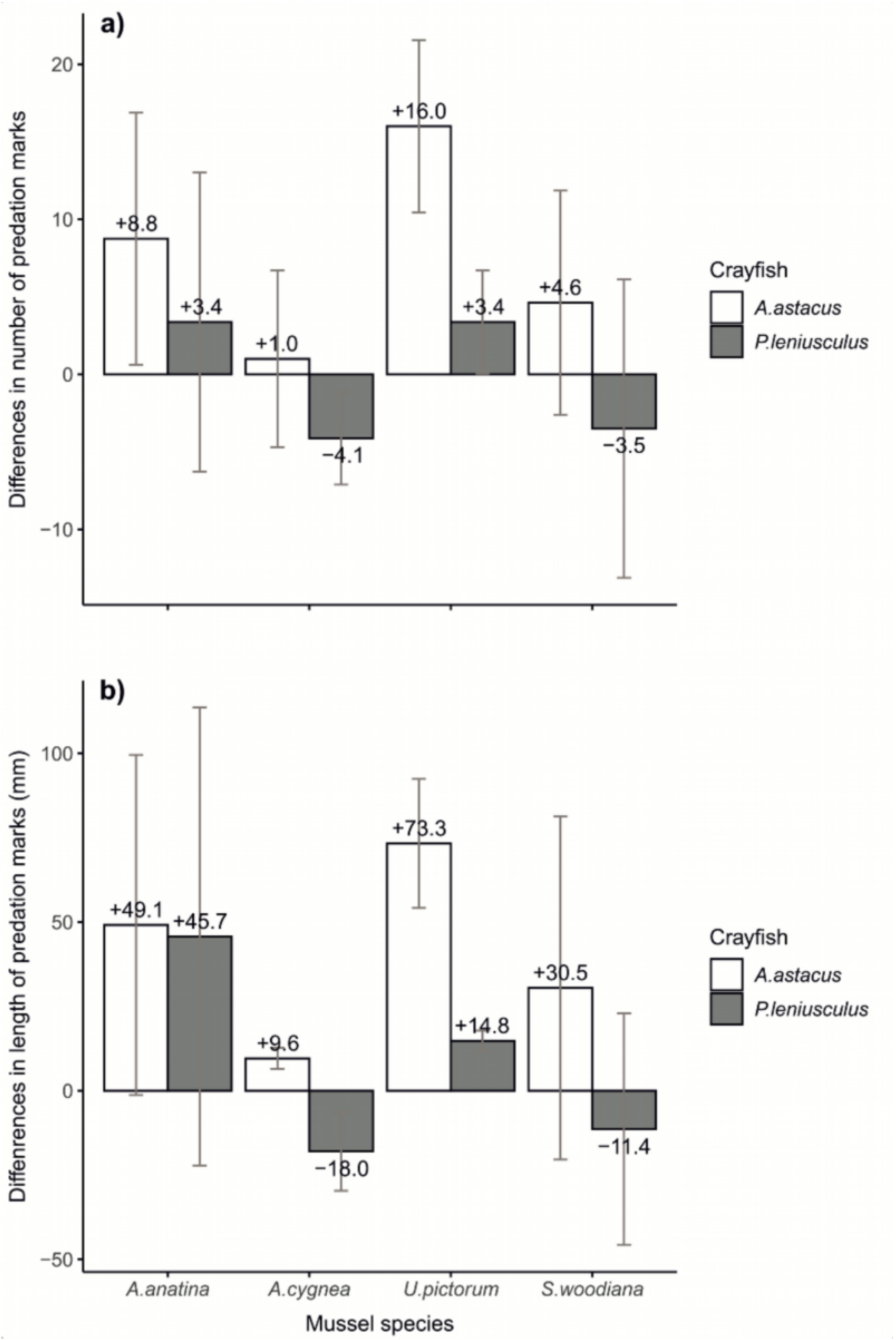

Fig. 15 Differences in the mean a) number and b) length of predation marks per mussel species between the first and the second part of the experiments with *A. astacus* and *P. leniusculus*.

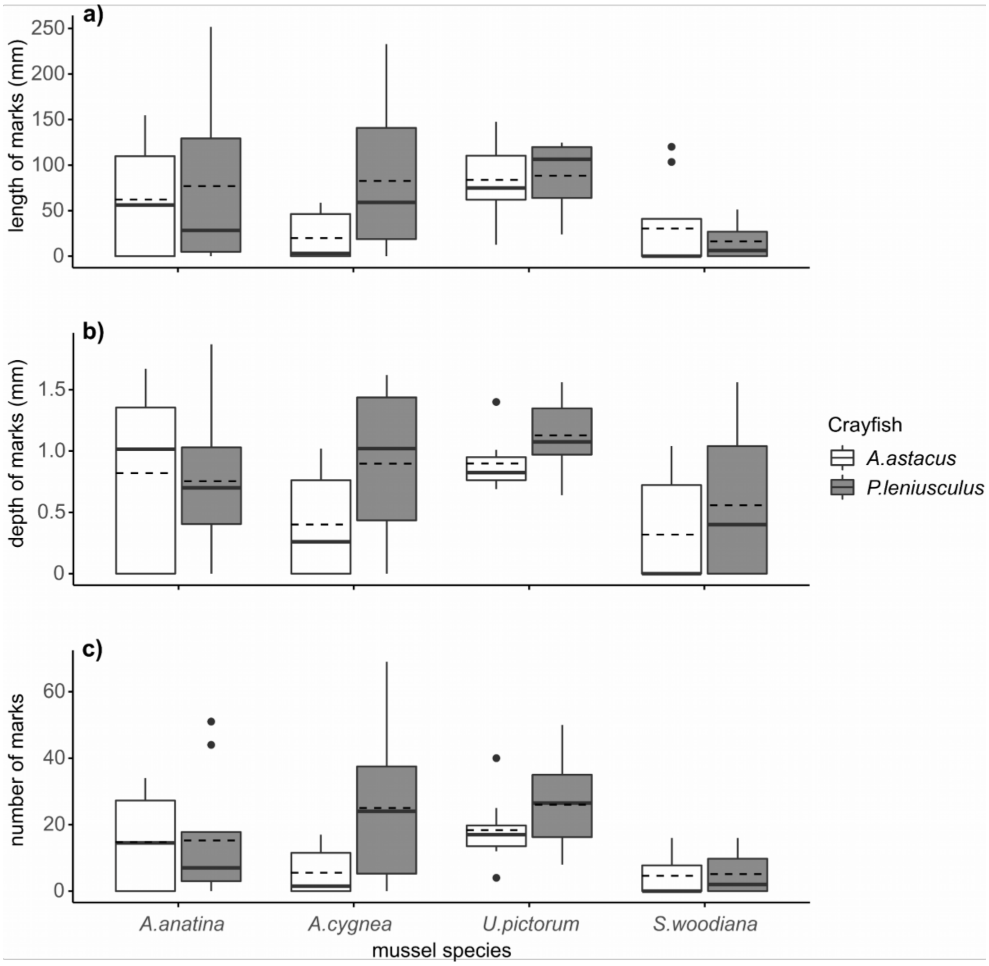

Fig. 16 Boxplots of a) the length, b) the depth and c) the number of the predation marks of each mussel species per crayfish species of the learning experiment. The horizontal lines indicate median values, dashed lines the mean values, boxes the 25th to 75th percentiles, whiskers the lowest and highest values within 1.5 times the values observed in the percentile boxes and black dots single cases exceeding 1.5 times the values observed in the percentile boxes.

Between the experimental parts, pairwise comparison showed significant differences in the number of marks (Model 3; $p < 0.001$) caused by *A. astacus*, but again not for marks caused by *P. leniusculus*. In the second part of the experiment of *A. astacus*, the mean number of damages as well as the length of the marks per mussel specimen strongly increased in *U. pictorum* (+ 16.0 marks per specimen; + 73.3 mm) and in *A. anatina* (+ 8.8 marks per specimen; + 49.1 mm) compared to the first part of the experiment. In the second part of the experiment with *P. leniusculus*, this increase was much lower in *U. pictorum* (+ 3.4 marks per specimen; + 14.8 mm) and in *A. anatina* (+ 3.4 marks per specimen; + 45.7 mm). In *A. cygnea* (- 4.1; - 18.0 mm) and *S. woodiana* (- 3.5; - 11.4 mm), both the number and the length of predation marks, decreased in contrast to the first part of the experiment with *P. leniusculus* (**Fig. 15**). Based on the mean number of marks, both crayfish species mostly preferred *U. pictorum* in the second part of the experiment, with the number of marks being four to five times greater in this species compared to *S. woodiana* where the lowest number of marks was found (**Fig. 16**; **Table S2**).

4.4.3 Field impact assessment

During our field investigation, we found a total of 196 mussel specimens in 8 out of 18 sampling frames (R10-R11 and R13-R18). With 127 individuals, most of these mussels were *U. crassus*, but we also found 31 *A. anatina* and 38 *A. cygnea* (**Table 7**). The presence of crayfish was confirmed at 16 of the 18 locations, whereby only the native *A. astacus* occurred at places with mussels. The invasive *P. leniusculus* was only found in the lower parts of the Mooshamer Weiherbach (R02-R06) where no living mussels are found. None of the found mussels showed any predation marks.

Table 7: Number of mussels and crayfish per species at the different locations in the Mosshamer Weiherbach. R18 was the most upper point after the outflow of the pond Mooshamer Weiher and R01 was the lowest point on the east side of the village Ascholding.

Location	*U. crassus*	*A. anatina*	*A. cygnea*	*A. astacus*	*P. leniusculus*
R18	9	12	28	2	-
R17	35	14	9	8	-
R16	14	3	1	8	-
R15	2	2	-	2	-
R14	51	-	-	14	-
R13	14	-	-	17	-
R12	-	-	-	5	-
R11	1	-	-	10	-
R10	1	-	-	7	-
R09	-	-	-	4	-
R08	-	-	-	-	-
R07	-	-	-	3	-
R06	-	-	-	-	9
R05	-	-	-	-	4
R04	-	-	-	-	4
R03	-	-	-	-	3
R02	-	-	-	-	8
R01	-	-	-	-	-
Total	**127**	**31**	**38**	**80**	**28**

4.5 Discussion

The findings of this study show that both the native *A. astacus* and the invasive *P. leniusculus* are able to learn how to prey on freshwater bivalves. Contrary to expectations, the more thick-shelled *U. pictorum* had higher damage rates compared to mussel species with thinner shells, indicating that other factors such as shell shape seem to be important in determining susceptibility. The lowest observed damage occurring in the non-native *S. woodiana* combined with the greater mussel predation damage caused by the invasive crayfish species suggests that synergistic interactions of both non-native species may lead to multiple stressors critical for native freshwater mussel populations of conservation concern, yet no such effect could be confirmed in the wild.

4.5.1 Effects of native and invasive crayfish

Across all our laboratory experimental parts and all used mussel species, damages could be observed. This was expected from previous studies that report mussel predation effects by crayfish (e.g., Glon et al. 2017; Klocker and Strayer 2004; Machida and Akiyama 2013; Meira et al. 2019; Perry et al. 1997; Schmidt and Vandré 2012; Sousa et al. 2019). To the best of our knowledge, no other study had yet applied a cross-experimental design with native and invasive crayfish as well as native and invasive unionids, allowing a direct comparison of predation effects.

As evident from the observed mussel damages in the co-exposure, *P. leniusculus* has greater effects than the native *A. astacus*. In general, *P. leniusculus* is known for its greater activity (Wutz and Geist 2013), faster growth and more aggressive behaviour (Söderbäck 1991) compared to the native *A. astacus*, probably further exacerbating its impact on both native crayfish and mussel populations. Several impacts of *P. leniusculus* on freshwater biodiversity are known. For example, invasive crayfish such as *P. leniusculus* have negative effects on benthic invertebrates (Usio et al. 2009) and hence on insectivorous fish (Guan and Wiles 1998). Their ecological impacts are greater compared to native crayfish (James et al. 2015). According to Nyström et al. (1996), *P. leniusculus* is also more efficient in grazing compared to the native *A. astacus*, which can result in strong decreases of macrophyte biomass. Gherardi (2007) even suspects that predation and competitive behaviour of non-indigenous crayfish species can also change interactions within communities resulting in trophic cascade effects. In addition to the direct effects on mussels, invasive crayfish such as *P. leniusculus* are geomorphic agents that mobilise sediment (Johnson et al. 2011) which can cause considerable damages to river banks by burrowing (Sibley 2000). The resulting increased sediment erosion can have negative impacts on water quality (Faller et al. 2016) and overall mussel habitat quality (Geist and Auerswald 2007), negatively affecting mussel populations.

4.5.2 Effects on mussel species

Our hypothesis that predation effects would be greatest in mussel species with thinnest and most delicate shells (i.e. *A. anatina* and *A. cygnea*) has to be rejected. Over all experimental parts, the thick-shelled *U. pictorum* was most strongly affected in terms of number of damaged specimens and the invasive *S. woodiana* was hardly affected, with *A. anatina* and *A. cygnea* in an intermediate position.

However, direct mortality was only observed in 5 out of 128 specimens used during the experiment and only in *A. anatina*. In addition, it could be observed during the experiment that crayfish lifted several specimens of *A. anatina* out of the sediment and moved them into the tubes. Even if this suggests that the lower weight of the mussels plays an important role in being attractive for crayfish, these results should be interpreted with caution since no predation marks could be found in two of the empty dead shells and mortality of *A. anatina* was also observed in mussels without treatment. Besides direct mortality over the short time of the experiment, other effects like excavating and “playing” with mussels result in shell clamping and consequently reduced filtration and energy intake as well as translocation to less favourable sites (e.g., inside crayfish shelters), even if this does not necessarily play a major role under natural conditions. Nevertheless, this could lead to a weakening of the mussels which will – together with the slight damages all around the shells that result in mussels no longer being able to close completely and being exposed unprotected to external influences – probably decrease fitness and increase the risk for mortality.

For our experimental design, we choose a conservative approach and only used adult mussels of similar size classes for inter-species comparison since shell size seems to influence predation susceptibility. zu Ermgassen and Aldridge (2011) showed that *P. leniusculus* increasingly harmed smaller specimens of *D. polymorpha* with a size range of 7 to 12 mm. A likely explanation is that mussels reach their size very quickly and begin to be unattractive as food for crayfish already at small sizes compared to adult mussels. However, the size of this species stays much smaller in comparison to the unionids used in our study and juvenile unionids stay burrowed into the substrate and thus are not easy to find or even accessible for crayfish in natural conditions (Ożgo et al. 2021). In addition, Olden et al. (2009) found in a study of signal crayfish predation on non-native mystery snails (*Bellamya chinensis*) a u-shaped relationship between snail size and prey value to the crayfish. Small snails are valuable because they are easier to handle, whereas very large snails are still valuable because the reward for breaking into their shell is high. Intermediate sized snails were the least valuable, because they are both hard to handle and consume but contain less food than the largest snails. Similar results were obtained by Machida and Akiyama (2013) who showed in experiments with two margaritiferid species from Japan and *P. leniusculus* that mortality resulting from predation only occurred in juvenile specimens (10.09–19.37 mm), but damage rate was higher in larger mussels (>50 mm), whereas Sousa et al. (2019) found higher damage rates and mortality in smaller specimens of *Margaritifera margaritifera* (22.0–39.7 mm) caused by *P. leniusculus*.

Over all experimental parts, a clear difference in predation preference was evident. Whilst the number of marks is likely an indicator for the intensity and number of crayfish attacks proportional to disturbance, the depth of marks is likely more strongly linked to the injury intensity. In line with our hypothesis, the first part of the experiment (without previous contact to mussels) revealed a tendency of thin-shelled *A. anatina* and *A. cygnea* being preferentially preyed upon by *A. astacus* and *P. leniusculus*, respectively. However, in the second part of the experiment both crayfish species mostly preferred the most thick-shelled *U. pictorum*, suggesting our original hypothesis has to be rejected. This is in contrast to the results of Meira et al. (2019) where signal crayfish preferred thin-shelled *A. anatina* over thick-shelled *Potomida littoralis* and *Unio delphinus* and invasive species *C. fluminea*. The authors attributed this to the thinner and more fragile shells and thus, an easier handling as well as a less energetically cost intensive predation. However, both results are difficult to compare since Meira et al. (2019) used two different mussel species present in southern Europe and a wider range of shell sizes (e.g., 35-126 mm for *A. anatina*; 44-78 mm for *P. littoralis* and 36-97 for *U. delphinus*). Based on our results of experiment part 1 with *A. astacus*, it can be assumed that the thinner shells and thus the lower weight makes these mussels more attractive for unexperienced crayfish. However, the observed damage patterns of our repeated exposure experiment with both crayfish species as well as our behavioural observations suggest that it is not the thickness of the shells that determines their susceptibility to crayfish predation, but that this is rather a matter of how the crayfish manage to fix the shells and can hold on to them to get access. In this respect, the more pointed shape of the shells as in *U. pictorum* allows easier access for crayfish mandibles. This may also explain the differences in sectional damages as, for example, in the posterior-dorsal part in *U. pictorum*. In *A. anatina* most of the damages were also found in the posterior-dorsal part which could be explained by the triangular shape of the hinge. Still, many damages were also found in the originally buried anterior-ventral part of this species. It was observed during the experimental parts that the crayfish used the first and second pairs of walking legs to excavate the mussels and to turn them to find a spot accessible for their mandibles. Perry et al. (1997) have also made similar observations in laboratory experiments with *Faxonius* spp. crayfish and zebra mussels.

Hence, our findings can also explain the low damage rates of the invasive *S. woodiana*. This species has a rounder shape and thus provides fewer angles to be fixed and attacked by the crayfish. Considering, however, that size can affect predation susceptibility, *S. woodiana* may also have a considerable advantage over native ones due its much greater maximum size and its faster growth.

Whilst our experimental approach allowed a standardised comparison between crayfish and mussel species, these findings cannot directly be transferred to field situations as, e.g., evident from the field validation at the Mooshamer Weiherbach. This may be explained by differences between the controlled laboratory exposure and realistic field settings including differences related to food choice, ambient environmental conditions, the greater variation of mussel sizes at the field site as well as the absence of *P. leniusculus* at site with mussel occurrence. Under natural conditions, mussel species show great differences in their burrowing behaviour (Ożgo et al. 2021; Zieritz et al. 2014) and thus may be less accessible for crayfish compared to aquaria-based exposures with limited substrate depth. In addition, invasive freshwater crayfish are omnivorous and often show opportunistic feeding behaviour (see Guan and Wiles 1998; Lewis 2002; Nyström et al. 1996). Therefore, if enough alternative and better accessible food sources are available, the effects on mussels may not be that large, since excavation may be too time consuming and energy costing for crayfish (Klocker and Strayer 2004). Furthermore, it is not common that both native and invasive crayfish species occur in the same system, only with *A. astacus* in the upper and *P. leniusculus* in the lower part of a stream as it was found in the Mooshamer Weiherbach. All of these factors individually or in synergy can explain why we did not find damaged mussel specimens. Nevertheless, there is already evidence for crayfish predation on another species of freshwater mussel which is more exposed to open water conditions in its adult stage in natural conditions (e.g., Schmidt and Vandré 2012).

Even though we cannot directly link shell damage of our results with population level effects in the wild, the findings of our experiment still clearly suggest that invasive crayfish can exert significantly more effects on freshwater mussel communities than co-evolved native species of crayfish. Moreover, since invasion of signal crayfish mostly results in a timely die-out of native crayfish (e.g., Vorburger and Ribi 1999), signal crayfish rarely face direct competition with native crayfish. This mechanism provides the opportunity for a fast establishment and expansion of *P. leniusculus*. Additionally, signal crayfish can reach high densities of 0.4 (Wutz and Geist 2013) to 2.2 (Guan 2000) specimens per m^2. In line with our results, this could rapidly lead to an increased predation pressure on native mussels.

4.5.3 Effects of the spread of *P. leniusculus*

Given the fact that *P. leniusculus* can reach higher densities than the native crayfish species (Guan and Wiles 1996), the expected increase in invasive crayfish densities as well as their distribution expansion (Kouba et al. 2014) will increase predation pressure since crayfish have a major impact on the benthic food web (Reynolds et al. 2013).

In addition, if signal crayfish colonise new habitats that were unsuitable for native crayfish, this will introduce a new stressor for mussels resulting in potentially irreversible species diversity shifts (Hobbs et al. 1989). Our results of the co-exposures suggest that with *P. leniusculus* appearing in new habitats, this could create high predation pressure on mussel populations that never had been in contact with crayfish. Moreover, *A. astacus* may be introduced into new refuges to conserve this species. James et al. (2015) propose that native and non-native crayfish are ecologically similar and accordingly native crayfish should not be translocated to ark sites, since they may cause impacts to benthic communities. Even if our results show a higher impact from invasive crayfish, the results of the learning part of the experiment indicate that a translocation of native crayfish could also be a threat for native mussel species after *A. astacus* learned to use mussels as prey.

However, all this should be interpreted with caution. Even though we observed clear mussel damage patterns directly linked to crayfish exposure, our experiment also shows that mortality of adult mussels was relatively low over this short period of time, suggesting that most adult mussels survive even prolonged exposure to crayfish in a confined space. Nevertheless, predation effects should not be ignored, particularly since they might be much higher on juvenile mussels. Also, considering the lifespan of mussels, for example over 28 years for Anodonta (Aldridge 1999), a cumulative predation and damage effect over time, which begins with the early life stages, can be expected.

Given that the invasive *S. woodiana* was significantly less affected in both experimental parts, and that *P. leniusculus* caused more damages than the native *A. astacus*, an occurrence of both invasive species could enhance the competitive ability of *S. woodiana*. Considering the known competitive advantages of *S. woodiana* over native mussel species, it can be assumed that an introduction of this species will already be disadvantageous for native mussel populations in absence of crayfish predation. In particular, possible outcompeting effects have already been shown related to reproduction success: The greater larval survival (Benedict and Geist 2021), the larger number of suitable hosts, the higher excystment rates and the faster development of *S. woodiana* (Douda et al. 2012; Huber and Geist 2019) as well as the greater glochidia output and the ability for breeding multiple times a year (Labecka and Czarnoleski 2021; Labecka and Domagala 2018) increase the reproduction success of *S. woodiana*. Contrary, the reduced transformation success rates of *A. anatina* on host fish previously infested with *S. woodiana* (Donrovich et al. 2017) reduce the recruitment of native mussel populations. Additionally, its wider tolerance towards changing environmental conditions, e.g., temperature, also could give this species an advantage in times of climate change (e.g., Bielen et al. 2016; Corsi et al. 2007; Douda et al. 2012).

If both *P. leniusculus* as well as *S. woodiana* invade the same system, the high impact in native species but the low impact *of P. leniusculus* on *S. woodiana* could potentially lead to multiple stressors for native mussels as has already been similarly reported, inter alia, in the upper Danube River (Brandner et al. 2013) or in laboratory experiment with invasive round goby and amphipod on native gammarids (Beggel et al. 2016). This may result in increased problems for, and local extinction of native mussel populations as well as the facilitated spread of invasive species.

5 A spatially explicit approach to prioritize protection areas for endangered freshwater mussels

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5.1 Abstract

In spite of their conservation importance, only a fraction of lakes and streams globally – including their catchments – are currently covered by conservation areas. To identify conservation gaps, assessing the spatial distribution of biodiversity in relation to conservation areas is a promising approach.

A gap analysis approach was used to evaluate the protection status of habitats of two endangered freshwater mussel species, *Margaritifera margaritifera* and *Unio crassus*, in the Federal State of Bavaria, Germany. First, ecological niche models (ENMs) were developed for both mussel species based on presence-only data in order to identify suitable habitats. Secondly, binary maps of suitable/unsuitable habitats for the species were used to compare different categories of currently protected areas in a gap analysis.

ENMs for *M. margaritifera* revealed a spatially restricted distribution with good model performance, whereas the spatial distribution of *U. crassus* was wider and model performance was weaker. For *M. margaritifera*, a higher percentage of suitable habitat is already under protection, whereas for *U. crassus* only half of the suitable habitats are under any sort of protection.

Our results suggest that suitable habitats of both species are not sufficiently protected. More effective conservation of *M. margaritifera* and *U. crassus* requires a separate management: increasing the respective protection categories of already protected habitats of *M. margaritifera* and incorporating an increased area of suitable habitats under legal protection for *U. crassus*.

Candidate's contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by the candidate with support from KI and KS. The first draft of the manuscript was written by the candidate and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

5.2 Introduction

Identifying and implementing protected areas are important strategies to counteract the increased rate of species extinctions and biodiversity loss. Systematic conservation planning can aid in effectively prioritizing and designing protected areas (Decker et al. 2017; Margules and Pressey 2000; Moilanen et al. 2009). While this approach has been widely applied to terrestrial and marine ecosystems, freshwater ecosystems are still underrepresented (Abell 2002; Herbert et al. 2010; Hermoso et al. 2016; Lopes-Lima et al. 2017; Nel et al. 2009; Nel et al. 2007). Given that freshwater ecosystems are considered the most threatened ecosystems on the planet, with a pressing need for their conservation and restoration (Geist 2011; Geist 2015; Geist and Hawkins 2016), spatial conservation prioritization using SDMs can be an important tool to make conservation planning successful (Moilanen et al. 2008; Moilanen et al. 2009).

The Federal Nature Conservation Act of 1977 (BNatSchG) is a major federal law for environmental protection and landscape conservation in Germany. The regulation includes the conservation and protection of native species and designations of protected areas for the conservation of species and natural landscapes. Additionally, along with conservation areas designated by BNatSchG, the European Union (EU) created the world’s largest protection network, the European Natura 2000 network (Council of the European Communities, 1992), which has main focus on the conservation of terrestrial biodiversity. These protected areas, which include Nature Reserve, Landscape Protection Area, National Park, Nature Park, Biosphere Reserve, and Special Area of Conservation, have different regulations and protections (**Table 8**). For example, Natura 2000 Network’s Special Area of Conservation, Nature Reserve, and National Park have strict regulations limiting modifications to nature and landscapes (**Table 8**). In those areas, land-use management minimizes destruction of natural habitats and anthropogenic activities in order to protect and preserve ecosystems. In contrast, Biosphere Reserves, Nature Parks, and Landscape Protection Areas have moderate regulations on land management, particularly regarding land-use and accessibility (Meyerholt 2010). The aim in those areas is to ensure sustainable land-use and regional development by maintaining the functional capacity of natural habitats. Although the European Union Water Framework Directive requires to meet other European Union

Table 8 Protection types, legislation, level of protection, designation of protected area, number of sites, total and mean areas (km^2), and total river segments (km) under the protection types in Bavaria.

Type of protected areas	Legislation	Level of protection	Designation of protected areas	Number of sites	Total area (km^2)	Mean area (km^2)	Total river segments (km)
Nature Reserve	§ 23 BNatSchG	Protection of landscape and ecological valuable habitats; land development is prohibited	Federal States of Germany	602	1,643.9	2.7	1064.4
Landscape Protection Area	§ 26 BNatSchG	Protection of landscape and ecological functions; land development is prohibited	Federal States of Germany	702	21,209.4	30.2	9759.7
National Park	§ 24 BNatSchG	Protection of natural processes and maintenance of natural dynamics; land development is prohibited	Federal States of Germany	2	450.5	225.2	107.6
Nature Park	§ 27 BNatSchG	Protection of landscape in order to maintain native species and their diversity; land development is not prohibited	Federal States of Germany	18	22,449.0	1247.2	8098.2
Biosphere Reserve	§ 25 BNatSchG	Protection of ecological and cultural landscape in order to maintain cultural diversity and biodiversity	Federal States of Germany after recognition by the UNESCO	252	3,273.1	13.0	831.0
Special Area of Conservation	European Union Habitats Directive (92/43/EEC), §§ 31-36 BNatSchG	Protection of habitat and species listed in annex I and II of the European Union Habitat Directive	Federal States of Germany	2,696	6,458.8	2.4	7006.3

directives' objectives (e.g., Habitat Directive) relevant to aquatic areas, many currently protected areas often do not sufficiently cover freshwater ecosystems and species such as freshwater mussels (Hermoso et al. 2015).

Freshwater mussels (Bivalvia: Unionoida) are amongst the most endangered aquatic organisms in the world (Bogan 1993; Lopes-Lima et al. 2017; Strayer et al. 2004). Over the last decades, severe declines in species richness and abundance of freshwater mussels have been observed worldwide (Bogan 1993; Haag 2012; Howard et al. 2015; Lydeard et al. 2004). Primary causes of population decline include habitat fragmentation and degradation, water pollution, introduction of invasive species, and climate change (e.g., Bodis et al. 2016; Dudgeon et al. 2006; Lydeard et al. 2004; Regnier et al. 2009; Strayer et al. 2004; Young and Williams 1983). The loss of mussel populations may cause long-term ecological consequences to freshwater ecosystems because freshwater mussels often dominate benthic animal biomass in rivers and provide various ecosystem services such as water filtration, nutrient excretion, and sediment stabilization (Boeker et al. 2016; Lummer et al. 2016; Richter et al. 2016; Vaughn 2018). The complex life history of freshwater mussels, where larvae (glochidia) are obligate parasites of fish, further complicate the conservation efforts of this group. As a result, research on the conservation of freshwater mussels has increased exponentially in the last 30 years (Lopes-Lima et al. 2014).

The freshwater pearl mussel, *Margaritifera margaritifera* (Linnaeus 1758), and the thick-shelled river mussel, *Unio crassus* (Philipsson 1788), are two mussel species widely distributed across north and central Europe; however, many populations suffer from dramatic declines in abundance due to a lack of recruitment (Geist 2010; Stoeckle et al. 2017). Therefore, both species are currently listed as “endangered” on the IUCN Red List (IUCN (International Union for Conservation of Nature) 2017) and protected under EU law (Annex II and IV for *U. crassus* and Annex II and V for *M. margaritifera* of the EU Habitats and Species Directive). Despite their shared conservation status, these species substantially differ in habitat requirements (Denic et al. 2014; Geist and Auerswald 2007) and host-use (Taeubert and Geist 2017; Taeubert et al. 2012a; Taeubert et al. 2012b). *Margaritifera margaritifera* is often considered as a habitat specialist. It occurs in cool, oligotrophic upland streams with bedrock, cobble, and gravel substrates, consistent flow, and low carbonate content (Bauer 1992; Geist 2010; Lopes-Lima et al. 2017). With a lifespan of more than 100 years, *M. margaritifera* is one of the longest living invertebrates

(Geist 2010). *Unio crassus* has a broader range of environmental tolerance than *M. margaritifera* (Stoeckle et al. 2017). The species occurs in oligotrophic headwater streams to more eutrophic downstream reaches of large rivers (Lopes-Lima et al. 2017); it also uses various microhabitats, hydrology, and substrate conditions often with high amounts of fine sediments and organic matter (Denic et al. 2014). Furthermore, *U. crassus* uses a wider variety of host fishes when compared with *M. margaritifera* (Stoeckl et al. 2015). As suitable hosts for the thick-shelled river mussel especially three fish species were detected in the upper Danube drainage, in particular the European minnow (*Phoxinus phoxinus*), the common chub (*Squalius cephalus*) and the three-spined stickleback (*Gasterosteus aculeatus*) (Taeubert, Gum, & Geist, 2012; Taeubert, Martinez, Gum, & Geist, 2012). Conversely, only the brown trout (*Salmo trutta* f. *fario*) seems to be a suitable host for the freshwater pearl mussel in this area (Geist et al. 2006; Taeubert and Geist 2017).

A previous study found associations of occurrence between mussel species and its fish hosts, and the importance of environmental factors on mussel occurrences (Inoue et al. 2017). The same study hypothesized that mussel distributions are nested within the host fish distributions and the presence of mussels at a given site is primarily driven by local abiotic conditions. However, the distribution of suitable habitats for *M. margaritifera* and *U. crassus* have not yet been modelled using ecological niche models (ENMs). Such information is important when implementing effective conservation strategies and prioritizing conservation areas. Furthermore, although current protected areas were often designated primarily on terrestrial ecosystems (e.g., Nel et al. 2009), it is important to know whether the current protected areas cover suitable habitat for aquatic fauna. For the implementation of effective conservation strategies for endangered mussel species, Lopes-Lima et al. (2017) recommend prioritizing species-specific conservation. This policy is recommended for *M. margaritifera* and *U. crassus* given their differences in habitat requirements and fish hosts (Inoue et al. 2017).

In light of the need for effective conservation of freshwater mussels, the objective of this study was to test whether or not the distributions of suitable habitats for the target species are sufficiently covered by currently protected areas. Ecological niche models for the two mussel species, *M. margaritifera* and *U. crassu*s, were developed and subsequently the coverage between protected areas and areas that are predicted to be suitable for each species, was compared.

5.3 Methods

5.3.1 Study area

The study area is located in the federal state of Bavaria in southeast Germany (**Fig. 17**). This area comprises four major river drainages: the Danube drainage (approx. 48,220 km^2), the Rhine drainage (approx. 20,309 km^2), the Elbe drainage (approx. 1,971 km^2), and the Weser drainage (approx. 48 km^2) (LfU (Bayerisches Landesamt für Umwelt) 2014). Only 1.8% of rivers in Bavaria are considered to be unmodified; most rivers have been extensively altered by human activities (LfU (Bayerisches Landesamt für Umwelt) 2014). These alterations include flow regulations, riparian modification, water retention, and technical flood protection. A geographic information system (GIS) layer of streams and rivers in Bavaria (**Fig. 17**) was obtained from the State Office for Environment in Bavaria (Bayerisches Landesamt für Umwelt, www.lfu.bayern.de), which included the German stream order system (LAWA 1993). Areas for ecological niche modelling were set within a 500-m buffer around the river segments because river width information was unavailable. Furthermore, using the 500-m buffer, the effect of riparian environments on mussel occurrences was examined because diffuse pollution and sediment erosion associated with land use in riparian zone have the potential to affect aquatic organisms and their habitat (Brim-Box and Mossa 1999; Inoue et al. 2017). The 500-m buffered river layer was created using ARCGIS 10.3.1 (ESRI, Inc).

5.3.2 Species records

Species occurrence data for *M. margaritifera* and *U. crassus* was obtained from the species conservation mapping database organized by the State Office for Environment in Bavaria (LfU (Bayerisches Landesamt für Umwelt) 2016). The occurrence data were collected from 1990 to 2016. Initially, the dataset included 1,371 georeferenced occurrence points (390 for *M. margaritifera*; 981 for *U. crassus*); however, a total of 71 occurrence points were discarded (four for *M. margaritifera*; 67 for *U. crassus*) since these points were located outside of river segments or recorded no live individuals. Therefore, 1,300 presence-only points (386 for *M. margaritifera*; 914 for *U. crassus*) were retained for the analyses.

Given that occurrence data often show strong sampling bias in sampling efforts (Fourcade et al. 2014), sdmtoolbox v1.0b (Brown 2014) in ARCGIS was used to reduce spatial autocorrelation in the occurrence data by selecting

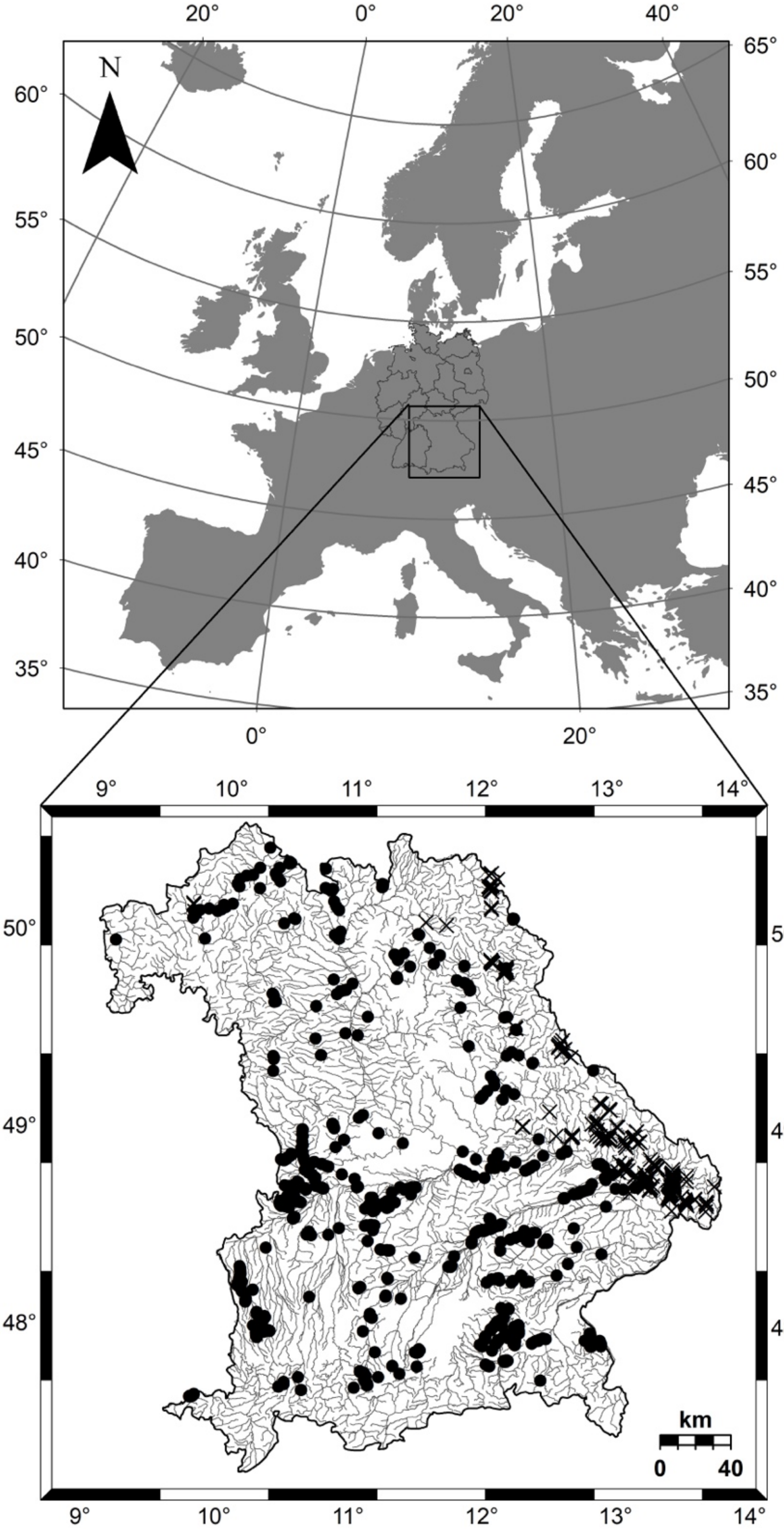

Fig. 17 Map of the Federal State of Bavaria, Germany, depicting the river systems and distribution of the 1371 occurrence points of *Margaritifera margaritifera* (black crosses) and *Unio crassus* (black circles) collected from 1990 to 2016

one record within a 2 km radius (Kramer-Schadt et al. 2013; Phillips et al. 2009). Briefly, occurrence data were spatially filtered by the 2-km radius and reduced to a single point within the 2 km Euclidian distance. After spatially rarefying occurrence points, 348 occurrence points (68 for *M. margaritifera*; 280 for *U. crassus*) were retained.

5.3.3 Environmental variables

The distribution of mussels can be influenced by a range of abiotic factors, including variability of climate, heterogeneity in riparian land cover, geology, and variability in water chemistry (Inoue et al. 2015; Morris and Corkum 1999; Österling and Högberg 2014; Strayer 1993). Initially 29 environmental variables that potentially influence the distribution of *M. margaritifera* and *U. crassus* were obtained (Table 2). These variables included 21 climatic variables, three landscape variables, two anthropogenic variables, and three physicochemical variables. For climate variables, 19 bioclimatic variables were obtained from WorldClim (http://www.worldclim.org; Hijmans et al. 2005), and annual potential evapotranspiration (PET in mm yr^{-1}) and aridity index (ranged from 0 to 1; very arid to very humid) from CGIAR-CSI (http://www.cgiar-csi.org, Zomer et al. 2008). The three landscape variables include a global land-cover layer obtained from GlobeLand30 Land Use and Land Cover (LULC) dataset (http://www.globallandcover.com), and the proportions of cropland and pastureland obtained from the Global Agricultural Lands dataset (Ramankutty et al. 2008). The two anthropogenic variables include human population density (number of persons $kilometre^{-2}$) and human footprint index (ranged from 0 to 100; most wild to least wild) obtained from the National Aeronautics and Space Administration (NASA) Socioeconomic Data and Applications Center (SEDAC, http://sedac.ciesin.columbia.edu). Due to a lack of consistent hydrological data in Bavaria, the three physicochemical variables include equilibrium phosphorous concentration in rivers estimated based on local lithology (equilibrium PO_4^{3-} mg L^{-1}) obtained from the European Commission's Joint Research Centre Water Portal (http://water.jrc.ec.europa.eu/), and the amount of nitrogen and phosphorus fertilizer inputs obtained from the Global Agricultural Lands (Potter et al. 2010).

Table 9 Abiotic covariates used in the ecological niche models (ENMs). Of 29 bioclimatic, landscape, anthropogenic, and physicochemical variables initially examined, 12 uncorrelated covariates were chosen for ENMs (Pearson correlation coefficient < 0.6; '×' in the 'Used' column)

Covariates	Description	Resolution	Used
Climate variables			
Annual mean temperature (°C)	Mean monthly temperatures of a year	1 km	
Mean diurnal range (°C)	The mean of the monthly temperature ranges	1 km	
Isothermality (%)	Proportion of temperature difference between mean daily range and annual range	1 km	x
Temperature seasonality (°C)	Standard deviation of temperature	1 km	x
Max temperature of warmest month (°C)	Maximum monthly temperature occurrence	1 km	
Min temperature of coldest month (°C)	Minimum temperature value across all months within a year	1 km	
Annual temperature range (°C)	Temperature range between coldest and warmest month	1 km	
Mean temperature of wettest quarter (°C)	Mean temperature for the three months with the highest cumulative precipitation	1 km	
Mean temperature of driest quarter (°C)	Mean temperature for the three months with the lowest cumulative precipitation	1 km	x
Mean temperature of warmest quarter (°C)	Mean temperature for the three months in the warmest quarter	1 km	
Mean temperature of coldest quarter (°C)	Mean temperature for the three months in the coldest quarter	1 km	
Annual precipitation (mm)	Sum of the precipitation values of each months in one year	1 km	
Precipitation of wettest month (mm)	Total precipitation prevailing during the wettest month	1 km	
Precipitation of driest month (mm)	Total precipitation prevailing during the driest month	1 km	
Precipitation seasonality (%)	Coefficient of precipitation variation	1 km	x
Precipitation of wettest quarter (mm)	Precipitation prevailing during the wettest quarter	1 km	
Precipitation of driest quarter (mm)	Precipitation prevailing during the driest quarter	1 km	
Precipitation of warmest quarter (mm)	Total precipitation prevailing during the warmest quarter	1 km	
Precipitation of coldest quarter (mm)	Total precipitation prevailing during the coldest quarter	1 km	
Annual potential evapotranspiration (mm yr^{-1})	Amount of mean annual potential evapotranspiration	1 km	x
Aridity index (AI)	Mean annual precipitation/mean annual potential evapotranspiration	1 km	x
Landscape variables			
Global landcover	Categorical data. Land cover types include: cultivated land (denoted as 10); forest (20); grassland (30); shrubland (40); wetland (50); water bodies (60); tundra (70); artificial surfaces (80); bareland (90); permanent snow and ice (100)	30 m	x
Area of cropland (%)	Proportion of land areas used as cropland (land used for the cultivation of food) in the year 2000	1 km	x
Area of pasture (%)	Proportion of land areas used as pasture land (land used to support grazing animals) in the year 2000	1 km	x
Anthropogenic variables			
Population density	Population density in persons per square kilometre in year 2000, adjusted to match UN country totals	1 km	x
Human footprint	A percentage the relative human Influence on the land's surface; value ranges from 0 to 100.	1 km	x
Physicochemical variables			
PO_4^{3-} concentration (mg L^{-1})	Estimated equilibrium phosphate in rivers, based on local lithology. Values refer to the year 2005	5 km	x
Nitrogen fertilizer (Tg N yr^{-1})	Nitrogen introduced as fertilizer	5 km	
Phosphorus fertilizer (Tg P yr^{-1})	Phosphorus introduced as fertilizer	5 km	

Using sdmtoolbox, all environmental layers were re-projected to the Gauss-Krüger coordinate system (Germany, zone 4). The spatial resolution was adjusted to 500 x 500 m, and the layers were masked with the 500-m buffered river layer. Multicollinearity among environmental variables was tested (≥ 0.6 Pearson correlation coefficient) and 12 uncorrelated environmental variables were randomly chosen and used for further analyses (**Table 9**).

5.3.4 Ecological niche modelling

The maximum entropy algorithm in MAXENT v3.3.3k (Phillips et al. 2006) was used to develop ecological niche models (ENMs) for *M. margaritifera* and *U. crassus* using the georeferenced occurrence records and environmental variables. Given the sporadic distribution of the species and that it is ecologically realistic to sample background points from the know occurrence area (Elith et al. 2011), a bias layer of Gaussian kernel density of the occurrence points was created with a bandwidth of 50 km to control for background sampling efforts. The bias layer and the following parameters were used to estimate habitat suitability scores in MAXENT: random test percentage of 0, regularization multiplier of 1, maximum number of background points of 10,000, and maximum iterations of 500. The ten-fold cross-validation method with random seed was used to replicate models.

5.3.5 Model evaluation

Area under the receiver operating characteristic curve (AUC) was used to evaluate the accuracy of models predicting distribution of suitable habitat (Liu et al. 2005; Phillips et al. 2006). Based on Araujo et al. (2005) adapted from Swets (1988), AUC values were categorized as follows: AUC value greater than 0.9 as ‘excellent’, AUC values between 0.8 and 0.9 as ‘good’, AUC values between 0.7 and 0.8 as ‘fair’ and AUC values less than 0.7 as ‘poor’.

Marginal response curves for each environmental variable were graphed and relative contributions of the environmental variables to each ENM were estimated. Based on the suitability threshold scores (0.4377 for *M. margaritifera*; 0.3143 for *U. crassus*) that were calculated from the 10th percentile of occurrence records (i.e., the suitability of the occurrence records below which 10% of occurrence points’ suitabilities; hereafter, 10th percentile threshold value), binary maps (suitable/unsuitable) for each species were generated using 10 Percentile Training Presence function in MAXENT. These threshold values constitute stricter criterion for converting a continuous suitability scores to binary one compared to using the lowest presence threshold scores. Therefore, values above the threshold were classified as suitable habitat and values below as unsuitable habitat using spatial analyst tools in ARCGIS.

5.3.6 Gap analysis

To identify gaps between current conservation areas and suitable habitats for *M. margaritifera* and *U. crassus* in Bavaria, a gap analysis was conducted (Burley 1988). First, current conservation areas in Bavaria, which are designated by BNatSchG and the European Commission’s Natura 2000 Network, were identified. The types of conservation areas included Nature Reserve (NR), Landscape Protection Areas (LPA), National Park (NATP), Nature Park (NP), Biosphere Reserves (BR), and Natura 2000 Special Area of Conservation (SAC) (**Table 8**). Note that some conservation areas are designated by multiple overlapping conservation types. The GIS layer of conservation areas was obtained from the State Office for Environment in Bavaria (Bayerisches Landesamt für Umwelt, www.lfu.bayern.de). The river layer was overlaid on the layer of conservation areas and river segments within each conservation type was extracted. A total length of river segments was calculated under each conservation category. Secondly, each species’ binary suitable/unsuitable layer was converted to polyline vector layers, and a total length of suitable/unsuitable habitat within Bavaria was measured. Lakes were classified as unsuitable, as neither of the two mussel species occurs in lentic habitats. Using the conservation area layers and each species’ suitable/unsuitable layer, protection gaps were evaluated by estimating proportions of suitable habitat that were not in the segments of each conservation category.

5.4 Results

5.4.1 Ecological niche modelling and model evaluation

The model performance of ENMs was different between the species. Mean AUC value for *M. margaritifera* was 0.936 (SD = 0.020) indicating excellent performance; however, AUC value for *U. crassus* was 0.677 (SD = 0.045) indicating poor model performance.

Table 10 Estimates of relative contributions (%) of the environmental variables to the MAXENT models for *Margaritifera margaritifera* and *Unio crassus*. AUC_{test} is area under the receiver operating characteristic curve that was used to evaluate model performance.

Variables	*M. margaritifera*	*U. crassus*
Aridity index	3.4	27.5
Isothermality (%)	0.1	0.3
Temperature seasonality (°C)	2.3	6.5
Mean temperature of driest quarter (°C)	11.6	3.7
Precipitation seasonality (%)	6.1	6.3
Area of cropland (%)	9.2	10.8
PO_4^{3-} concentration (mg L^{-1})	61.5	15.7
Global landcover	1.5	4.3
Human footprint	3.1	1.7
Area of pasture (%)	0.8	5.1
Population density	0.3	2.7
Annual potential evapotranspiration (mm $year^{-1}$)	0.0	15.4
AUC_{test}	0.936±0.020 SD	0.677±0.045 SD

The best predicting variable of habitat suitability for *M. margaritifera* was equilibrium phosphorus concentration in rivers (61.5%), followed by mean temperature of driest quarter (11.6%), the proportion of cropland (9.2%), and precipitation seasonality (6.1%) (**Table 10**). Strong negative correlation between phosphorus and the occurrence of *M. margaritifera* (**Fig. 18d**) indicated a narrow tolerance range to phosphorus levels. The best predictors of suitable habitats for *U. crassus* were aridity index (27.5%), equilibrium phosphorus concentration in rivers (15.7%), potential evapotranspiration (15.4%), percentage of cropland (10.8%), and temperature seasonality (6.5%) (**Table 10**). Predicted habitat suitability for *U. crassus* increased with higher phosphorus values (**Fig. 19d**) indicating a higher tolerance than *M. margaritifera*.

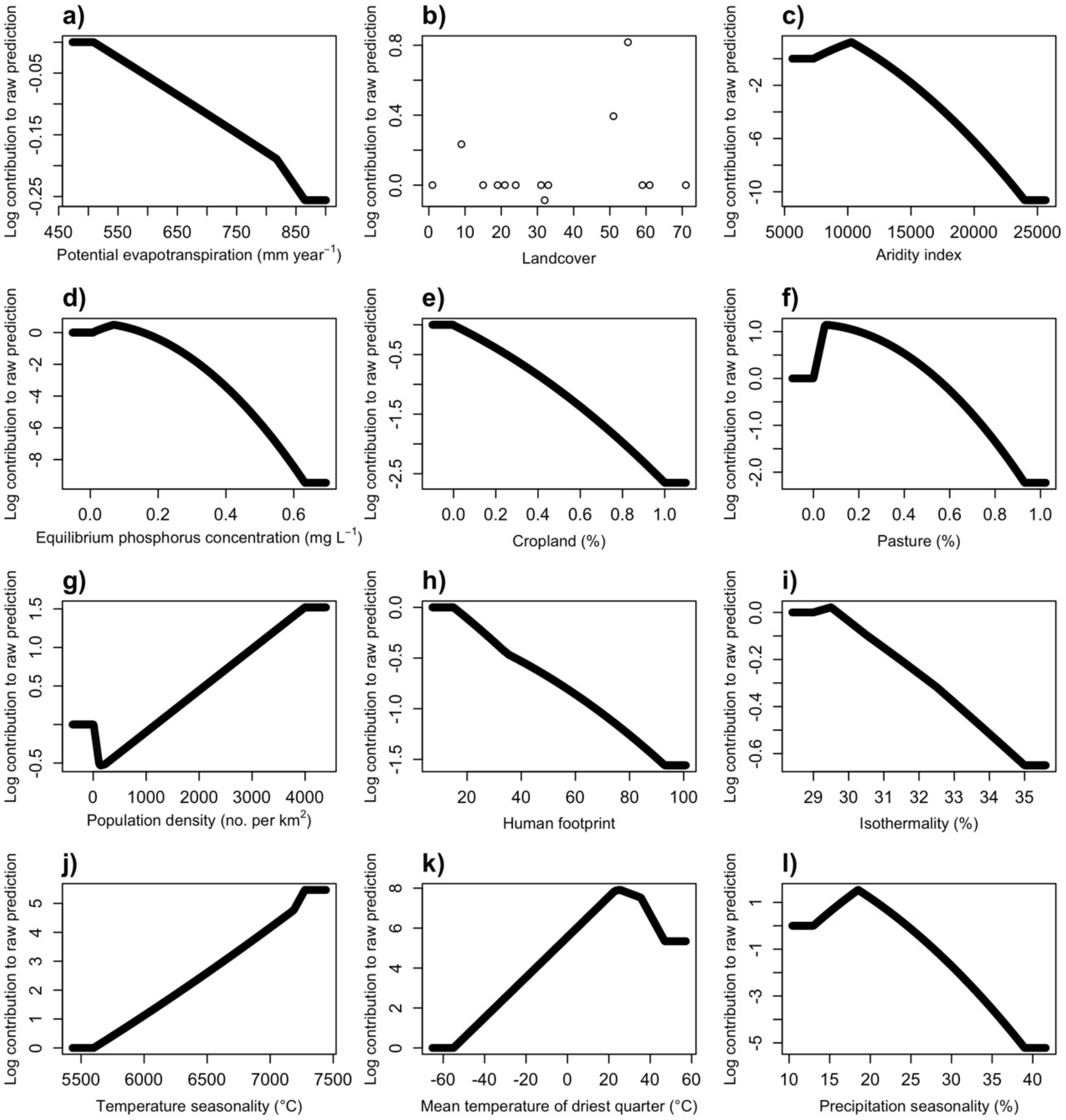

Fig. 18 Responses of *Margaritifera margaritifera* to each environmental variable (marginal response curves) showing how each environmental variable affects the prediction of habitat suitability.

The 10^{th} percentile threshold values (0.4377 for *M. margaritifera*; 0.3143 for *U. crassus*) were used to convert suitability scores for each species into binary predictions of suitable/unsuitable habitats (**Fig. 20**). The total lengths of suitable river segments for *M. margaritifera* were 2,211.5 km (segments ranged from <0.1 to 49.9 km; mean = 2.0 km; median = 0.9 km), which represent 7.9% of total river segments in Bavaria. For *U. crassus*, 52.8% of total rivers in Bavaria were suitable (16,273.3 km in total; ranged from <0.1 to 97.6 km; mean = 3.8 km; median = 1.6 km). Generally, suitable habitats for *M. margaritifera* and *U. crassus* did not overlap (**Fig. 20**). Suitable habitats for *M. margaritifera* were spatially restricted to east and northeast Bavaria (**Fig. 20a**). A small portion of

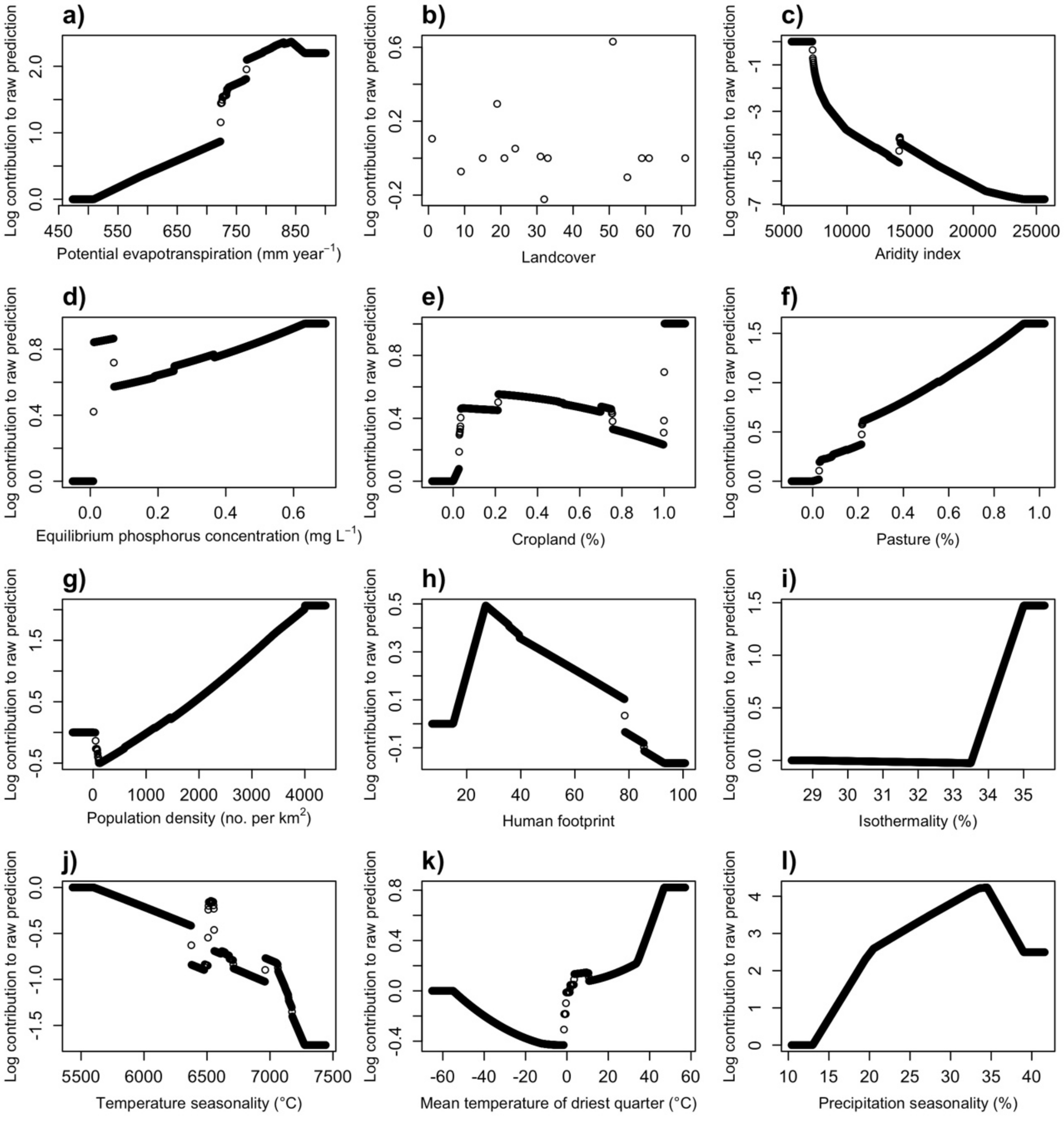

Fig. 19 Responses of *Unio crassus* to each environmental variable (marginal response curves) showing how each environmental variable affects the prediction of habitat suitability.

northwest Bavaria also had suitable habitat for *M. margaritifera*. The spatial extents of predicted suitable habitats were much wider for *U. crassus* than those of *M. margaritifera* (**Fig. 20b**). The models predicted that alpine rivers in the extreme south of Bavaria were unsuitable for both species (**Fig. 20**).

5.4.2 Gap analysis

Two of the most dominant conservation types in Bavaria were the Landscape Protection Areas (21,209.4 km^2 in total; 9,759.7 km in total river segments) and Nature Parks (22,449.0 km^2 in total; 8,098.2 km in total river segments), followed by the Natura 2000's Special Areas of Conservation (6,458.8 km^2 in total; 7,006.3 km in total river segments) (Table 1). Landscape Protection Areas, Natura 2000's Special Areas of Conservation, Nature Reserves and Nature Parks are distributed across Bavaria, but National Parks were located only in east Bavaria and there are only two areas classed as Biosphere Reserves, one in the north and one in the south of Bavaria.

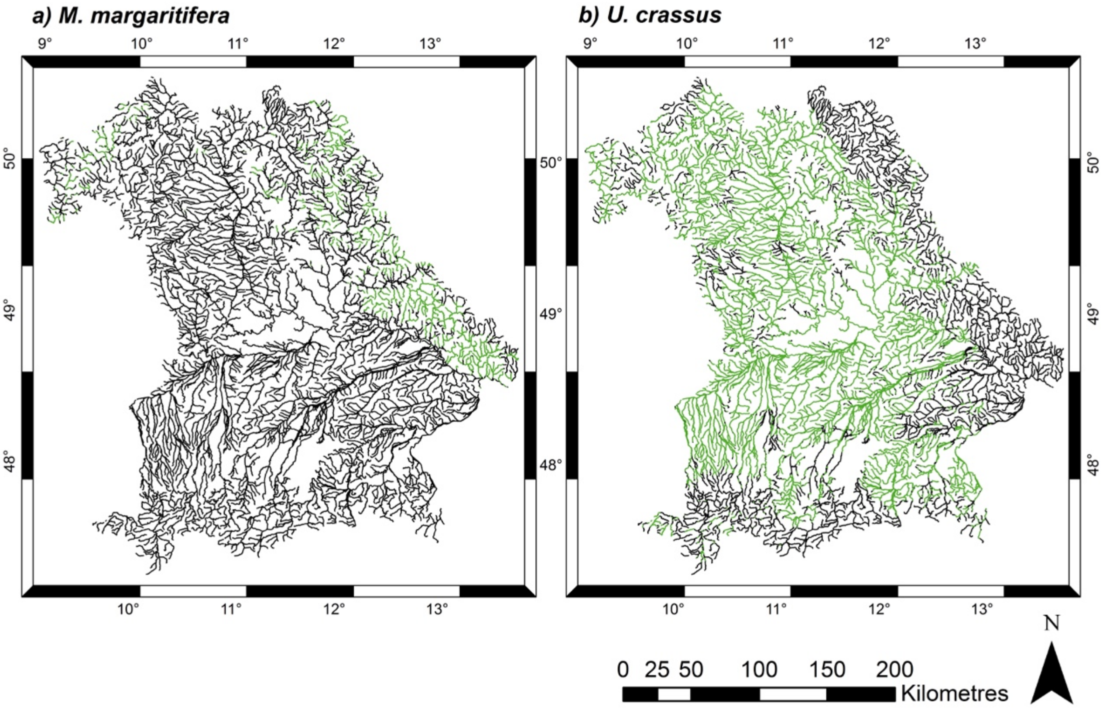

Fig. 20 Binary maps of the MAXENT model for (a) *Margaritifera margaritifera* and (b) *Unio crassus*. Values below the 10th percentile threshold values (0.4377 for *M. margaritifera;* 0.3143 for *U. crassus*) were defined as unsuitable habitat (black) and values above as suitable habitat (green).

The majority of predicted suitable habitats of *M. margaritifera* (86.7% of the total suitable areas) were found within some sort of conservation area, while only half of the suitable areas of *U. crassus* (50.5%) were within conservation areas (Fig. 5). The majority of predicted suitable habitat of *M. margaritifera* was covered by the Landscape Protection Areas (67.7% of total suitable habitat) and Nature Parks (76.9%); the total exceeds 100% because some of these conservation areas overlap. The Natura 2000's Special Areas of Conservation covered 29.8% of the total suitable habitat of *M. margaritifera*. Similarly, these conservation areas were dominant for the predicted suitable habitats of *U. crassus* (31.5% in the Landscape Protection Area; 23.4% in the Nature Parks; 24.4% in the Natura 2000's Special Area of Conservation; **Fig. 21**). For both species separately, the Nature Reserve, Biosphere Reserve, and National Park covered less than 5% of total suitable habitats (**Fig. 21**).

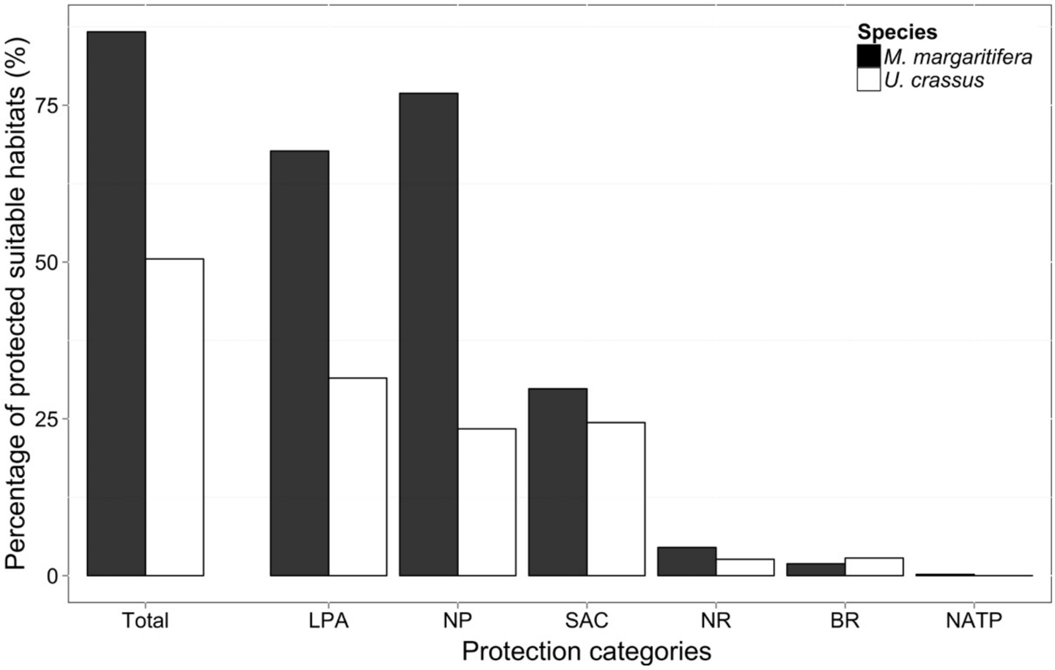

Fig. 21 Percentage of suitable habitats for *Margaritifera margaritifera* (black) and *Unio crassus* (white) within the protected area types: landscape protection area (LPA), nature park (NP), special area of conservation (SAC), nature reserve (NR), biosphere reserve (BR) and national park (NATP).

5.5 Discussion

In this study, ecological niche models were developed for two European mussel species of high conservation priority, *M. margaritifera* and *U. crassus*, based on different uncorrelated environmental variables. ENMs provided a basis for a gap analysis, which allowed quantification of the amount of protected suitable habitats within currently protected areas. While a large extent of the predicted suitable habitats of *M. margaritifera* is already covered by protected areas, the predicted suitable habitats of *U. crassus* are widespread throughout Bavaria and only sporadically covered by protected areas.

Model performance of ENMs differed between *M. margaritifera* and *U. crassus*, with better performance in *M. margaritifera*. The poor performance of the model of *U. crassus* was likely due to its broader ecological niche and life history plasticity (e.g., use of multiple host fish species, occurrence in both carbonate-rich and siliceous habitats), which is also reflected in its wider distribution. Models of a species with limited distributional range tend to reach higher AUC values than models of widespread species (Elith et al. 2006; Hernandez et al. 2006). Although the distribution of *U. crassus* was previously thought to be strictly limited by water quality and habitat (e.g., Buddensiek et al. 1993; Hochwald 1990; Hochwald 1997; Hus et al. 2006; Zettler and Jueg 2007), recent studies suggested that this species has a much higher tolerance to various environmental conditions (e.g., Denic et al. 2014; Douda 2010; Lewin 2014; Stoeckl and Geist 2016). Relatively low contributions of the environmental factors to the ENM suggests that no single environmental variable was a dominant factor in defining the habitat suitability of *U. crassus* (**Fig. 19**; **Table 10**). Alternatively, however, the predicted suitable habitats of *U. crassus* may be legacy effects that result in suitable, but unoccupied habitat. Recent improvements of habtiat from historic habitat degradation (e.g., water pollution) may not be reflected in mussel assemblage in the models due to long population response times. Further study incorporating additional environmental variables is needed to help improve future model performance and better predict the suitable habitats of freshwater mussels. These include characteristics of microhabitats and physicochemical conditions, hydrological and hydraulic conditions, and substrates; however, such variables are currently unavailable in the state of Bavaria. Furthermore, it is recommended groundtruthing the predicted suitable habitats to verify the presence of mussel species.

The high AUC value of the ENM of *M. margaritifera* can be explained by its highly specific habitat requirements. In contrast to *U. crassus*, *M. margaritifera* distribution is restricted to oligotrophic streams (Bauer 1988; Taeubert and Geist 2017). The marginal response curves of *M. margaritifera*'s ENM showed that the amount of equilibrium phosphorus concentration in rivers and the percentage of cropland surrounding rivers negatively correlated with habitat suitability for *M. margaritifera* (**Fig. 18**). This matches the expectation and indicates that *M. margaritifera* has a strong preference to low levels of natural phosphate concentration and is sensitive to modification of riparian habitats, which can often contribute to eutrophication. Croplands particularly, are often considered the major diffuse source of phosphorus input into rivers through fertilizer (Carpenter et al. 1998). The results of the current study are congruent with a previous study (Inoue et al. 2017).

While over 50% of suitable habitat for each species is within protected areas, most of these areas have the lowest level of environmental protection (i.e., Landscape Protection areas and Nature Parks) (**Table 8**). For example, the mission of the Landscape Protection Areas and Nature Parks is to protect and conserve cultural landscapes and promote recreation and tourism. Only activities that modify the character of the landscape are prohibited in these protection areas; thus, the regulation does not provide sufficient protection for fauna and flora in these areas. Conversely, the Nature Reserves and National Parks have strict regulations for protecting their natural environment as these protected areas are designated to minimize human influence on the area to preserve and protect habitat and biodiversity. However, only a small fraction of the predicted suitable habitats of both species lies within these protected areas. Furthermore, although the Nature Reserve sites are the third most abundant in Bavaria, the mean area of each site is rather small (2.73 km^2; **Table 8**). With regards to the National Parks, each park has large area (mean area = 225.23 km^2; Table 1); however, there are currently only two National Parks in Bavaria that do not overlap with the current distribution range of *U. crassus* and *M. margaritifera*. Finally, since the establishment of the Natura 2000's Special Areas of Conservation, many newly protected areas have been added in Germany. However, the mean area of these protected areas is 2.4 km^2 (**Table 8**) despite the fact that about a quarter of the total suitable habitats for both species lies within these areas.

The small percentage of highly regulated protected areas may not sufficiently contribute to the aim of sustainable conservation of both species and protection of its preferred habitats. In many cases, protected areas only cover short stream segments. Given that streams are longitudinally linked systems in which ecosystem-level processes in downstream areas are linked to those in upstream areas (Vannote et al. 1980), populations in suitable habitats may be at risk if they are adjacent to low quality areas. The information of habitat connectivity and protection gaps can be used to further designate new protected areas. Freshwater mussels are considered to be ideal target species of conservation, with some species simultaneously fulfilling the criteria of indicator, flagship, keystone and umbrella species (Geist 2010). Therefore, protecting freshwater mussels by increasing connectivity of their habitats would improve conservation of other aquatic organisms in the region.

Based on the results of the ecological niche modelling and the observed differences in protected area coverage for the two mussel species, different conservation management implications can be deduced. In the case of *M. margaritifera*, given that the majority of populations are already associated with some kind of protected area, albeit typically weak levels of protection, the most useful strategy is to increase protection by upgrading the respective protection categories that have stricter protection of landscape. This is likely to mitigate further habitat degradation and population loss because *M. margaritifera* populations are negatively impacted by even slight changes in the stream substrate conditions (Geist and Auerswald 2007) and stream nutrient levels (this study; Bauer 1988; British Standards Institution 2017; Inoue et al. 2017). In contrast, the greater tolerance of *U. crassus* to substrate conditions and nutrient input, as well as the greater spectrum of host fish species, will in most cases not require an extremely strict legal protection category. Instead, the comparatively low coverage of current *U. crassus* populations with any means of legally protected area management should be a major concern. Thus, increasing the number of any protected areas should be the main priority for this species.

This study provides valuable information for possible management directions of mussel conservation in Bavaria, with the same approach being applicable for wider geographic scales. In recent years, new populations of freshwater mussels have been found primarily due to the improvement of survey methods and occurrence mapping as well as knowledge of habitat requirements for the target species. Implementing gap analyses identifies protection gaps for newly found populations and provides comprehensive coverage for the protection needs of mussels in Bavaria. It needs to be acknowledged that legal protection of suitable habitats can only partly contribute to conservation. Additional restoration of degraded habitats should also be followed, ideally following a systematic and step-wise approach (Geist 2015; Geist and Hawkins 2016). A holistic approach should be followed and is likely to be most successful over the long term since protected area management alone cannot be successful if not accompanied by means of active habitat and population conservation and restoration.

6 General discussion

This thesis provides novel insights into the effects of functional interactions of invasive *S. woodiana* and *P. leniusculus* with native freshwater bivalves (Chapters 3 & 4). It also uses a spatially explicit approach to assess conservation gaps in the protection of two highly threatened freshwater bivalve species (Chapter 5).

Both invasive species, the Chinese pond mussel *S. woodiana* and the signal crayfish *P. leniusculus*, which were suspected of having a negative impact on native freshwater bivalves, but without sufficient scientific evidence, have now proven to pose a serious threat to European freshwater unionids. On the one hand, it has been shown that *S. woodiana* is already widespread in Bavarian water systems (Chapter 3) and thus often overlaps with occurrences of native freshwater bivalves, where the already known negative effects of this invader can affect native bivalve populations. On the other hand, cross-experiments with native and invasive crayfish species and native and non-native bivalves revealed that *S. woodiana* is also less prone to predation by invasive crayfish *P. leniusculus* (Chapter 4), giving this species another major advantage over its native counterparts, especially when both species invade the same system. In addition, signal crayfish, which is already widespread in European freshwater water systems, has proven to be more problematic in terms of predation than native noble crayfish (Chapter 4). However, a learning effect in the use of bivalves as a food source could be demonstrated for both crayfish species.

The gap analysis of Chapter 5, which was based on two ecological niche models of the highly threatened freshwater pearl mussel *M. margaritifera* and thick-shelled river mussel *U. crassus*, respectively, revealed that the actual conservation management of these species is deficient, since habitats of both species, which should be protected sufficiently, seem not to be adequately covered by protected areas (Chapter 5). However, it has been shown that such spatially explicit approaches can be powerful tools for identifying conservation gaps and for setting priorities in conservation planning.

6.1 Invasive bivalve species

Sinanodonta woodiana is non-native to Europe and, compared to others, a relatively young invader, as it was first discovered in Romania in 1979 (Sarkany-Kiss 1986). Since then, this species is spreading across Europe, Central and North America and Indonesia (Sousa et al. 2014). In the eastern and southern part of Europe the distribution of this species is well documented, whereas, up to now, systematic information for Germany was still missing (see Konecny et al. 2018). However, as a generalist species, it is likely that this non-native bivalve will continue to invade many more European aquatic systems if its further spread is not stopped or at least reduced. As shown in the first study (Chapter 3), first evidence of the distribution of this relatively recent invader in the Bavarian part of Germany was overdue, and this species does not appear to be a problematic threat limited to a specific location. Moreover, the results revealed, that this species is already widespread and established in many water systems that are also inhabited by other native freshwater bivalves, albeit not systematically documented until now. Additionally, its spread, especially in the immediate vicinity of fish ponds, can mainly be attributed to fish stockings as have already been shown by others (e.g., Sárkány-Kiss et al. 2000; Spyra et al. 2012; Tomovic et al. 2013; Urbańska et al. 2019). However, due to the high number of suitable host fish species (Douda et al. 2012; Huber and Geist 2019; Sárkány-Kiss et al. 2000; Urbańska et al. 2021) and its broad ecological niche shown in Chapter 3 and by Urbańska et al. (2021), this species may have a high dispersal rate when released in a natural water system. This can also be suggested, if one follows the spread of *S. woodiana*, e.g., in the Danube. There, the downstream spread of this species from Hungary to the Danube Delta in Romania took place in less than 10 years, and its most upstream occurrence was found in 1998 in Austria (Sárkány-Kiss et al. 2000). However, now that *S. woodiana* has been found in the Rothsee (Chapter 3), it can be expected that this species will also occur in the Main-Danube-channel in Germany, using such artificial connections to colonize other drainage systems. This shows the potential for fast spread of this species and suggests that it will continue to expand its range rapidly, especially as many more water systems than Danube and Main have been artificially connected.

Besides *S. woodiana*, four other invasive bivalve species, are recognized in European water systems so far: *C. fluminea*, *C. fluminalis*, *D. polymorpha* and *D. r. bugensis* (Lopes-Lima et al. 2017). These species that have invaded European freshwaters for a long time are well studied and their effects on ecosystems as well as on economics are well-known (Geist et al. 2023; Sousa et al. 2014). *Dreissena polymorpha*, e.g., which has already invaded Europe in the 18th and 19th century (Lopes-Lima et al. 2017), is considered one of the most worse invader (Nentwig et al. 2017), which predominantly has enormous economic consequences (Aldridge et al. 2004). Another dreissenid, *D. r. bugensis*, has also invaded Europe, although somewhat delayed compared to *D. polymorpha*. Contrary to *S.* woodiana, which was brought to Europe by infested fish (Konecny et al. 2018), both dreissenid species were deliberately introduced by fouling hulls or in ballast water of ships and rapidly spread throughout Europe. After a first colonization, they have also fast spread along connected channels and rivers, but they also made big jumps attached to recreational boats or fishing gear. Today, both species are already widespread and common in many freshwater systems of Europe (Karatayev and Burlakova 2022).

Compared to species such as *D. polymorpha*, which seem to have almost reached their full ecological distribution potential in the northern hemisphere (Alix et al. 2016; Quinn et al. 2014), non-native *S. woodiana* will continue to expand its range in the future, resulting in an increasing threat to native bivalves (Sousa et al. 2014; Urbańska et al. 2021), as it has recently been detected for the first time in many aquatic systems in combination with its broad ecological niche (Chapter 3). While this species currently appears to be problematic only for native species with a broader ecological niche, such as *A. anatina*, *A. cygnea* or *U. crassus*, respectively, habitat overlap with highly specialized species such as *M. margaritifera* is still lacking (this study; Geist et al. 2023). This may be explained by temperature limitations, as *S. woodiana* seems not to be able to successfully reproduce at low water temperatures (Bespalaya et al. 2018; Spyra et al. 2016; Urbańska et al. 2019). However, with climate change, condition of such habitats could change, facilitating an invasion by *S. woodiana*. With increasing water temperatures as a result of the ongoing climate change, even water systems that do not currently appear to be suitable for *S. woodiana* recruitment could be successfully invaded in the future (Urbańska et al. 2021). SDMs, such as those presented in Chapter 5, could be used to assess the risk of invasion by this species under the expected climate scenarios. However, such models are usually based on environmental predictors and on observed data of species distribution (Jeschke et al. 2022), which are often missing for invasive species. Even for such prominent species like the dreissenids or *C. fluminea*, which have been widely discussed both in the scientific community and in politics, many studies have only reported single observations. Though, a systematic monitoring has not yet been established neither in Germany nor in Europe. Nevertheless, it is generally crucial to have any basic ecological data of the distribution of such non-native species, not least in order to understand their ecological niche and thus to predict a potential risk these non-natives could pose on native species if their habitats overlap.

6.2 Impacts on native biodiversity

When invading a new system with occurring native bivalves, non-native species can impair native bivalve fauna by several mechanisms (see Section 1.2). This has already been shown for some non-native bivalve species, which were found to have strong negative impacts on native bivalve populations (Sousa et al. 2014). However, so far, the focus in freshwater bivalve conservation was on effects of invasive bivalve species such as dreissenids and corbiculids, which are already well characterized (e.g., Ferreira-Rodríguez et al. 2018; Karatayev and Burlakova 2022; Strayer and Malcom 2014; Strayer and Smith 1996), while effects of other invaders such as *S. woodiana* or invasive crayfish on native unionids are less studied. However, since at least the arrival of *S. woodiana* is a more recent issue, information on its distribution or interaction with native fauna was still lacking. Nevertheless, it is essential to obtain long-term data series on the distribution of such invaders and on their interactions with native and other invasive fauna in order to assess the long-term effects on habitats and native species (Karatayev and Burlakova 2022). As one of the most prominent examples of a long-term study from the Hudson River in New York shows, significant reductions in turbidity have been observed due to high filtration activities of the invasive *D. polymorpha*, in parallel with reported declines in native species (Strayer et al. 2011; Strayer et al. 2014; Strayer et al. 2019a; Strayer and Malcom 2014; Strayer and Smith 1996; Strayer et al. 2019b).

As shown in Chapter 3 of this study and by Geist et al. (2023), habitat overlap between invasive *S. woodiana* and native unionids is more likely to occur with more generalist species, such as *Anodonta anatina*. Competitive effects will therefore mainly affect these species. However, with climate change, other habitats may be opened up for *S. woodiana* invasion, which may also lead to stronger impacts on more specialized species such as *M. margaritifera*. However, as such specialized native species often already suffer from many other threats, at least from the changing habitat conditions, they are more vulnerable to locally extinction, as these species often only have a small geographical range (Groom et al. 2006). However, conservation management has mainly focused on such rare species. Based on the good knowledge of the distribution and ecological niche of endangered native bivalves, climate models could be useful tools to predict future risk of such invaders.

Spread and distribution of invasive crayfish in Europe are already well known and the mechanisms these invaders can affect native crayfish populations are well studied

(Holdich 2003). Signal crayfish, e.g., which are already widespread in many European water systems, are more aggressive than native noble crayfish leading to strong competitive effects (Söderbäck 1991). Additionally, since invasive crayfish are often immune to the crayfish plaque, but being a likely vector when invading new systems, total die-offs of native crayfish populations have been observed (Vorburger and Ribi 1999). However, effects on other aquatic fauna such as native unionids were only assumed, although not sufficiently studied. For native freshwater pearl mussels, predation by invasive signal crayfish has long been suspected (Schmidt and Vandré 2012). Sousa et al. (2019) have shown the first evidence of signal crayfish predation on freshwater pearl mussels in systematic experiments. Even for other bivalve species, predation by invasive crayfish has been shown (Machida and Akiyama 2013; Meira et al. 2019). However, some authors assumed that predation susceptibility is based on shell size or on shell thickness (Meira et al. 2019; Sousa et al. 2019). As the results of Chapter 4 show, even larger shells can be damaged by crayfish and thicker shells do not preserve from predation. The painter's mussel (*Unio pictorum*), which has the thickest shells of all mussel species used in the experiment, was one of the most affected species. It can be suggested that predation susceptibility can be better explained by the shells shape, since shells of *U. pictorum* are more elongated, which seems to facilitate the crayfish to attack the shells with its mandibles. With predation being one of the four main topics in freshwater bivalve conservation, further research is needed to better understand factors affecting mussel autecology (Aldridge et al. 2023). However, predation by other fauna, such as mammals, is known to be a major threat to native freshwater bivalves, leading to severe population declines and changes in species composition (Diggins and Stewart 2000). And although the experimental results of Chapter 4 could not be confirmed in the field, mainly due to the lack of overlap between signal crayfish and native bivalves, predation by invasive crayfish should be considered as an additional threat in future conservation management of native unionids

However, results of Chapter 4 also suggest that pre-existing signal crayfish colonization may promote subsequent *S. woodiana* colonization. Similar effects have already been shown for habitats invaded by *D. polymorpha*, which are often subsequently invaded by other Ponto-Caspian species such *D. r. bugensis* (Haltiner et al. 2022; Hetherington et al. 2019), potentially resulting in invasional meltdown (Simberloff and Von Holle 1999). Even other invertebrate species have been shown to be involved in such invasional meltdown events, as evidenced by occurrences in the Danube (Beggel et al. 2016; Brandner et al. 2012), Great Britain (Gallardo et al. 2015) or the North American Great Lakes

(Ricciardi 2001). However, impacts to native bivalves have been shown for both studied invasive species (see Chapters 3 & 4), which suggests that it is highly problematic for native unionids if both invasive species invade the same freshwater system.

6.3 Management recommendations

Based on the results of this thesis, both invaders, *S. woodiana* and *P. leniusculus*, which have previously received little attention in their interactions with native bivalves, need to be considered important players in freshwater ecosystems. These species have unique distribution and population trends, as well as impacts on the ecosystem that must be taken into account (see Chapters 3 & 4). While more was known about the impact of *S. woodiana* on native aquatic fauna (e.g., Benedict and Geist 2021; Donrovich et al. 2017; Douda and Čadková 2018; Douda et al. 2017b; Douda et al. 2012; Labecka and Domagala 2018; Reichard et al. 2012; Taskinen et al. 2021; Urbańska et al. 2021), there was still a lack of understanding of its recent distribution. In contrast, *P. leniusculus* was already known to be widespread in European freshwaters, however, its impact on freshwater bivalves was little understood.

Additionally, as shown in Chapter 5, recent conservation management of endangered bivalve species is inefficient. Gap analysis for both highly endangered unionids species, *M. margaritifera* and *U. crassus*, respectively, revealed that suitable habitats of both species are not sufficiently covered by protected areas. However, protected areas are usually less affected by human activities and thus less prone to be invaded by non-native species (Foxcroft et al. 2011; Lonsdale 1999; Pysek et al. 2003). In addition, undisturbed native bivalve communities have a higher biotic resistance to invasion by non-native species, as this was shown in a long-term study of the undisturbed Szeszupa River in Poland, where low densities and a stable abundance and distribution have been reported over the last 35 years (Ożgo et al. 2021). To keep protected areas as strategic refugia for native species (Gallardo et al. 2017), focus of conservation management should be based on optimization and prevention of such areas. Implementing tools for conservation prioritization could therefore help to improve conservation planning (Moilanen et al. 2008; Moilanen et al. 2009). Given that conservation of intact habitats should be preferred over restoration (Geist 2015), actual protection areas should be adjusted and their total area should be increased to offer adequate protection for those endangered native species. Additionally, as the results have shown, it is necessary to bring conservation and management of invasive species together in order to effectively protect and manage freshwater ecosystems.

Given the already widespread distribution of *S. woodiana*, which has also been shown to have significant competitive effects on native mussel populations, and the fact that current protected areas do not fully match the suitable habitats of endangered native mussels, this problem is likely to be further exacerbated by climate change. As shown in Chapter 3, the methodology used is well suited to providing important insights into the distribution and ecology of non-native species. However, such results should not only be used for individual scientific studies, but using the methodology described in Chapter 3, these results could be combined with other results to provide a complete picture of this invasive species. Even though the results of Chapter 3 already provide a comprehensive picture of the remarkable distribution of *S. woodiana* in Bavaria and at the same time indicate some potential vectors, such tools could also be highly useful for the whole of Europe. However, given the broad ecological niche identified, such systematic monitoring should also include a wide range of water systems, as this was done for Bavaria (Chapter 3), which also requires more effort. Approaches such as the ecological niche models presented in Chapter 5 are powerful tools that have already been used in conservation management to show relationships between, for example, ecological niches and conservation status for highly threatened species. However, the application of such models to invasive species is currently hampered by a lack of available information on ecology or actual distribution.

This all highlights the need for further scientific research and state-level monitoring to better understand and manage such invasive species. From a scientific perspective, there is a need for a more systematic collection of occurrences of non-native species such as *S. woodiana*. This should include not only presence or absence data, but also verification of the species' distribution, potential overlaps with native species, and possible overlaps with protected areas. Frequently, non-native species spread rapidly across space and are often characterized by boom-bust dynamics (Cerwenka et al. 2014; Strayer et al. 2017), causing different effects at different times and locations. These effects may include the sudden discharge of large amounts of nutrients from the decomposing soft tissues of these populations after a mass mortality event (McDowell and Sousa 2019). Therefore, such information will be crucial for understanding the extent of the problem and for developing effective management strategies. It is necessary to routinely track invasive species (Pergl et al. 2020) to understand their habitats, their ecosystem functions, and, not least, their impacts on native species (Geist 2011). Conducting studies on non-native species over a prolonged period is crucial for identifying the species, times, and locations that require management interventions the most (Pergl et al. 2020). When combined with control, mitigation, or restoration measures, these long-term studies can enhance the evaluation of management actions, leading to more evidence-based decision-making in management of invasive species, which can help to eliminate ineffective actions and to accelerate the development of effective approaches (Geist and Hawkins 2016).

Ultimately, it is important to note that addressing the issue of invasive species in conservation management goes beyond the realm of science and also requires the provision of funding and political decision-making, where the focus has traditionally been on native species. In contrast to protected native species, for which at least regular state-wide monitoring is carried out regularly (e.g., Stoeckl et al. 2020), providing sufficient data on occurrence (see Chapter 5) and ecological niches, such systematic monitoring is still lacking for non-native species. Non-native species are often only noticed when they are already established over large areas. In addition, the impact on native biodiversity is often recognized only after a very long delay (Crooks 2005), and early management is not possible due to the lack of such knowledge. The creation of a common database, including invasive species monitoring results, and institutions such as the Bavarian Mussel Coordination Office that is mainly focusing on native unionids, but could also extent their activities on invasive species, could greatly improve our understanding and management of invasive species. However, such decisions are depending on the available funding and political will.

6.4 Outlook

This thesis has highlighted the actual and future challenges in freshwater bivalve conservation in Europe dealing with invading non-native species as an important threat for native freshwater bivalve fauna. The results presented in the previous chapters provide crucial knowledge on effects and interactions of invasive aquatic species and native unionid bivalves. It was shown that, on the one hand, *S. woodiana* is already widespread and thus has a large overlap with native freshwater bivalves, which was unexpected. On the other hand, predation on native bivalves by already widespread invasive crayfish has been demonstrated, and even a learning effect of the crayfish using bivalves as a food source has been observed.

However, the approach presented in this thesis should be extended and applied to other invasive species, as the used methods were effective in understanding the impact of the two invasive species tested. However, the results presented are static and can only be seen as a snapshot in time. In the face of ongoing global change, future studies should focus on such effects in context of increasing temperatures, as this could at least open up new habitats for an invasion by non-native species such as *S. woodiana.* In addition, experiments with invasive crayfish, such as those presented in Chapter 4, should be extended to other invasive crayfish species that are also already common in European freshwaters, and to interactions with other native species such as macroinvertebrates, to also investigate predatory effects on other native fauna beyond the well-studied disease transmission.

Approaches such as the ecological niche models presented in Chapter 5 exemplarily for two endangered unionid species could be a powerful tool for estimating the dispersal potential of a newly invaded non-native species such as *S. woodiana*. Systematic monitoring, as shown for *S. woodiana* in Chapter 3, should be used to define the ecological niches of such species and to provide input variables on occurrences for the construction of ecological niche models of invasive species as a tool for invasion management in the future. In addition, such models can even be merged with spatially modelled habitats of threatened species to predict potential future habitat overlap and thus threats for native species, facilitating conservation management. However, as shown for native unionids, most current conservation areas are not well adapted to fit the needs of native species and therefore provide little protective effects or high resistance to invasions. Protected areas need to be expanded or re-aligned also considering non-native species. Thus, conservation efforts need to be focused on both conservation of native and management of invasive species. Furthermore, funding is required to support the research and monitoring necessary to better understand and manage the impacts of invasive species on freshwater ecosystems.

Based on all of the above, it is clear that the current management of conservation and of invasive species is inadequate. To address the conservation gaps identified in the previous chapters, it is necessary, firstly, to implement better monitoring also including invasive species, secondly, to assess functional interactions between invasive and native species using experimental set-ups, and thirdly, to combine the management of invasive species with the conservation management of native species.

Publication List

The following papers were included in this thesis*:

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Dobler AH, Geist J (2022) Impacts of native and invasive crayfish on three native and one invasive freshwater mussel species. *Freshwater Biology* 67(2): 389-403.

Dobler AH, Hoos P, Geist J (2022) Distribution and potential impacts of non-native Chinese pond mussels *Sinanodonta woodiana* (Lea, 1834) in Bavaria, Germany. *Biological Invasions* 24: 1689-1706.

*The candidate's contribution to each main chapter is presented below the respective abstract.

Papers with shared authorship or co-authorship (not included in this thesis):

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Appendix

Table S1 Sizes of the four different mussel species used for both exposure experiments with the potential predators *A. astacus* and with *P. leniusculus* († *indicates non-native species to Europe)*

		A. astacus		*P. leniusculus*†	
mussel species	n	Experiment part 1	Experiment part 2	Experiment part 1	Experiment part 2
A. anatina	32	7.84 cm ± 0.59 SD	8.16 cm ± 0.55 SD	8.59 cm ± 0.67 SD	8.34 cm ± 0.55 SD
A. cygnea	32	8.09 cm ± 0.43 SD	7.99 cm ± 0.75 SD	8.63 cm ± 0.35 SD	9.14 cm ± 0.68 SD
U. pictorum	32	8.54 cm ± 0.46 SD	8.26 cm ± 0.70 SD	8.75 cm ± 0.86 SD	9.04 cm ± 0.42 SD
S. woodiana†	32	8.84 cm ± 0.59 SD	7.98 cm ± 0.58 SD	9.22 cm ± 0.65 SD	8.74 cm ± 0.58 SD

Table S2 Mean number of predation marks of each mussel species resulting from the two experimental parts with *A. astacus* and *P. leniusculus*.

		A. astacus		*P. leniusculus*	
Mussel species	n	Experiment part 1	Experiment part 2	Experiment part 1	Experiment part 2
A. anatina	32	6.0 ± 6.8 SD	14.8 ± 15.0 SD	11.9 ± 10.6 SD	15.3 ± 20.3 SD
A. cygnea	32	4.5 ± 12.7 SD	5.5 ± 7.0 SD	29.1 ± 26.5 SD	25.0 ± 23.6 SD
U. pictorum	32	2.4 ± 5.0 SD	18.4 ± 10.6 SD	22.6 ± 11.0 SD	26.0 ± 14.3 SD
S. woodiana	32	0.0 ± 0.0 SD	4.6 ± 7.2 SD	8.6 ± 16.0 SD	5.1 ± 6.5 SD