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Cocktail of invaders in European inland waters – ecological characteristics, interactions and consequences

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**Doctoral thesis by
Sara Roje**

Czech Republic, Vodňany, 2021

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1. Biological invasions in freshwater ecosystems

Freshwater ecosystems have unique biodiversity compared to marine and terrestrial ecosystems (Dudgeon et al., 2006). They occupy less than 1% of the Earth's surface but support approximately one-tenth of the world's species and one-third of all vertebrates (Strayer and Dudgeon, 2010). These species include around 17,800 fishes (Fricke et al., 2020) and almost 700 crayfish (Crandall and De Grave, 2017). Moreover, they provide a wide range of valuable services to human populations. The increased demand for freshwater resources had led to a crisis from both a human and a biodiversity standpoint (Vörösmarty et al., 2010). While the conservation status and distribution of freshwater taxa are less known in comparison to terrestrial species (Darwall et al., 2011). There is growing evidence that freshwater taxa are at greater risk of extinction than those in terrestrial or marine ecosystems (Sala et al., 2000; Clausnitzer et al., 2009; Cumberlidge et al., 2009) making freshwater conservation a priority concern for the future.

Non-native species, some of which become invasive after establishment, are animals that are intentionally or accidentally introduced into areas outside of their natural range for nutritional needs and health control or for commercial, recreational and biological purposes. The increase in international trade, travel, and transport – particularly in the second half of the 20th century – led to many species being intentionally or unintentionally introduced outside of their native ranges and the number of species being introduced to new regions is still rising (Pyšek et al., 2010; Hanafiah et al., 2013; Seebens et al., 2017, 2021). As a consequence, biological invasions are currently a significant threat to freshwater biodiversity and ecosystem function worldwide (Strayer and Dudgeon, 2010; Catford et al., 2012; Moorhouse and MacDonald, 2015; Tricarico et al., 2016). They also have overwhelming economic costs (Diagne et al., 2021). Currently, the estimated annual economic impact of alien species in Europe is between 12 and 20 billion euros (Kettunen et al., 2008; Scalera, 2010) and this figure could be even higher as the potential economic and environmental effects for almost 90% of the alien species found on the continent are unknown (Vilà et al., 2010).

Many European inland waters (hereafter, EIW), in particular large rivers that serve as dispersal pathways (such as Loire, Rhine, Elbe, Danube, or Volga), are already colonized by many high-impact aquatic invasive species (hereafter, AIS) and these species have substantially altered the native communities. In these localities, AIS often dominate the benthic biomass which significantly influences the energy flow and forms alternative trophic networks (Leuven et al., 2009; Godard et al., 2012; Rewicz et al., 2014). Many shipping channels connect large European rivers making it easy for AIS to disperse through the network of navigation channels to other major rivers and their catchments. They can also spread by natural migration from places where they have been introduced. Further invasions by subsequent species may then alter the interactions between alien and native species leading to alien-only ecosystems, where aliens interact or co-exist with each other (Gherardi et al., 2009). When this occurs, multiple high-impact invaders can simultaneously influence the invaded ecosystems and communities, or one invasive can control another, so impacts cannot increase. For this reason, the relationships between established non-native species, remaining native species, and the new invaders should be considered and evaluated in conjunction (Kumschick et al., 2015). Interactions between natives and aliens, as well as between different AIS, may also vary across physical gradients that can even reverse the dominance patterns (Chucholl et al., 2008; Kestrup and Ricciardi, 2009). The vulnerability of particular EIW to biological invasions is also different. It is affected by many other factors such as negative alterations of the aquatic environment (pollution, habitat destruction, droughts, temperature fluctuations, etc.), which usually support the establishment, spread and success of AIS (Strayer, 2010; Catford et al., 2012). Such intricate problems can only be solved by using complex insight and well-defined approaches specifically adapted for each scenario (Kumschick et al., 2015).

Finally, aquatic ecosystems face huge risks from invasive species because of the ever-present threats to biodiversity and the increased human demand for water resources. There is a general consensus that some alien species will continue to spread and be significant drivers to the degradation of aquatic ecosystems and biodiversity loss (Sala et al., 2000; Gherardi et al., 2009).

1.1. Pathways of invaders in Europe

Freshwater inland waterways have a crucial role in transporting merchandise through Europe, covering more than 37,000 km, these waterways connect hundreds of cities and industrial regions. The European network of inland waterways connects catchments of southern European seas (e.g., Mediterranean, Black, Azov, and Caspian) to northern European seas (e.g., North, Wadden, Baltic, and the White Sea). This network consists of navigable rivers and a large number of constructed canals. The vast majority of invaders in European freshwaters originate from North America and the Ponto-Caspian region, others come from South America, Africa, Oceania - Australia, Asia, and the rest of AIS are intracontinental European species outside of their natural range (Nunes et al., 2015). Constructed inland canals have connected previously isolated waterbodies and enabled introductions, translocations and migrations of a high number of AIS within Europe (Bij de Vaate et al., 2002; Galil et al., 2007; Panov et al., 2009). In Europe, four main corridors are recognized: northern, central, southern and western (Bij de Vaate et al., 2002; Panov et al., 2009). Aside from intentional introductions, AIS can spread due to transportation in ships via ballast water, aquaculture related introductions, farming of AIS, ornamental or aquarium trade, escape/release, commercial or recreational fishing, organic pollution, river engineering, hydropower development, tourism, spread by aquatic birds and other animals and biological research (Copp et al., 2010; Patoka et al., 2016). The introduction of AIS is mostly detrimental for native species, causing changes in the food web, behavior and population. However, not all introduced alien species have been recognized as causing harm everywhere, as they could serve as cultural icons in different areas of the world. Some of them even become keystone species protected under law.

Human impact due to economy and demography reflects on species and habitats in various ways by increasing propagule pressure, pathways of introduction, eutrophication and the intensity of anthropogenic disturbance all of which influence the outcome of invasions (Perdikaris et al., 2012; Patoka et al., 2016; Gebauer et al., 2018). Therefore, alien species richness often positively correlates with human density and activity (McKinney, 2001; Stohlgren et al., 2006). These variables have been suggested to be more important than environmental conditions or climate (Pyšek et al., 2010). However, even regions with low human impact are not resistant to invasion (Deutschewitz et al., 2003; Wu et al., 2010; Pyšek et al., 2013).

1.2. Influence of the environmental and ecological factors

Success or failure of new species introductions depends on the biological attributes of the invaders and the recipient habitat characteristics, including both biotic and abiotic factors (Brown, 1989; Moyle and Light, 1996; Sakai et al., 2001).

Biotic components include all living organisms (autotrophs and heterotrophs – plants, animals, fungi, bacteria) which directly or indirectly affect organisms in the environment by their mutual interactions, the waste remains, parasitism, disease and predation. The main biotic factors influencing an invasive species in a new habitat are native species richness or diversity, vacant niches, absence of enemies and the competitive abilities of resident species (Mack et al., 2000). Other important biotic factors affecting invasive species are the sizes of predators and prey (Thorp et al., 2018) and the combined effects of multiple predators (Wasserman et al., 2016).

The diversity of both native and invasive species is dependent on the ecosystem's physical factors, which can either promote invasibility or inhibit it. Components such as water, light, wind, soil, humidity, minerals and gases affect the organism's ability to survive, reproduce and exist in the environment. Physical factors include the region's climate, nutrient levels and disturbance regime which are defined by the frequency of episodic events (Moyle and Light, 1996). Changes in climate could have the most significant impact on the abundance of invasive species. Abiotic factors play a role in defining the vulnerability of a given habitat to AIS. The most important abiotic factors influencing predator-prey interactions are temperature and habitat structure (Vucic-Pestic et al., 2011; Alexander et al., 2012).

1.3. Impact of invaders

Bioinvasions of freshwater ecosystems have many known and potential impacts on community structure and ecosystem function (Havel et al., 2015). Invasive species have a large and diverse range of impacts in Europe. This diversity of impacts is mainly driven by the diversity of species and makes it difficult to make generalized statements about types of impacts (Pyšek and Richardson, 2010; Keller et al., 2011). The introduction of alien species is broadly recognized to be one of the main threats to biodiversity and causes of animal extinctions (MEA, 2005). AIS can have dramatic impacts on ecosystems (Bellard and Jeschke, 2016; Vimercati et al., 2020), as once they are established, they can be near impossible to remove and can be extremely expensive to control (Pimentel et al., 2005). The impact of serious invaders is rarely restricted to a single ecosystem service and freshwater invaders exhibit the widest range of differential impacts (Vilà et al., 2010). The trend to farm alien species has caused irreversible ecological impacts (Naylor et al., 2001; Gozlan, 2008; Keller et al., 2011). Invaders affect native species via competition, modification of habitats, hybridization, concomitant disease or parasite introduction and predation. Competition of AIS for food or space with native species disrupts the food web. AIS can cause native population decline because they usually have robust predatory habits; this is also correlated with other drivers of environmental change, such as habitat modifications (Rewicz et al., 2014). Invaders are well known to restructure freshwater food webs (Zanden et al., 1999). AIS are causing quantitative changes in community structure by becoming the dominant species. Generally, AIS are driving changes in the energetic budget of the invaded ecosystem by removing keystone species and primary producers. Furthermore, they often transport diseases that can be dangerous and lethal. Some species are introduced for biocontrol to reduce population sizes of other species (Fuller et al., 1999). Sometimes AIS can encounter and mate with closely related species, mixing the genetic code and creating hybrid zones (Hovick and Whitney, 2014). Although, hybridization does not necessarily result in the loss of species diversity. Some invaders are also able to cope and survive in more contaminated areas with higher levels of pollution than native species (Karatayev et al., 2009), showing more aggressiveness for survivability. Alien invaders can also directly impact human health in various ways including injuries, allergies, new contaminants (bacteria, toxins) and via their role as intermediate hosts to human parasites. They also have indirect impacts such as the use of chemicals to control AIS and making changes to ecosystems, which in turn, makes the invaded area less suitable for recreational human use and can damage cultivation/aquaculture affecting human well-being in developing countries (Souty-Grosset et al., 2018). In Europe, the number of AIS increased by 76% between 1970 and 2007 (Figure 1) (Butchart et al., 2010), applying exponential pressure on native ecosystems, particularly in inland waters. Ongoing invasions over the last two decades, by Ponto-Caspian crustaceans, molluscs, fish and other organisms, have been reported from the middle and upper sections of the Danube River, the Rhine River and other parts of the world (Strayer and Dudgeon, 2010; Borcherding et al., 2011; Kornis et al., 2012; Beggel et al., 2016).

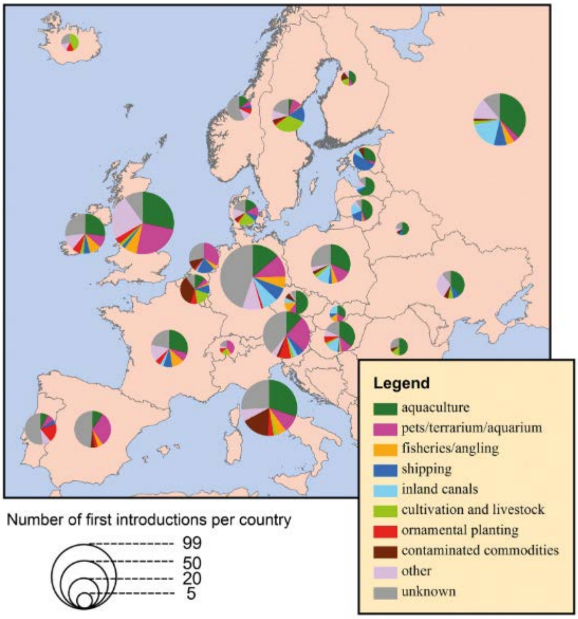

Figure 1. *The proportion of initial introductions of freshwater alien species in Europe through different pathways of introduction per recipient country. The size of the pie chart represents the number of species that were initially introduced by a different introductory pathway in a specific country (From Nunes et al., 2015).*

1.4. Introduction of AIS

Bioinvasion is the often rapid expansion of a species into regions where it had not previously existed, always as a result of human interference (voluntary or accidental). In the process of invasion different barriers must be overcome to move from one stage to the next: Blackburn et al. (2011) proposed a unified framework that combines previous stage-based and barrier models providing terminology and categorization for populations at different points in the invasion process (Figure 2). Human actions are the primary means of the intentional or unintentional introduction of invasive species. Secondary invasions are invader-facilitated invasions, where the invasion success of one alien species is facilitated by another alien species (O'Loughlin and Green, 2017). An invasion process can be divided into three stages: arrival, establishment and integration (Vermeij, 1996). The characteristics of adapting to the new area depend on the species itself and its dispersal capacity, environmental tolerance, feeding behavior, life-history traits and reproductive mode. The influence that invasive alien species have on native communities is often reflected in the changes induced in the food web structure and trophic hierarchy, which affect the flows of energy, matter and nutrients through the ecosystem (Genovesi et al., 2017). Not all alien species are detrimental to their new environment, and often they themselves have difficulties with growth and reproduction. Simberloff and Von Holle (1991) proposed that once established, some invaders may alter habitat conditions in favor of other invaders, thereby creating a positive feedback system that accelerates the accumulation of invading species. Some species spread and reproduce excessively, feeding on native species or out-competing them for habitat and resources, they may also harbor parasites and diseases that are lethal to native wildlife or dangerous to human health.

Some of the species were intentionally introduced into inland waters for economic purposes, but some were escapees from aquaculture facilities. AIS also causes damage that costs billion euros to the European economy every year. Major groups are considered essential for generating income through aquaculture, commercial and sport fisheries (e.g., salmonids, cyprinids and more recently, sturgeons). However, introductions and intrastate translocations have occasionally resulted in intraspecific competition, genetic introgression or even extirpation of self-recruiting species (Perdikaris et al., 2012). Indeed, AIS are recognized as one of the main drivers of species extinction and global biodiversity loss (https://www.cbd.int/invasive/).

Information on introduction pathways is currently scattered across many databases that often use different categorizations to describe similar pathways. Pathway patterns derived from the combined and individual data sets show that the intentional pathways "Escape" (aquaculture, pet, ornamental, etc.) and "Release" (biocontrol, fisheries, conservation, etc.) are most important for plants and vertebrates, while for invertebrates, algae, fungi, and micro-organisms unintentional (parasites, vessels, canals, natural dispersal, etc.) transport prevail (Saul et al., 2017). Although many vectors are responsible for species introductions, the rising volume of air and ship transport has been identified as the primary driver of invasions (Lodge, 2006). The role of canals connecting different watersheds (also known as invasion corridors) has significantly increased the recent spread of alien species in EIW (Rakauskas et al., 2016).

Many aquatic invaders such as amphipods, crayfish, fish and bivalves were imported for human necessity and deliberately released into streams, rivers, and water bodies. Other species were introduced via the pet trade, which often contain "hitch-hiking" species and pathogens in the water they are transported in (Rixon et al., 2005; Chucholl, 2013; Mrugała et al., 2014; Patoka et al., 2015).

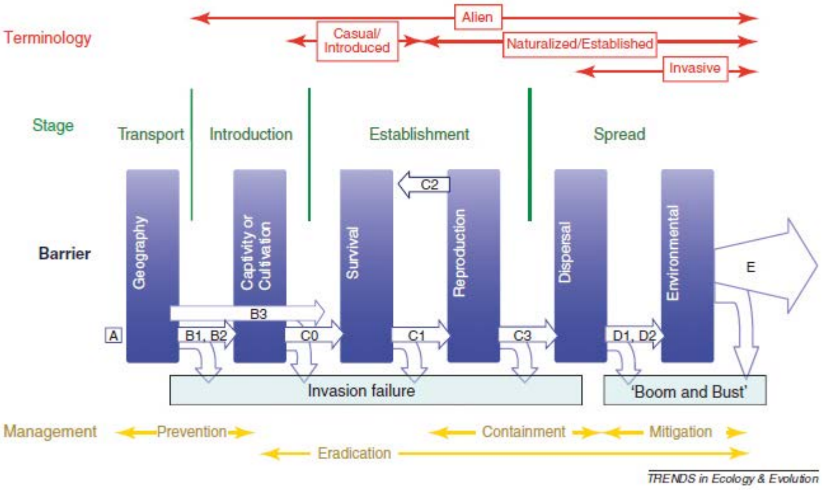

Figure 2. *A proposed unified framework for biological invasions that recognizes that the invasion process can be divided into a series of stages and that in each stage there are barriers that need to be overcome for a species or a population to pass on the next stage. Different species are referred by different terms in the terminology depending on where in the invasion process they have reached and different management interventions apply at the different stages. Different parts of this framework emphasize views of invasion that focus on individual, population, process or species. The unfilled block arrows describe the movement of species along the invasion framework with respect to the barriers, and the alphanumeric codes associated with the arrows relate to the categorization of species with the respect to the invasion pathway: A/Not transported beyond limits of native range, B1/Individuals transported beyond limits of native range, and in captivity or quarantine, B2/Individuals transported beyond limits of native range and in cultivation, B3/Individuals transported beyond limits of native range, and directly released in novel environment, C0/Individual released into the wild in location where introduced with no reproduction and capability for surviving for a significant period, C1/Individuals surviving in the wild in location where introduced but no reproducing, C2/Individuals surviving in the wild in location where introduced with reproduction occurring but population not being self-sustaining, C3/Individuals surviving in the wild in location where introduced with reproduction occurring and self-sustaining population, D1/Self-sustaining population in the wild, with individuals surviving a significant distance from the original point of the introduction, D2/Self-sustaining population in the wild, with individuals surviving and reproducing a significant distance from the original point of the introduction, E/ Fully invasive species, with individuals dispersing, surviving and reproducing at multiple sites across a greater or lesser spectrum of habitats and extent of occurrence (From Blackburn et al., 2011).*

1.5. Spread of diseases via invaders

Invasive species may introduce parasites and pathogens to new areas, bringing them into contact with a large pool of potential new hosts, thereby threatening native species (Krueger and May, 1991; Keller et al., 2011). For example, the crayfish plague pathogen *Aphanomyces astaci* Schikora, introduced with the North American crayfish in the mid-19th century, has devastated native European crayfish populations (Holdich and Reeve, 1991). Alien crayfish species are carriers of crayfish plague and create new transmission pathways for the zoospores of the disease in Europe and elsewhere. Additionally, native parasites may also play a significant role in the invasion process by benefiting the intruder via the mechanism termed

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the ‘enemy release hypothesis’ (Sax and Brown, 2000; Mitchell and Power, 2003), whereby lower infectivity in invaders than in the native hosts provides a competitive advantage for the aliens. Other examples are rainbow trout as hosts for the salmon parasite *Gyrodactylus salaris* Malmberg, 1957 and the parasite fauna of the invasive round goby being transmitted to native species (Kvach and Skóra, 2007; Ondračková et al., 2010; Francová et al., 2011). Furthermore, toxic algae bloom caused by some alien phytoplankton (*Alexandrium* species, etc.) can affect some dreissenid species, as they are filter-feeders and have been shown to create new pathways for transferring contaminants (e.g., Hg, Cd, PCBs, botulism toxin) (Southward Hogan et al., 2007; Carrasco et al., 2008).

AIS can also cause health problems in humans such as allergies, poisoning and skin damage. For example, the Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards, 1853 in its native range is a host for the lung fluke parasite, causing disease of the lungs and other body parts, the North American crayfish can be a host for trematodes that are potential parasites to humans, the sharp shells of zebra mussels can inflict cuts, and the round goby can accumulate toxins such as heavy metals and cyanotoxins which can be transferred to human food (Keller et al., 2011).

1.6. Economic losses

AIS are numerous and widely distributed and budgets are limited as they have dramatic impacts on multiple sectors of the economy, such as agriculture, fisheries, and forestry (Holmes et al., 2009; Paini et al., 2016; Ali Ahmed et al., 2021). Biological invasions can result in both intended and unintended costs on society, with their damage often being dependent on how effectively they are controlled (Perrings, 2002; Ricciardi et al., 2017). Prevention and rapid response to new invasions are the most cost-effective means to avoid or mitigate the economic damage caused by AIS. AIS continue to incur high financial costs, with an estimated between 12 and 20 billion € per year in the E.U. (Kettunen et al., 2008). The sectors of agriculture, forestry, fisheries and healthcare are the main economic sectors where alien species lead to substantial costs.

Overall, over 14,000 alien species are listed in the European database DAISIE (Inventory of alien invasive species in Europe) with the economic impacts recorded for 13% and the ecological impacts for 11% (Vilà et al., 2010). “The project DAISIE”, run between 2005 and 2008, is regarded as the most significant database for alien species containing information on 12,122 invasive species in Europe with 2,440 experts investigating the threats of biological invasions across Europe. Two decades ago, The International Union for the Conservation of Nature (from now, IUCN) made a list of “100 of the world’s worst” invasive alien species in Europe, in order improve communication and awareness of invaders globally. Quantification of the economic impacts of invasive species have been made at several scales in Europe (Kettunen et al., 2008; Haubrock et al., 2021). DAISIE, among other tools as the EASIN database, is commonly used to retrieve data on alien invasive species. Still, there are huge, significant gaps in the current knowledge of economic costs due to AIS. Direct economic costs have resulted from the invasion of zebra mussels due to the maintenance and repair of powerplants, industrial facilities, navigation buoys and dock pilings in the case of harbors, boats, and shipping. The InvaCost database (full database and descriptive files; version 3 at https://doi.org/10.6084/m9.figshare.12668570) contains information on all retrievable scientific articles and cost damages which are summarized for five families of bivalves: Cyrenidae, Dreissenidae, Mytilidae, and Unionidae (Haubrock et al., 2021), and the collective worldwide cost was amounted to 63.6 billion US dollars between 1980–2020. According to currently unpublished data by Haubrock et al. (2021), economic costs of bivalves in freshwater ecosystems represent 99% of the total costs incurred in North America, with 13 entries from zebra mussels that invaded most of the waterways in central and western Europe well before mid-20th century. In turn, less than 1% of globally reported costs of invasive bivalves were estimated from Europe. The InvaCost database also reports costs on crustaceans and other aquatic species, from which fish Euroasian ruffe *Gymnocephalus cernua* Linnaeus, 1758 and the gastropod known as the golden apple snail *Pomacea canaliculata* Lamarck, 1819 were listed in the top 10 most costly aquatic invasive alien species (Cuthbert et al., 2021).

2. Examples of important aquatic invaders groups

Following scientific and grey literature and databases (e.g., journals, etc.), we summarize the amphipod, crayfish, fish, bivalve molluscs and other impactful important AIS marked as very invasive in Europe (Table 1; Table 2; Table 3; Table 4 and Table 5). Selected species are most frequently listed as important aliens, forbidden or conversely alien but economically significant.

2.1. Invasive amphipod species

Representatives of amphipod crustaceans are regularly identified as high-impact freshwater invaders (Devin et al., 2004; Cuthbert et al., 2020). They can be found in a broad spectrum of freshwater habitats and are widely distributed with a wide range of environmental tolerances (e.g., low oxygen). They are known for outcompeting other gammarid species through competition and predation. As they are predominantly omnivores, they reduce species richness and community diversity by feeding on organic debris derived from the surface environment (Väinölä et al., 2008).

Amphipods can carry alien parasites and could transfer them to native gammarids and local fish. Amphipods can be transported attached to aquatic birds such as migratory waterfowl, surviving long-distance flights to other waterbodies (Rachalewski et al., 2013a).

Benthic amphipods have been widely established in Europe and North America outside of their native ranges (Table 1), here not only do they affect the macroinvertebrates and fish communities (e.g., Kinzler and Maier, 2003; Kelly and Dick, 2005; Berezina, 2007), they also dominate the diets of the bigger predators, e.g., crayfish and fish (perch, eel, pike, etc.) (Kelleher et al., 2000; Neveu, 2001). Amphipods are almost impossible to remove from the invaded area once it has been colonized.

Table 1. *Examples of invasive amphipod species selected based on scientific literature and databases, their native and invasive ranges, main pathways of introduction, and references.*

TAXON	NATIVE RANGE	INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Chelicorophium curvispinum G.O. Sars, 1895 Caspian mud shrimp	Ponto-Caspian basin	Europe	Fish and mussel host, inland canals, shipping	(Dedju, 1967; Ricciardi and Rasmussen, 1999; Bij de Vaate et al., 2002; Hänfling et al., 2011; DAISIE, 2018; EASIN, 2018)
Dikerogammarus bispinosus Martynov, 1925	Ponto-Caspian basin	Austria	Inland canals, ballast water	(Hänfling et al., 2011; EASIN, 2018; CABI, 2021; FAO, 2021)
Dikerogammarus haemobaphes Eichwald, 1841 Demon shrimp	Ponto-Caspian basin	Baltic and the North Sea drainages	Inland canals, ballast water, hull fouling	(DAISIE, 2009; Hänfling et al., 2011; EASIN, 2018; CABI, 2021; FAO, 2021)
Dikerogammarus villosus Sowinsky, 1894 Killer shrimp	Ponto-Caspian basin	Baltic and the North Sea drainages, Europe	Inland canals, ballast water, hull fouling	(Pöckl et al., 2003; Füreder and Pöckl, 2007; Hänfling et al., 2011; DAISIE, 2018; EASIN, 2018)
Echinogammarus ischnus Stebbing, 1899	Ponto-Caspian basin	Baltic and the North Sea drainages	Inland canals, ballast water	(Jażdżewski, 1980; Bij de Vaate et al., 2002; Hänfling et al., 2011; CABI, 2021)
Echinogammarus trichiatus Martynov, 1932	Ponto-Caspian basin	Baltic and the North Sea drainages, Dnepr reservoirs	Inland canals, ballast water	(Bij de Vaate et al., 2002; Füreder and Pöckl, 2007; Hänfling et al., 2011; Rachalewski et al., 2013b)
Echinogammarus warpachowskyi G.O. Sars, 1894	Ponto-Caspian basin	Baltic drainage, reservoirs, lakes in Ukraine	Inland canals, ballast waters	(Bij de Vaate et al., 2002; Hänfling et al., 2011)
Gammarus lacustris G.O. Sars, 1863	Ponto–Caspian basin	Baltic and the North Sea drainages	Aquarium trade	(Hänfling et al., 2011; EASIN, 2018)
Gammarus tigrinus Sexton, 1939	East coast of North America	The British Isles, Baltic, and the North Sea drainages	Ballast water	(Grigorovich et al., 2000; USGS, 2005; Holdich and Pöckl, 2007; DAISIE, 2009; Hänfling et al., 2011)
Gmelinoides fasciatus Stebbing, 1899	Lake Baikal	Baltic drainage, lakes of North-western and Central Russia	Food for fish production	(Panov and Berezina, 2002; DAISIE, 2009; Hänfling et al., 2011)
Obesogammarus crassus G.O. Sars, 1894	Ponto-Caspian basin	Baltic drainage, lakes and reservoirs in Western Russia	Food for fish production, inland canals	(Jażdżewski, 1980; Bij de Vaate et al., 2002; DAISIE, 2009; Hänfling et al., 2011)
Pontogammarus robustoides G.O. Sars, 1894	Ponto-Caspian basin	Europe	Inland canals, shipping	(Hänfling et al., 2011; DAISIE, 2018; EASIN, 2018)

The target species of the present Ph.D. thesis is only one of the mentioned amphipods, the killer shrimp *Dikerogammarus villosus* Sowinsky, 1894.

Killer shrimp

The killer shrimp is listed as one of Europe's top 100 invaders (Nentwig et al., 2018). The introduction of killer shrimp in European freshwaters is, to date, recognized as a threat to the integrity of invaded ecosystems. This Ponto-Caspian gammarid spread after the opening of the Rhine-Main-Danube Canal and has been established in German rivers (Koester et al., 2018), including the Danube, Moselle, and Rhine (Bij de Vaate et al., 2002). Ponto Caspian species spread through the so-called „southern corridor" from the Danube River through human-made canals (Berezina and Ďuriš, 2008). Killer shrimp have now spread through the lentic and lotic environments of Italy (Casellato et al., 2006; Tricarico et al., 2010; Mancini et al., 2021), France (Devin et al., 2001), the Netherlands (Velde et al., 2002), Great Britain (MacNeil et al., 2010), Switzerland, as well as toward the Baltic region (Rewicz et al., 2017).

The invasive success of killer shrimp is attributable to several life history characteristics (Bruijs et al., 2001) which include a broad ecophysiological tolerance (Devin et al., 2004; Pöckl, 2007), rapid growth rate, high fecundity (Kley and Maier, 2006; Rolla et al., 2020), effective anti-predator strategies and strong competitive ability (Kobak et al., 2016).

The predatory behavior of killer shrimps is recognized as a crucial determinant of its ecological impact on other benthic invertebrates, including native amphipods (Dick and Platvoet, 2000; Kley and Maier, 2006). A significant decrease in the abundance of native gammarids was recorded in the presence of killer shrimp (Haas et al., 2002; Kley and Maier, 2006). In the laboratory, killer shrimp consume a wide range of freshwater macroinvertebrates (Rewicz et al., 2014), even in the presence of predatory fish which could be indicative to its success as an invasive species (Richter et al., 2018). This wide dietary range has also been seen in the field using stable isotope analyses (Riel et al., 2006; Hellmann et al., 2015; Mancini et al., 2021).

The stable isotope analyses suggest that killer shrimp can occupy the same trophic level as some predatory fish species and amphibians (Marguillier, 1998; Warren et al., 2021). Killer shrimp are also vectors of the microsporidian *Cucumispora dikerogammari* Ovcharenko and Vita (parasitic fungal species) (Ovcharenko et al., 2010) that can spread to other native gammarids as well as reduce their longevity (Bacela-Spychalska et al., 2012).

The study by Velde et al. (2000) refers to killer shrimp as an excellent example of successful invaders, characterized by their short life span and generation time, rapid growth rate with early sexual maturity, high fecundity, ability to reproduce asexually, gregarious behavior, ability to repopulate depopulated habitats, size compared to their relatives and omnivorous diet. Due to these characteristics, killer shrimp poses a threat to native macroinvertebrates taxa (mainly to other amphipods) in freshwater ecosystems throughout Europe.

Invasive crayfish species

According to the research of Baumart et al. (2015), some species adapt strategies that diminish competitive pressures, enabling them to co-exist in the same environment. Species with similar ecological traits (i.e., prey items, shelter preference, and predators) usually compete for resources in natural environments (Louhi et al., 2014), but adaptive crayfish invaders can easily shift their tropic niches in response to a stronger invader (Veselý et al., 2021).

Many crayfish species have been introduced worldwide for human food, forage fish, bait or via the pet trade (Hobbs et al., 1989; Patoka et al., 2014). Several crayfish species have become highly successful invaders in freshwater habitats worldwide (Strayer, 2010; Lodge et al., 2012), causing substantial ecological impacts (Twardochleb et al., 2013) especially in habitats where native crayfish are naturally lacking (Rodríguez et al., 2005).

Crayfish are adaptable omnivores that feed on algae, macrophytes, benthic invertebrates, fishes and fish eggs, with reports of terrestrial feeding and even one example of an individual feeding on a human corpse (Pascali et al., 2020). Alien crayfish species often reach high densities (Bobeldyk and Lamberti, 2008). They have diverse ecological impacts on several parts of the food web (Hobbs et al., 1989; Lodge et al., 2000; Gherardi et al., 2009). They can move great distances either naturally or by human-mediated transfers (Panov et al., 2004). Crayfish that were transported by being attached to the wheels of a vehicle over a long distance have also been reported (Banha et al., 2014). Recently-hatched crayfish can also be transported by attaching to moving animals like ducks for distances of up to 6.1 km, far enough for them to be transported to another aquatic system (Águas et al., 2014; Anastácio et al., 2014). Besides the aforementioned transfer pathways, some crayfish species have been translocated for economic reasons such as aquaculture, enhancing fish production, for the pet and restaurant trades, and some have subsequently become established in the wild (Vodovsky et al., 2017; Vogt, 2018).

Numerous non-indigenous crayfish have now been reported in many European countries (Kouba et al., 2014; Patoka et al., 2016; Weiperth et al., 2019). The notorious history of the non-indigenous crayfish invasion in Europe started in 1890 with the introduction of the **spiny-cheek crayfish** *Faxonius limosus* Rafinesque, 1817 when approximately 100 specimens from the USA were released into a 0.1 ha fish farm pond northeast of Berlin, Germany. Many introductions followed throughout Europe, as this species was expected to replace the indigenous noble crayfish *Astacus astacus* Linnaeus, 1758 whose populations decreased due to the oomycete *Aphanomyces astaci* Schikora pathogen that causes the crayfish plague. Crayfish plague is the leading cause of the decline of susceptible native crayfish in Europe because the native crayfish species show very high mortality rates when exposed to this pathogen (Holdich et al., 2009). Within the last few decades, the crayfish plague resurged as one of the major threats to indigenous crayfish in Europe, mostly due to the spiny-cheek crayfish.

The **signal crayfish** *Pacifastacus leniusculus* Dana, 1852 was introduced for aquaculture and has spread rapidly throughout Europe, causing mass mortalities of native crayfish (McNeill et al., 2010; Filipová et al., 2013). In 1970, about 2,000 individuals were introduced in Scandinavia and since then, indigenous crayfish species have been threatened by infection, direct competition and the negative impacts caused to the native ecosystem and community – as signal crayfish altering trophic interactions, interference competition or indirectly through habitat modification (Snyder and Evans, 2006). Signal crayfish is one among the invasive crayfish that presents a real threat to the rest of native crayfish populations as they can occupy the same habitats in brooks and rivulets (Souty-Grosset et al., 2006).

Red swamp crayfish *Procambarus clarkii* Girard, 1852 is listed in the top 100 worst invasive species in Europe and it is widely distributed in western parts of Europe (Holdich et al., 2009; Kouba et al., 2014; Weiperth et al., 2019). The evidence says that even though the species is considered to be restricted to warmer waters, it can still flourish in colder climates and at higher altitudes (Chucholl, 2011). The red swamp crayfish is responsible for the most extensive range of impacts, such as crayfish plague dissemination, competition, predation on native species, habitat modifications, food web impairment, herbivory and macrophytes removal (Savini et al., 2010; Banha and Anastácio, 2011; Souty-Grosset et al., 2016). It increases its success rate by consuming terrestrial food sources when necessary and having high adaptability to extreme conditions including droughts and pollution (Souty-Grosset et al., 2018; Kouba et al., 2016; Haubrock et al., 2019).

One way or another, the introduction of non-native crayfish in Europe has caused a significant reduction in the population size and density of native crayfish while also damaging the entire ecosystem (Lodge et al., 2000).

Table 2. *Examples of invasive crayfish species selected based on scientific literature and databases, their native and invasive ranges, main pathways of introduction and references.*

TAXON	NATIVE RANGE	INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Faxonius immunis Hagen, 1870 Calico crayfish	North America	Europe	Escape from bait bucket, pet trade	(EASIN, 2018; CABI, 2021; FAO, 2021)
Faxonius juvenilis Hagen, 1870 Kentucky River crayfish	North America	Europe	Escape from aquaculture	(EASIN, 2018; CABI, 2021; FAO, 2021)
Faxonius limosus Rafinesque, 1817 Spiny-cheek crayfish	North America	Europe, Africa, USA	Escape from aquaculture and bait bucket, food for fish	(DAISIE, 2009; EASIN, 2018; CABI, 2021; FAO, 2021)
Faxonius virillis Hagen 1870 Virile crayfish	North America	Europe	Escape from aquaculture and bait bucket	(EASIN, 2018; CABI, 2021; FAO, 2021)
Pacifastacus leniusculus Dana, 1852 Signal crayfish	North America	Europe, Asia	Escape from aquaculture and bait bucket, food for fish production	(Gherardi et al., 2009; EASIN, 2018; CABI, 2021; FAO, 2021)
Procambarus acutus Bouvier, 1897 White River crayfish	North America	Europe	Escape from aquaculture and bait bucket, laboratory release	(EASIN, 2018; CABI, 2021; FAO, 2021)
Procambarus clarkii Girard, 1852 Red swamp crayfish	Mexico, USA	USA, Europe, Asia, Africa	Pet trade, escape from aquaculture, fisherman transport	(Gherardi et al., 2009; EASIN, 2018; CABI, 2021; FAO, 2021)
Procambarus virginalis Lyko, 2017 Marbled crayfish	(North America)	Europe, Madagascar, Japan	Escape from aquarists, pet trade	(EASIN, 2018; CABI, 2021; FAO, 2021)

In Europe, five non-native crayfish species are regulated by the EU Regulation 1143/2014 (E.U., 2016) on invasive alien species: spiny-cheek crayfish, signal crayfish, red swamp crayfish, calico crayfish *Faxonius immunis* Hagen, 1870 and marbled crayfish *Procambarus virginalis* Lyko, 2017 (Table 2). They are on the list of Invasive Alien Species of Union concern to constrain their further spread across Europe (E.U., 2016). The target species of the present Ph.D. thesis are two of the mentioned crayfish species, marbled crayfish and signal crayfish.

Marbled crayfish

Marbled crayfish establishment, presence and spreading is an excellent example of the successful introduction of an ornamental non-indigenous species. Due to numerous new records of this species along with its appearance in the German pet trade, the marbled crayfish is now very frequent in many parts of the world (Hossain et al., 2018; Vogt, 2018). The majority of marbled crayfish records are from Germany (Martin et al., 2010), following other countries such as Croatia (Cvitanić, 2017), Czech Republic (Patoka et al., 2016), Belgium (Scheers et al., 2021), Estonia (Ercoli et al., 2019), France (Grandjean et al., 2021), Hungary (Lőkkös et al., 2016), Italy (Vojkovská et al., 2014), Malta (Deidun et al., 2018), Romania (Pârvulescu et al., 2017), Slovakia (Janský and Mutkovič, 2010; Lipták et al., 2017) and Ukraine (Novitsky and Son, 2016) with the numbers growing nationally and continentally. The marbled crayfish has even been recorded in Madagascar and Japan (Kawai et al., 2009; Jones et al., 2009; Chucholl and Pfeiffer, 2010; Kouba et al., 2014). There are also records from the Netherland and Sweden, but the species is yet to establish viable populations in these countries (Bohman et al., 2013; Koese and Soes, 2021). This species consists entirely of females that reproduce parthenogenetically without the need of a male. For this reason, only one individual is needed to establish a viable population (Scholtz et al., 2003). It has impressive overwintering ability, being able to survive at ~2.5 C for three months under laboratory conditions (Veselý et al., 2015) as well as by established populations. It is estimated that in a controlled environment, the marbled crayfish can complete seven reproduction cycles during its 2 to 3 years lifespan, with a generation time of about 6–7 months (Vogt, 2010). The amount of juveniles increases with each cycle as larger females can produce more eggs (Vogt, 2011). The time period spent carrying the eggs differed in field surveys through Europe: in Romania during April and May (Pârvulescu et al., 2017), Germany from June to October (Chucholl and Pfeiffer, 2010), Croatia in June and September (Cvitanić, 2017) and in the Czech Republic and Ukraine in September (Patoka et al., 2016; Novitsky and Son, 2016). This suggests that reproduction may occur from spring until autumn, which is most probably related to water temperature (Vogt, 2011). Under laboratory conditions, the maximum number of juveniles in one clutch was 427 (Lipták et al., 2017) and 349 (Hossain et al., 2019), while a maximum of 647 eggs was recorded in the Slovak section of the Danube and 724 eggs in a single marbled crayfish clutch in Germany (Chucholl and Pfeiffer, 2010). So, it seems that the number of eggs could exceed 700 eggs per individual. Aside from its fast reproduction, marbled crayfish also utilizes a wide range of food sources and has a strong impact on the food web structure in the ecosystem (Lipták, 2019).

The marbled crayfish is also an important food source for predatory fish, meaning it affects multiple trophic levels (Lipták, 2019) and it has also been confirmed as a carrier of the crayfish plague (Tilmans et al., 2014). The marbled crayfish is regarded as relatively drought-resistant (Kouba et al., 2016) and can complete terminal phases of embryogenesis, including hatching, as well as post-embryogenic development, but only in highly humid conditions (Guo et al., 2019). The marbled crayfish is also able to cope with other invasive crayfish (Chucholl and Pfeiffer, 2010; Hossain et al., 2020), it has a wide ecological amplitude and can migrate over land (Chucholl et al., 2012).

Signal crayfish

The signal crayfish is native to north-western North America but was first introduced to Europe in Sweden in the 1960s (Maitland and Adams, 2001) to supplement stocks affected by the crayfish plague (Holdich and Rogers, 1999) and has subsequently been introduced to many countries both legally and illegally. Since this species was found out to be ecologically comparable to the noble crayfish whose populations were decimated by crayfish plague, signal crayfish were released into open waters and were observed to be thriving in streams as well as ponds. However, compared to the noble crayfish, they can survive at higher temperatures and are more tolerant to pollution (Kozák et al., 2015). However, their temperature tolerance is limited and this is linked to its inability to establish itself in some regions, e.g., Southern Iberia (Capinha and Anastácio, 2011). The signal crayfish is generally immune to the crayfish plague and can act as a carrier similarly to its North American relatives. Although, it can also succumb to the effects of the plague when subject to stress (Smith and Söderhäll, 1986; Alderman et al., 1990).

A study by Ercoli et al. (2021), who investigated the food usage of adult and juvenile signal crayfish, showed that there is no significant difference between the diets of adults and juveniles, both having the same potential food sources (other crayfish, detritus, macroinvertebrates, and periphyton). This research suggests that adults and juveniles exhibit seasonal feeding habits, which are probably due to ecological behavior rather than food resource availability. Both cause similar effects on macroinvertebrate communities in Italian stream ecosystems.

Signal crayfish have invaded 29 European territories, becoming the most widespread invasive alien species in Europe (Kouba et al., 2014). Their illegal introductions are constantly reported across Europe and they are noticeably spreading throughout Scandinavia (Skov et al., 2011).

Signal crayfish females can usually carry 100–400 eggs, but some large females can have over 500 eggs (Savolainen et al., 1997). Compared to noble crayfish, they mature earlier – males in 2 years and females in 3 years. Their higher fecundity, quicker maturation and their ability to inhibit cold upper stretches in a stream is a serious threat to native crayfish populations (Buřič et al., 2020a). Signal crayfish can also affect native biota via habitat modification due to their burrowing behavior (Dorn and Mittelbach, 1999), resulting in habitat degradation and bioturbation (Maitland and Adams, 2001; Turley et al., 2017). The ability to affect stream communities has also been confirmed as they have been shown to lower community richness of invertebrates (e.g., Plecoptera, Crustacea, Hydracarina, Hirudinea, and Tricladida) when they are present (Crawford et al., 2006).

Signal crayfish can move overland and can even travel several hundred meters in one night (David, 2003). In a study by Banha and Anastácio (2014), signal crayfish were capable of surviving air exposure for an extended period of ~21.5 h, whilst also being able to move an average of 17.5 m per day (Anastácio et al., 2015) sometimes individuals had complete immobility whilst others moved much further moving 461 m in just half a day. When compared to other invaders, signal crayfish showed the highest potential to establish dominance on similarly sized crayfish in interspecific interactions (Fořt et al., 2019).

2.3. Invasive fish species

Invasive freshwater fish are groups of non-native species primarily introduced for the purpose of aquaculture and related activities. Alien cyprinids and salmonids are well acclimatized to European freshwater ecosystems. It is known that alien fishes can disrupt food webs from the apex or the center. Often, the introduction and establishment of non-native fish into new habitats has enormous effects on the behavior, distribution, and abundance of native species and ecosystem function (Strayer, 2010). The most apparent effects of introduced fish species include the near-disappearance of large, active prey species and behavioral changes such as avoidance of microhabitats frequented by fish in the daytime. Further direct changes to the community and ecosystem could be due to cascading effects such as losing the most vulnerable species (Simon and Townsend, 2003). Because predatory fish often control the community structure of lakes and streams (Brooks and Dodson, 1965; Power, 1990) and game fish have been widely introduced by fishing agencies, a lot of attention has been placed on exploring the community impacts of fish. Several studies have demonstrated that fishing activities contribute to the long-distance dispersal of invasive fauna (Banha and Anastácio, 2015).

According to the review of Rakauskas et al. (2016), Prussian carp *Carassius gibelio* Bloch, 1782, Chinese sleeper *Perccottus glenii* Dybowski, 1877 and monkey goby *Neogobius* *fluviatilis* Pallas, 1814 are the three most invasive species in the northern branch of the central European invasion corridor (Table 3). One of the examples of fish that has both beneficial and detrimental effects is the mosquitofish *Gambusia affinis* Baird and Girard, 1853 and *Gambusia holbrooki* Girard, 1859, as they were widely introduced to suppress larval mosquitoes, yet caused a lot of negative impacts on native species of insect, fish and amphibian (Fuller et al., 1999) (Table 3).

Table 3. *Examples of invasive fish species selected based on scientific literature and databases, their native and invasive ranges, main pathways of introduction, and references.*

TAXON	NATIVE RANGE	INTRODUCED/ INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Ameiurus melas Rafinesque, 1820 Black bullhead	North America	Europe	Escape from pools, game fish	(Page and Burr, 1991; DAISIE, 2018; EASIN, 2018; FISHBASE, 2021)
Ameiurus nebulosus Linnaeus, 1819 Brown bullhead	North America	North America, Europe, Asia, Pacific islands	Escape from aquaculture	(DAISIE, 2018; EASIN, 2018; FISHBASE, 2021)
Carrasius auratus Linnaeus, 1758 Goldfish	Asia	Worldwide	Ornamental fish	(DAISIE, 2018; EASIN, 2018; FISHBASE, 2021)
Carassius gibelio Bloch, 1782 Prussian carp	Asia	Europe	Escape from aquaculture, release from aquarium or pets trade, game fish	(Semenchenko et al., 2011; CABI, 2019; FISHBASE, 2021)
Clarias gariepinus Burchell, 1822 African catfish	Africa	Europe, Asia	Escape from aquaculture, live food trade	(DAISIE, 2018; EASIN, 2018; FISHBASE, 2021)
Ctenopharyngodon Idella Valenciennes, 1844 Grass carp	China to eastern Siberia, USA	Worldwide, Europe and Asia	Escape from aquaculture, weed control	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Gambusia affinis Baird and Girard, 1853 Western mosquitofish	North and Central America	Worldwide	Release for biocontrol	(FISHBASE, 2021)
Gambusia holbrooki Girard, 1859 Eastern mosquitofish	Eastern and Southern America	Worldwide	Release for biocontrol	(Sanz et al., 2013)
Hypophthalmichthys molitrix Valenciennes, 1844 Silver carp	Asia	Worldwide	Escape from aquaculture (accidentally escaped, or was deliberately introduced)	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Hypophthalmichthys nobilis Richardson, 1845 Bighead carp	China	Worldwide, Europe	Escape from aquaculture	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Ictalurus punctatus Rafinesque, 1818 Channel catfish	North America	South America, Europe, Asia	Escape from aquaculture, release as game fish, inland canals	(EASIN, 2018; Haubrock, 2018; FISHBASE, 2021)

TAXON	NATIVE RANGE	INTRODUCED/ INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Lepomis gibbosus Linnaeus, 1758 Pumpkinseed	North America	West and Central Europe, Iberian Peninsula	Release as game fish	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Micropterus salmoides Lacepède, 1820 Largemouth bass	North America	South America, Europe, Asia, and Pacific islands	Escape from aquaculture, and also use as game fish	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Neogobius fluviatilis Pallas, 1814 Monkey goby	Ponto-Caspian region	Europe	Inland canals, shipping	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Neogobius melanostomus Pallas, 1814 Round goby	Ponto-Caspian region	Europe, North America	Inland canals, shipping	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Oncorhynchus mykiss Walbaum, 1792 Rainbow trout	North America	Worldwide	Escape from aquaculture	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Oreochromis niloticus Linnaeus, 1758 Nile tilapia	Africa	Worldwide	Escape from aquaculture	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Perccottus glenii Dybowski, 1877 Chinese sleeper	Asia	Europe	Escape from aquaculture, release from the aquarium	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Pseudorasbora parva Temminck and Schegel, 1846 Stone moroko	North and Central America	Europe, Asia	Contaminant of aquarium species, ornamental fish	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Salvelinus fontinalis Mitchill, 1814 Brook trout	North America	Worldwide	Escape from aquaculture, release as game fish	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)
Squalius cephalus Linnaeus, 1758 European chub	Europe	Worldwide	Release as game fish	(EASIN, 2018; CABI, 2019; FAO, 2021; FISHBASE, 2021)

Currently, five alien gobiids are present in European freshwater ecosystems: round goby, monkey goby, western tubenose goby *Proterorhinus semilunaris* Heckel, 1837; racer goby *Babka gymnotrachelus* Kessler, 1857 and bighead goby *Ponticola kessleri* Günther, 1861 (Harka and Bíró, 2007; Roche et al., 2013). The target species of the present Ph.D. thesis is the round goby as a representative of this group.

The **round goby** is a small aggressive bottom-dwelling fish and a member of the Gobiidae family. Its native range is the Ponto-Caspian region, but it is found throughout European and North American waterbodies as it was transported in ballast tanks of both inland and transoceanic ships (Jude et al., 1992; Skóra and Stolarski, 1993; Brown and Stepien, 2008). After being introduced, it has established self-sustaining populations and spread through a combination of natural dispersal, commercial shipping, and the activities of fishermen within invaded waterbodies (Charlebois et al., 2001; Johnson et al., 2005; Kornis et al., 2012). Its recent distribution in Europe includes nearly all navigable rivers; the Dnieper, Dniester, and Don Rivers, the Moscow River (Kornis et al., 2012), the Vistula River (Sapota, 2004), the Oder River (Czugała and Woźniczka, 2010), the Rhine (Borcherding et al., 2011), the Elbe river (Buřič et al., 2015; Roche et al., 2015) and the Danube (Simonovic et al., 2001; Eros et al., 2005), as well as many of their tributaries.

It differs in appearance from other gobies as it has a round black spot on the first dorsal fin (Stranai and Andreji, 2004). In contrast to the other bottom-dwelling fish group-bullheads (genus *Cottus*), the pelvic fins of all Ponto-Caspian gobies are fused to form a suctorial disc. This species displays marked sexual dimorphisms. Both sexes have an erectile urogenital papilla, while the male papilla is longer and more pointed than the broad and blunt papilla of the female (Kornis et al., 2012).

The round goby is a generalist benthic feeder with a broad diet spectrum that includes zooplankton, benthic invertebrates (e.g., amphipods, crayfishes), and the eggs and larvae of fish (Kornis et al., 2012). The diet composition of the round goby is mainly influenced by habitat, time of day and year, and body size (Janssen and Jude, 2001; Diggins et al., 2002; Walsh et al., 2007; Skabeikis and Lesutienė, 2015). The round goby can rapidly adapt to locally abundant food sources (Carman et al., 2006). Many studies are done on the diet composition and food preference of the round goby (Walsh et al., 2007; Mack and Andraso, 2015; Skabeikis and Lesutienė, 2015) which have found that round gobies undergo a dietary shift from arthropods to bivalves as they grow. This shift is accompanied by changes in pharyngeal morphology which means that the round goby has strong pharyngeal teeth that can effectively crush mollusc shells in habitats where molluscs appear, while no changes in teeth were detected in the habitats that lacked hard-shelled food (Johnson et al., 2005; Thompson and Simon, 2014; Andraso et al., 2017). Round goby prey consumption has been observed to change with size, showing a correlation between goby size and mollusc size (Skora and Rzeznik, 2001; Phillips et al., 2003; Johnson et al., 2008). With the absence of molluscs, round gobies obtain most of their energy from amphipods, chironomids, and caddisflies in comparison to other invertebrates (Pennuto et al., 2010). Molluscs and gastropods are the most consumed in the localities with high abundances of the round goby (Kipp et al., 2012; Skabeikis et al., 2019). In the study by Fitzsimons et al. (2006), round goby, sculpins and crayfish were shown to pose a threat to the eggs and larvae of other fish species and this has also been confirmed in multiple field and laboratory studies (e.g. Chotkowski and Marsden, 1999; Steinhart et al., 2004; Roseman et al., 2006).

In general, the round goby prefers rigid substrate and is usually most abundant in rocky habitats with numerous spaces (Ray and Corkum, 2001). Such environments provide structures that are crucial for reproduction (Meunier et al., 2009) and hiding from predators (Belanger and Corkum, 2003). During the spawning season, the round goby prefers shallower waters over deep (Kornis et al., 2012). During winter, the round goby migrates offshore and has been recorded at depths exceeding 100 m (Walsh et al., 2007).

As documented above, the invasion of the round goby represents a high potential for competition with native species. It is more effective at food acquisition (Bergstrom and Mensinger, 2009) and shelter competition (Dubs and Corkum, 1996; Balshine et al., 2005). Janssen and Jude (2001) documented the local extinction of the mottled sculpin *Cottus bairdii* Girard, 1850 in several parts of Lake Michigan only four years after the introduction of the round goby. Also, Kessel et al. (2016) detected a rapid decline in the abundance of the native river bullhead *Cottus perifretum* Freyhof, Kottelat and Nolte, 2005 after the appearance of the round goby. However, it depends on several factors such as native fish abundance, water temperature, food resources, and suitable habitat. Sometimes the effect on native bottom-dwelling species is not evident (Jurajda et al., 2005).

Besides competition for food and habitat, the round goby can cause recruitment failure of native fishes via direct predation of their eggs and larvae (Chotkowski and Marsden, 1999; Fitzsimons et al., 2006).

2.4. Invasive bivalve species

One of the essential classes of freshwater invaders are bivalve molluscs that suspension-feed on phytoplankton and seston, graze on periphyton, or browse vascular plants. Bivalve molluscs can develop massive populations in all kinds of freshwaters, consuming large amounts of primary producers and changing their composition. Modifications of the primary producers can affect nearly every part of the ecosystem (Strayer, 2010). Molluscs represent primary consumers, which can disrupt the food web from its base. Molluscs were introduced into new areas primarily due to commercial routes and the intensification of intercontinental traffic. Invasiveness varies widely between mollusc species and depends on their biology, vectors, availability of ecological niches, compatibility with new habitats, compatibility with new hosts (if reproduction is through parasitic larvae) and habitat integrity (Cianfanelli et al., 2007). Some invasive molluscs can count on rapid growth and reproduction for fast population recovery following disturbance (McMahon, 2002), which enhances their role as colonizers. Bivalves can exist in polluted water and sediments and toxic elements (e.g., copper, zinc, manganese, chromium, cadmium, lead, mercury, arsenic, etc.) can accumulate in their soft tissues and making them dangerous for human consumption (Sarma et al., 2013).

The **zebra mussel** *Dreissena polymorpha* Pallas, 1771 is one of the world's worse invasive alien species (Lowe et al., 2000) (Table 4). It is considered to be an invasive freshwater species with significant adverse impacts, modifying ecosystem structure and function changes and contributing to economic losses (Banha et al., 2016). Thanks to ballast waters, the zebra mussel has spread from the Ponto-Caspian region through European shipping canals (Bij de Vaate et al., 2002). Zebra mussel populations are often so large that they dominate heterotrophic biomass in large volumes of water. They can significantly reduce the amount of phytoplankton biomass, augmenting water transparency (Holland, 1993). The ecological impacts of the zebra mussel are numerous. It has a high-temperature tolerance and high spawning ability, it has spread in Europe and America, outnumbering the indigenous mussels and causing significant environmental changes. They compete with the indigenous mussels for food and space, but the main threat is the extinction of indigenous unionids through epizootic colonization (Baker and Hornbach, 1997). The negative impacts of zebra mussels go beyond ecosystems, they also cause a huge amount of damage by colonizing human structures, which creates problems for various humans activities (e.g., water supply, pipes of hydroelectric and nuclear power plants, public water supply plants, industrial facilities and fouling on the boats). Birds can transport zebra mussel larvae, but they are also frequently relocated by fishing boats (Banha et al., 2016).

Another highly invasive bivalve species in aquatic habitats is the **Asian clam** *Corbicula fluminea* O. F. Müller, 1774 (Caffrey et al., 2011; Lucy et al., 2012; Barbour et al., 2013) (Table 4). Asian clam invasion success and dispersion relies more on its life history traits (e.g., rapid growth, fast sexual maturation, high fecundity, short life span and extensive dispersal capacities with the help of human activities) than on its physiological tolerance (McMahon, 2002). Revisions of several studies showed that the invasion of the Asian clam has negatively impacted native bivalve abundance and diversity in European and North American freshwater ecosystems (Araujo et al., 1993; Strayer, 1999; McMahon, 2002). With its high burrowing and bioturbation activity, when abundant, it may displace and reduce available habitats for juvenile unionids and sphariids (Vaughn and Hakenkamp, 2001). Additionally, its suspension and deposit-feeding strategy may also negatively impact unionid juvenile recruitment (Hakenkamp and Palmer, 1999). Furthermore, its dense populations can ingest large numbers of unionid sperm, glochidia and newly metamorphosed juveniles (Strayer, 1999). It can compete for food resources with sphaeriids and juvenile unionids since they have higher filtration rates on a per biomass basis than sphaeriids and unionids and consequently have the potential to limit planktonic food available to native bivalves (McMahon, 1991). Negative impacts from the Asian clam include biofouling water channels and raw water systems of factories and power stations as well as creating problems for sand companies. Moreover, this invasive species can be a vector for new parasites and disease into the invaded ecosystem and can bioaccumulate and biomagnify contaminants (Sousa et al., 2008).

The **Chinese pond mussel** *Sinanodonta woodiana* Lea, 1834 is a large-sized representative of the Unionidae family and is an invader with a complex life cycle that is present in the flowing and standing waters of most of Europe and North America (Beran, 2008; Bogan et al., 2011) (Table 4). It is believed that the primary pathway of the introduction of Chinese pond mussel to Europe was accidentally alongside introduced Asian fish species of carp; silver carp *Hypophthalmichthys molitrix* Valenciennes, 1844; bighead carp *Hypophthalmichthys nobilis* Richardson, 1845; and grass carp *Ctenopharyngodon idella* Valenciennes, 1844. The Chinese pond mussel is known to seriously threaten the native population of bivalves from the family Unionidae as a direct competitor for food and space with native species, while another important factor is competition for fish hosts (Fabbri and Landi, 1991) as the larvae of this species develop on the gills and fins of fish. It is demonstrated that the Chinese pond mussel is able to reduce seston loads to levels that are comparable to native freshwater mussel species, without detectable changes in its filtration regime (Douda and Čadková, 2018).

Table 4. *Examples of invasive bivalve species selected based on scientific literature and databases, their native and invasive ranges, main pathways of introduction, and references.*

TAXON	NATIVE RANGE	INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Corbicula fluminalis O. F. Müller, 1774	Native to Asia	Europe, USA	Escape from aquaculture, shipping	(DAISIE, 2018; EASIN, 2018)
Corbicula fluminea O. F. Müller, 1774 Golden freshwater calm	Southern and eastern Asia (eastern Russia, Thailand, Philippines, China, Taiwan, Korea, and Japan), Australia, and Africa	North America, South America, Europe	Escape from aquaculture shipping release as aquarium species	(DAISIE, 2018; EASIN, 2018)
Dreissena polymorpha Pallas, 1771 Zebra mussel	Drainage basins of the Black, Caspian, and Aral Seas	Europe, North America, Asia	Contaminant in aquaculture-inland canals, shipping	(DAISIE, 2018; EASIN, 2018)
Dreissena rostriformis Deshayes, 1838 Quagga mussel	estuarine region of the rivers the Dnieper and Southern Bug	Europe, USA	Shipping	(DAISIE, 2018; EASIN, 2018; CABI, 2019)
Sinanodonta woodiana Lea, 1834 Chinese pond mussel	East Asian unionid mussel from the Amur River and Yangtze rivers	Established worldwide	Trade of contaminated commodities, aquaculture release, introductions	(DAISIE, 2018; EASIN, 2018)

However, no target species is included in the present Ph.D. thesis. The use of zebra mussel and Asian clam was planned for experiments during the internship in Portugal. However, the internship had been cancelled due to protective measures against the global pandemic SARS-CoV-2.

2.5. Other important aquatic invasive species in Europe

Table 5. *Examples of other important aquatic invasive species selected based on scientific literature and databases, native and invasive ranges, main pathways of introduction, and references.*

TAXON	NATIVE RANGE	INVASIVE RANGE	MAIN PATHWAYS	REFERENCES
Amphibalanus improvisus Darwin, 1854 Bay barnacle	North America Atlantic coast	Atlantic coast of Europe, Baltic, and the Black Sea	Hull fouling, fisheries, trade, ballast waters	(DAISIE, 2009; Hänfling et al., 2011)
Argulus japonicus Thiele, 1900 Japanese fish louse	East Asia	Worldwide	Fisheries and trade – accidental	(USGS, 2005; DAISIE, 2009; Hänfling et al., 2011)
Atyaephyra desmaresti Millet, 1831 Freshwater shrimp	Southern Europe	Central Europe	Canal	(Brink and Velde, 1985; Hänfling et al., 2011)
Eriocheir sinensis H. Milne-Edwards, 1853 Chinese mitten crab	China	Europe, North America	Ballast water	(Hänfling et al., 2011)
Hemimysis anomala G.O.Sars, 1907 Bloody-red mysid	Ponto-Caspian basin	Baltic and the North Sea drainages	Canal, ballast water	(Bij de Vaate et al., 2002; USGS, 2005; Füreder and Pöckl, 2007; DAISIE, 2009)
Jaerea istri Veuille, 1978	Ponto-Caspian basin	North Sea drainages	Canal	(Bij de Vaate et al., 2002; DAISIE, 2009; Grabowski and Szlauer-Łukaszewska, 2012)
Lymnosis benedeni Czerniavsky, 1882	Ponto-Caspian basin	Baltic and the North Sea drainages	Intentional, canal	(Bij de Vaate et al., 2002; DAISIE, 2009)
Pomacea canaliculata Lamarck, 1819 Golden apple snail	South America	Spain	Escape, aquarium release	(Nentwig et al., 2018)
Potamopyrgus antipodarum Gray, 1843 New Zeland mud snail	New Zealand	Europe	Aquarium release	(Halabowski et al., 2020)
Salvinia molesta D. Mitch – aquatic moss	Brazil	Spain, Netherlands, Great Britain	Aquarium release	(Luque et al., 2014)
Trachemys scripta elegans Wied-Neuwied, 1839 Red eared slider	North America, Florida	Europe	Aquarium release	(IUCNGISD, 2021)

3. Monitoring and management of freshwater invaders

Preventing the arrival of AIS is a major priority in managing biological invasions. The combination of multiple invaded ecosystems and high ecological and economic costs has led to the development of national and international teams, projects, databases, and applications with a special interest in the monitoring of aquatic invaders and their management. LIFE program financed projects dealing with the control and eradication of AIS from 1992 and from 1992 to 2002 in that time more than 100 projects were founded. Three major AIS databases widely used by the researchers and policymakers are the IUCN's Global Invasive Species Database (IUCNGISD, 2021) containing information on 2,413 alien invasive species, DAISIE (European Invasive Alien Species Gateway, http://www.europe-aliens.org/) and EASIN (European Alien Species Information Network, https://easin.jrc.ec.europa.eu/). A study by Saul et al. (2017) assessed the integration of available pathway information from different databases into a single data repository and analyzed it to support countries and institutions in order to meet major targets in environmental policy. The standard pathway categorization scheme was recently adopted by the Convention on Biological Diversity (Aichi Biodiversity Target 9). The combined data set includes pathway information for 8,323 species across major taxonomic groups (plants, vertebrates, invertebrates, algae, fungi, etc.) and environments (terrestrial, freshwater, and marine) (see Saul et al., 2017).

An example of projects the "AQUAINVAD-ED" that included experts in invasion biology, ecology and biotechnology that aimed at filling the current knowledge gaps by using novel molecular techniques combined with the power of data sourcing (citizen science) to develop innovative methods for early detection, control and management of AIS (AQUAINVAD-ED, 2017; Tricarico et al., 2017; Haubrock, 2018). The Environmental Impact Classification of Alien Taxa (EICAT) systematically summarises and compares the detrimental impacts that alien species have on native biota, but only compares alien species based on their highest impact magnitudes (Volery et al., 2021), showing negative or positive environmental impacts (Vimercati et al., 2020). The project "MoBI-aqua – cross-border monitoring of biological invasions for conservation of native aquatic biodiversity" should also be mentioned because the results presented in this thesis are part of that project, German – Czech cooperation on the monitoring of invasive species. This project has developed a specific action plan for the region as well as a public mobile application to encourage the involvement of citizens (MoBI aqua, 2021).

The aims of the above-mentioned projects are to educate members of the public, specifically, owners of alien species (hobbyists, suppliers, traders), as an escape or intentional release can cause problems, and fishermen for potentially spreading invaders by intentional fish stocking, and transport as contaminants on their equipment. Increasing public awareness and the direct involvement of the public into biodiversity conservation are extremely important for preventing invasions in the future (Buřič et al., 2020b). Problems with invasive species should be communicated through informative campaigns from aquarists to the public, as current knowledge on the incidence and consequences of bioinvasions is poor. For example, Scheers et al. (2021) proposed a crayfish surveillance system using a combination of active surveillance from systematic surveys by regional public authorities or scientific institutions and passive management using citizen science with local managers, anglers, naturalists, and aquatic species enthusiasts.

4. Objectives of Ph.D. Thesis

The experimental work in this thesis consists of field studies (monitoring of populations and sampling) as well as laboratory work (ecological studies and the interactions of different species). The main focus is on the comparison of different invasive aquatic organisms, their ecological characteristics and their interactions. These species were found co-existing in several monitored localities along the Elbe River and its tributaries during habitat and biodiversity screening from the project "MoBI-aqua – cross-border monitoring of biological invasions for conservation of native aquatic biodiversity" a result of cross-border cooperation between the Institute of Hydrobiology – Technical University of Dresden and the Faculty of Fisheries and Protection of Waters from 2017–2020. The initial plan was to include studies on 1) *food intake and evacuation rate in killer shrimp*, 2) exploring the interactions of killer shrimp and the early developmental stages of crayfish, 3) *observe the ethological interactions of bivalves and crayfish*, 4) looking at basic patterns of interactions of round goby and crayfish, 5) to compare the space usage and basic behavior of the round goby and the European bullhead, and to study 6) *round goby and European bullhead interactions*. The objectives 1), 3) and 6) marked by italics are unfortunately not presented in this thesis, mainly due to measures from the pandemic covid-19 [SARS-CoV-2], (1 and 3) causing the cancelation of abroad internships in Portugal (bivalve – crayfish interactions) and creating difficulties with sample extraction and analysis in TU Dresden, Germany (food intake of killer shrimp). The work on the last objective (*6*) was also delayed (again in co-operation with TU Dresden) and is now being prepared for publication (a manuscript will be ready in the upcoming months).

The rest of the objectives (2 and 4) was hence focused on killer shrimp, marbled and signal crayfish, and round goby. The main aim was to observe how the aforementioned aquatic invaders interact and how they influence each other when they co-exist. Another objective (5) was to explore the differences in space usage, water flow preference or tolerance and the basic behavior of the invasive round goby and the native European bullhead.

Here, the three objectives (2, 4 and 5) are presented as independent chapters. The effect of killer shrimp on the early developmental stages of crayfish and the effect of crayfish on killer shrimp is described in chapter 2, the interactions between the round goby and crayfish are described in chapter 3 and the behavioral differences between the round goby and the European bullhead are described in chapter 4.

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CHAPTER 2

PILFERER, MURDERER OF INNOCENTS OR PREY? THE POTENTIAL IMPACT OF KILLER SHRIMP (*DIKEROGAMMARUS VILLOSUS*) ON CRAYFISH

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RESEARCH ARTICLE

Pilferer, murderer of innocents or prey? The potential impact of killer shrimp (*Dikerogammarus villosus*) on crayfish

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Abstract

Freshwater ecosystems worldwide are facing the establishment of non-native species, which, in certain cases, exhibit invasive characteristics. The impacts of invaders on native communities are often detrimental, yet, the number and spread of non-native invasive species is increasing. This is resulting in novel and often unexpected combinations of non-native and native species in natural communities. While the impact of invaders on native species is increasingly well-documented, the interactions of non-native invaders with other non-native invaders are less studied. We assessed the potential of an invasive amphipod, the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894), to cope with other established invaders in European waters: North American crayfish of the Astacidae family—represented by signal crayfish *Pacifastacus leniusculus* (Dana, 1852), and the Cambaridae family—represented by marbled crayfish *Procambarus virginalis* Lyko, 2017. The main goal of this study was to investigate if killer shrimp, besides their role as prey of crayfish, can significantly influence their stocks by predating upon their eggs, hatchlings and free-moving early juveniles. Our results confirmed that killer shrimp can predate on crayfish eggs and hatchlings even directly from females abdomens where they are incubated and protected. As marbled crayfish have smaller and thinner egg shells as well as smaller juveniles than signal crayfish, they were more predated upon by killer shrimp than were signal crayfish. These results confirmed that the invasive killer shrimp can feed on different developmental stages of larger freshwater crustaceans and possibly other aquatic organisms.

Keywords Freshwater · Crustacea · Amphipod · Invasive species · Interaction · Predation

Introduction

Crustaceans play an important role as prey and consumers in aquatic ecosystems, transferring energy from lower to higher trophic levels. However they are also very successful invaders and the increased introduction, establishment and dispersal of non-native crustaceans in Europe has the potential to wreak havoc on freshwater foodwebs (Strayer 2010; Hänfling et al. 2011). They play an irreplaceable role in food chains, both as prey and as consumers transferring energy from lower to higher trophic levels (MacNeil et al. 1997; Dorn and Wojdak 2004; Väinölä et al. 2008; Lodge et al. 2012). In Europe, there are many native species of freshwater crustaceans, but the last decades have seen the increased introduction, establishment and dispersal of non-native crustacean species (Gherardi 2007). Many of these alien species have become invasive pests, even more frequently than usually hypothesised (Jeschke and Strayer 2005; Jeschke 2008), and their occurrence threatens native biota (Ricciardi et al. 2017; Jeschke and Heger 2018).

Freshwater ecosystems, especially large rivers, are increasingly subject to multiple invasions where several groups of invasive alien species (IAS) occur simultaneously in space and time (Gebauer et al. 2018). They compete for space, food, predate on natives and can sometimes transmit diseases and parasites (Ricciardi et al. 2011; Jeschke and Heger 2018). Increased spread of IAS in European waters results not only in contact and competition among native and non-native invasive species, but also among invaders from different biogeographic regions (Ricciardi et al. 2011). In addition, the presence of invasive species in an ecosystem

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can facilitate invasion by other species (invasional meltdown hypothesis), and increase the likelihood of their survival and negative ecological impacts (Simberloff 2006). Their effects on ecosystems can be divergent but simultaneously additive or even multiple. One way or another, IAS can inhibit, displace or even eradicate each other (Ricciardi and Atkinson 2004).

Decapods and amphipods are particularly successful and diverse orders of crustaceans, especially the non-native representatives of these groups in Europe (Gherardi 2007; Hänfling et al. 2011). Crayfish are the most abundant decapod invaders in European freshwaters, with at least eleven non-native species (Kouba et al. 2014; Weiperth et al. 2017). The vast majority of these species are classed as invasive, especially those originating from North America (Holdich et al. 2009; Kouba et al. 2014). They are characterized by the ability to withstand extreme conditions (Haubrock et al. 2019; Veselý et al. 2015), high fecundity, aggressiveness, activity, fast maturation, high population densities and/or the ability to transmit diseases deadly to native crayfish (Buřič et al. 2013; Kotovska et al. 2016; Svoboda et al. 2017; Vodovsky et al. 2017). In amphipods, species originating from the Ponto-Caspian basin are the most problematic IAS of the main river catchments across Europe (Bij de Vaate et al. 2002). In general, species of the genus *Dikerogammarus*, with the flag bearer species *D. villosus* (Sowinsky, 1894) known as the killer shrimp, are the most reported invasive amphipods in European freshwaters (Bij de Vaate et al. 2002; Pöckl 2009; Rewicz et al. 2014). Killer shrimp is a voracious predator, preying on a wide spectrum of benthic macroinvertebrates including insect larvae, leeches, isopods, other amphipods, and juvenile crayfish (Krisp and Maier 2005; Buřič et al. 2009; Boets et al. 2010; Rewicz et al. 2014). Predation on fish eggs is also reported (Taylor and Dunn 2017). Moreover, killer shrimp have been observed injuring or even killing other macroinvertebrates without consuming them, which illustrates their aggressive nature and potential impact on prey populations (Dick and Platvoet 2000).

Killer shrimp often co-exist and interact with several invasive crayfish species, e.g. the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817), representatives of the families Astacidae and Cambaridae (Gherardi 2007). Killer shrimp have been observed predating on early independent crayfish juveniles (Buřič et al. 2009), but their impact on other life stages remains unclear. Thus in our present study, we hypothesized a negative impact of killer shrimp on different developmental stages of crayfish (eggs, hatchlings, first independent stage of juveniles) even those actively protected by mothers. Species in the family Astacidae, such as the signal crayfish and native European noble crayfish *Astacus astacus* (Linnaeus, 1758), typically have larger eggs and early developmental stages (Kozák et al. 2009; Kouba et al. 2010), while species in Cambaridae, such as the clonal marbled crayfish *Procambarus virginalis* Lyko, 2017, are small bodied crayfish with smaller eggs and smaller juveniles (Kouba et al. 2014; Patoka et al. 2016). This study will elucidate if small amphipods like killer shrimp can negatively affect populations of larger decapods (including both mentioned families) via predation on their actively protected early developmental stages.

Materials and methods

Animals acquisition and maintenance

Killer shrimp

Killer shrimp adults were collected at the lower reaches of the Czech section of the River Elbe (coordinates: 50.655 N, 14.043 E) from shallow rocky habitats in April 2015. Individuals were hand collected by walking upstream and turning over stones, shaking the bottom substrate and using hand nets to capture individuals escaping or carried by the water flow. Killer shrimps were transported in polyethylene bags with water to the experimental facility of the Research Institute of Fish Culture and Hydrobiology (RIFCH) in Vodňany, where they were identified to species level using morphological characters (Eggers and Martens 2001) and acclimated to laboratory conditions before beginning the experiment. Killer shrimps were placed individually in 100 ml boxes half-filled with aged tap water. Water was exchanged daily and killer shrimps were fed once per day with one chironomid larvae, except on the day prior to the start of the experiment. The mean body weight of killer shrimps used in the experiments is reported below.

Marbled crayfish

Marbled crayfish was used as a suitable model species comparable with other cambarids (Hossain et al. 2018) like spiny-cheek crayfish or calico crayfish *Faxonius immunis* (Hagen, 1870) which are known to co-occur with killer shrimp in European rivers. Marbled crayfish were obtained from our own experimental culture held at RIFCH. Females with attached eggs were selected from the culture stock and held individually to avoid interference by other crayfish. They were acclimated to laboratory conditions in 2500 ml plastic boxes with 2000 ml of aged tap water at 20 °C before the experiments. The temperature was maintained by storing the boxes in an adjustable temperature incubator with 12 h light and 12 h dark photoperiod. The females were used in four different ways:

1. 38 females (carapace length, CL = 22.7 ± 4.0 mm, measured from the tip of rostrum to the posterior edge of cephalothorax, weight = 4.3 ± 2.5 g) were stripped of eggs to estimate fecundity. Eggs were carefully stripped firstly from the 3rd pair of pleopods using entomological forceps and placed in a Petri dish with a small amount of water and counted. Then, the rest of attached eggs were stripped to another Petri dish with a small amount of water and counted. Data obtained were used in a regression analysis to obtain an equation for linear regression to estimate total fecundity from eggs sampled from the 3rd pair of pleopods (as recommended by Hossain et al. 2019).

   The eggs were then used to test the ability of killer shrimp to destroy and eat marbled crayfish eggs (see below). The sample of 50 eggs was weighed using an analytical scale (Mettler, Toledo, USA) to the nearest 0.1 mg to calculate average egg weight (2.4 mg). The rest of the eggs were terminated by hot water and discarded.
2. 16 ovigerous females (CL = 25.2 ± 4.3 mm, weight = 5.7 ± 3.6 g) were used to test the ability of killer shrimp to destroy and eat marbled crayfish eggs directly from the female's pleopods in spite of maternal care and protection. Prior to the experiment, all eggs from the 3rd pair of pleopods were carefully stripped from each female using entomological forceps and counted to estimate initial total fecundity.
3. 25 ovigerous females (CL = 24.3 ± 3.9 mm, weight = 5.3 ± 2.6 g) were incubated until hatched juveniles reached the 3rd developmental stage (DS)—the stage of independence in cambarid crayfish (Andrews 1907; Vogt et al. 2004). Juveniles were carefully detached using entomological forceps and then used to test the ability of killer shrimp to overpower and eat marbled crayfish early juveniles. The sample of 50 juveniles was individually weighed using an analytical scale (Mettler, Toledo, USA) to the nearest 0.1 mg to calculate the average individual weight (3.9 ± 0.6 mg).
4. For the last experiment, 70 randomly selected mature marbled crayfish (CL = 22.6 ± 2.8 mm, weight = 3.4 ± 1.3 g) were taken from our own culture and maintained in the same way as ovigerous females. These were used to assess marbled crayfish predation on mature killer shrimp individuals.

Signal crayfish

Signal crayfish were caught in April 2015 from the pond system near Velké Meziříčí (49.379 N, 16.082 E) using baited traps. After transfer to the experimental facility of RIFCH, they were placed into flow through channels. Female crayfish with attached eggs were sorted and held individually to avoid interference by other crayfish. These females were acclimated to laboratory conditions in 2500 ml plastic boxes with 2000 ml of tap aged water at 15 °C before the experiments. The temperature was maintained by storing the boxes in an adjustable temperature incubator with 12 h light and 12 h dark photoperiod. The females were used in different ways:

1. 22 females (CL = 39.9 ± 4.3 mm, weight = 21.0 ± 6.6 g) were used to estimate the correlation between egg count on the 3rd pair of pleopods and total fecundity. Eggs were stripped, weighed, counted and managed as described above for marbled crayfish.
2. 16 ovigerous females (CL = 40.0 ± 5.0 mm, weight = 20.8 ± 7.0 g) were used to test the ability of killer shrimp to destroy and eat signal crayfish hatchlings (juveniles in the 1st DS; Andrews 1907) directly from a female's pleopods in spite of maternal care and protection. Juveniles in the 1st DS were used because we observed low predation rates on signal crayfish eggs (see below). Prior to the experiment, all hatchlings from the 3rd pair of pleopods were carefully stripped from each female using entomological forceps and counted to estimate initial total fecundity.
3. 18 ovigerous females (CL = 41.5 ± 4.1 mm, weight = 23.3 ± 6.3 g) were incubated till hatched juveniles reached the 2nd DS—the stage of independence in astacid crayfish (Andrews 1907). Juveniles were carefully detached using entomological forceps and then used to testing of killer shrimp ability to overpower and eat signal crayfish early juveniles (see below an experimental set-up part). The sample of 50 juveniles in the 2nd DS was individually weighed using an analytical scale (Mettler, Toledo, USA) to the nearest 0.1 mg to count the average individual weight (22.5 ± 2.9 mg).
4. For the last experiment, 70 randomly selected young mature signal crayfish (sex ratio 1:1, CL = 30.5 ± 2.7 mm, weight = 8.1 ± 2.2 g) from the same source as females above were used for predation on mature killer shrimp individuals (see below an experimental set-up part).

Experimental set-up

Killer shrimp as predators of unprotected crayfish eggs

The feeding rates of killer shrimp (mean individual weight 65.5 ± 28.1 mg and 62.2 ± 19.2 mg in signal and marbled crayfish experiments, respectively) were quantified by using seven egg densities (1, 3, 6, 10, 15, 20 and 25 eggs per experimental arena) with 8 replicates per each egg density and species. The experimental temperature was set at 15 and 20 °C for signal and marbled crayfish, respectively. 15 °C corresponds to the temperature at which signal crayfish can develop and hatch and 20 °C corresponds to the suitable temperature for marbled crayfish reproduction and the culture conditions of the experimental stock. The experiments were conducted in a Velp Scientifica-FOC 215e incubator with the light regime 12 h of light and 12 h of darkness. Boxes of 500 ml volume (bottom area 7.5×6 cm, 300 ml of aged tap water) were used as experimental arenas. Stripped eggs were introduced in the experimental arenas 1 h before the experiment. One individual killer shrimp was then introduced to each arena. Each killer shrimp was starved for 24 h prior to the experiment to standardize hunger level. The number of remaining undamaged and damaged eggs (chopped) in each arena was recorded after 24 h. In the case of eggs, no control stock without killer shrimp was made, because of the inactive nature of eggs.

We distinguished between eaten eggs (N_e), and damaged (chopped) eggs. We then analysed the effects of prey density and prey size (species). For prey species, a logistic regression between initial prey density (N_0) and the proportion of prey eaten (N_e/N_0) was computed to identify the shape of the functional response:

$$\frac{N_e}{N_0} = \frac{\exp\left(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3\right)}{1 + \exp\left(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3\right)} \tag{1}$$

where P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, estimated by the maximum likelihood (Juliano 2001). If $P_1 < 0$, the proportion of prey killed declines monotonically with the initial density of prey, matching a type II functional response. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey killed is a unimodal function of prey density, corresponding to a type III functional response (Juliano 2001). When our results indicated type II functional response we estimated functional response parameters using the type II Rogers random predator equation (Rogers 1972) that accounts for prey depletion during the experiment:

$$N_e = N_0\left(1 - \exp\left(-a\left(t - hN_e\right)\right)\right) \tag{2}$$

where N_e is number of prey eaten, N_0 is initial prey density per litre, a is the consumer attack rate (a, L.day^{-1}), h is the consumer handling time (h, day.prey^{-1}) and t is the duration of experiment in days. Before fitting the Rogers model to our experimental data, we used Lambert W function to solve Eq. 2 for N_e (for further details see Bolker 2008).

$$N_e = N_0\frac{W\left(ahN_0e^{-a\left(t-hN_0\right)}\right)}{ah}. \tag{3}$$

We then tested whether the attack rate and handling time of each predator was influenced either by type of prey or predator species by comparing the overlap in the 95% confidence intervals (CI) of the parameter estimated values. We assumed differences among treatment to be significant if CI of the parameter estimates did not overlap (Sentis et al. 2012, 2013). We calculated 95% CI using the standard errors for the estimates of each model parameter.

To determine the maximal number and biomass of eggs that killer shrimp are able to destroy or consume, we used the results from the three highest egg densities (15, 20, 25 eggs per arena), at which killer shrimp never killed or ate all the available prey. We calculated the average number of eaten prey, attacked prey (including eaten and attacked but not eaten prey together) and the biomass of prey eaten and expressed it as the percentage of individual predator body weight.

Killer shrimp as predators of juvenile crayfish

Functional response of killer shrimp (weight of 80.8 ± 31.4 mg and 87.1 ± 31.0 used for signal and marbled crayfish respectively) was quantified by measuring their feeding rate at seven densities of independent crayfish juveniles (1, 3, 6, 10, 15, 20 and 25 juveniles per experimental arena) for each crayfish species. As in the egg experiment, two experimental temperatures were used (15 and 20 °C) in accordance with the conditions experienced in the terminal stages of egg incubation and postembryonal development. Stripped independent juveniles (in the 2nd and 3rd developmental stage for signal and marbled crayfish, respectively) were introduced to the experimental arenas one hour prior to predators. After this acclimation period, killer shrimps were released into the arenas. The number of remaining prey and dead prey in each arena was recorded after 24 h. Nine and eight replicates were made for signal and marbled crayfish juveniles. In addition, replicates without consumers (five and three for signal and marbled crayfish, respectively) were conducted at each prey density to control for potential "natural" mortality of prey. We then used the same models as described above for crayfish eggs.

To determine the maximal number and biomass of juveniles that killer shrimp are able to destroy or consume, we again used the results from the three highest densities (15, 20, 25 per arena) at which killer shrimp never killed or ate all the available prey. We calculated the average number of eaten prey, attacked prey (including eaten and attacked but not eaten prey together) and the biomass of prey eaten and expressed it as the percentage of individual predator body weight.

Killer shrimp vs. ovigerous crayfish

In total, 14 carrying females were tested per crayfish species. For marbled crayfish we used ovigerous females, while in signal crayfish we used females carrying 1st DS juveniles (Andrews 1907) since we found low predation of signal crayfish eggs in the previous set-up. As before, experiments were run at 15 and 20 °C for signal and marbled crayfish respectively, maintained by a Velp Scientifica-FOC 215e incubator with a regime of 12 h of light and 12 h of darkness. Boxes with a volume of 2500 ml (bottom 25.4 × 17.3 cm, bottom area 0.044 m^2, 2000 ml of aged tap water) with a shelter (halved ceramic plant pot) and gravel substrate were used as experimental arenas.

Before stocking the experimental arenas, all eggs/juveniles were stripped from the females' 3rd pair of pleopods and counted to estimate the initial total fecundity (see above) in both control and amphipod-exposed females. Seven females from each species were individually placed in arenas together with a stock of killer shrimps and, as a control, seven females were placed in arenas without killer shrimps. Killer shrimp stock consisted of 16 animals per arena (weight of 76.8 ± 32.4 mg and 81.5 ± 26.7 mg used for signal and marbled crayfish, respectively), equivalent to a density of ~ 360 individuals per m^2. This corresponds with densities of killer shrimp found in natural conditions (e.g. MacNeil et al. 2010) but is much less than the highest recorded densities: 4000–10,000 individuals per square meter (Van Riel et al. 2006; Gallardo et al. 2012).

Experimental arenas were then placed in the incubators for 72 h. After this period all females were removed and all eggs/juveniles were gently stripped from their pleopods and counted. The remaining killer shrimps in the experimental arena were also counted. The estimated initial fecundity was then compared with actual counts in control and amphipod-exposed females.

Crayfish as predators of killer shrimps

Functional response of mature crayfish (both signal and marbled crayfish) was quantified by measuring their feeding rate at seven densities of mature killer shrimps (1, 3, 6, 10, 15, 20 and 25 killer shrimps per experimental arena). Individual killer shrimps had a mean weight of 95.4 ± 26.4 mg and 91.3 ± 31.5 mg for signal and marbled crayfish experiments, respectively. To account for small differences in crayfish body weight, the predator pressure on prey is expressed as number of prey eaten per gram of predator. The experimental temperature of 20 °C was used for both species, maintained by Velp Scientifica-FOC 215e incubator with light regime 12 h of light and 12 h of darkness. Killer shrimps were introduced to the experimental arenas one hour prior to consumers. After this acclimation period, crayfish were released into the arenas. The number of remaining prey and dead prey in each arena was recorded after 24 h. Eight replicates of each density were made for both signal and marbled crayfish. In addition, three replicates without consumers were conducted for each prey density to control for potential "natural" mortality of prey. We then modelled functional response as described above.

To reveal the maximal numbers of prey and biomass which each crayfish species is able to destroy or eat, we used the results from the tests with the three highest densities of killer shrimp (15, 20, 25 per arena), in which crayfish never killed or ate all prey offered. We calculated the average number of eaten prey, attacked prey (including eaten and attacked but not eaten prey together) and the biomass of eaten prey and expressed it as a percentage of individual predator body weight.

Statistical analyses

The statistical analyses were conducted in R and Statistica 13. Analysis of functional response of organisms and their parameters was performed in R (R Core Team 2016). To compare differences in estimated and observed fecundity in both control and amphipod-exposed carrying females we used paired *t* tests. A Mann Whitey non-parametric test was used to assess differences in estimated and observed fecundity between amphipod-exposed crayfish and the control group, and to test for differences in the relative biomass of prey eaten (relative to the individual predator biomass) by signal and marbled crayfish. For all the statistical test we used $\alpha = 0.05$.

Results

Killer shrimp as predators of crayfish eggs

Only 10 of 54 killer shrimps were able to open and consume the eggs of signal crayfish, i.e., only 18.5% of individuals. These 10 killer shrimps were able to eat up to 2 eggs and damage 3 eggs (damaged uneaten eggs) at maximum, respectively. Damaged eggs were observed in all densities, eaten eggs only in densities 6, 10, 20, and 25. In all cases we observed the consumption of all egg-stalks, the connecting parts between the female pleopods and eggs. There was no relationship between prey density and number of prey eaten so the relationship was not investigated by functional response approach.

Only one killer shrimp from 54 (i.e. 1.9%) did not damage and eat any marbled crayfish eggs (prey density 3). The

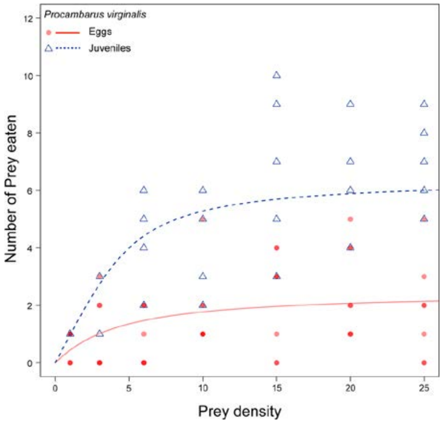

Fig. 1 Prey type dependent functional responses. Individual replicates (dots = eggs, triangles = juveniles) overlaid by prediction of the most parsimonious model (full and dashed line). Red = eggs, blue = juveniles

maximum amount of eggs consumed and damaged was 5 and 11, respectively. The smallest killer shrimp that successfully damaged and even consumed the egg was equal to the size of the smallest killer shrimp used (35 mg). The functional response curve corresponded to the Holling type II (P1 = -4.03; SE = 1.07; $P < 0.001$) (Fig. 1). Calculated attack rate and handling time are presented in Fig. 2 and Table 1. Those values indicate the lower effort to find and attack immobile prey but its more difficult handling.

At the three highest prey densities, the number of eaten marbled crayfish eggs and damaged eggs was significantly higher than that of signal crayfish (eaten eggs Z = -3.50, $P < 10^{-3}$; damaged eggs Z = -5.82, $P < 10^{-6}$). There was no difference in the relative biomass eaten between the two species. The average number of eaten prey, attacked prey and the prey eaten biomass expressed as the percentage of predator body weight is shown in Table 2.

Killer shrimp as predators of crayfish juveniles

Only 13 of 63 killer shrimps were able to predate on signal crayfish independent juveniles (2nd DS), i.e. only 20.6% of individuals. We therefore did not find a significant relationship between prey density and number of prey eaten, and did not calculate functional response. These 13 killer shrimps were able to eat up to 3 juveniles and kill 4 juveniles (killed and uneaten) at maximum, respectively. Eaten and/or killed juveniles were observed in all densities tested. The smallest

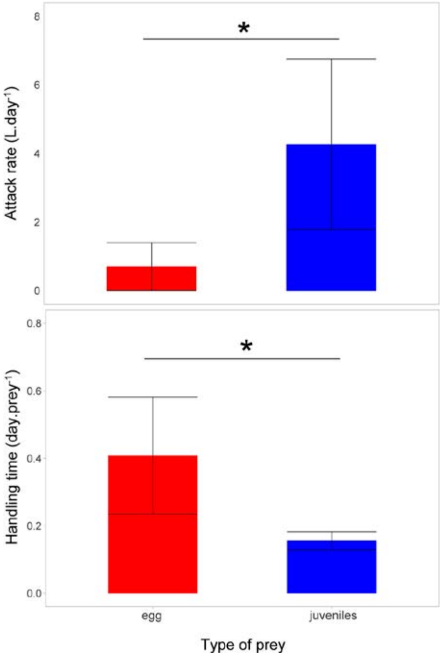

Fig. 2 Comparison of killer shrimp (*Dikerogammarus villosus*) attack rates and handling times between marbled crayfish (*Procambarus virginalis*) eggs and juveniles. Data shown as mean ± 95% confidence interval. Significant differences (revealed by comparing the overlap in the 95% confidence intervals (CI) of the parameter estimated values in accordance to Sentis et al. 2012, 2013) are marked by asterisk

Table 1 Handling time (h) and attack rate (a) of killer shrimp *Dikerogammarus villosus* preying on marbled crayfish (*Procambarus virginalis*) juveniles and eggs.

	Estimate	SE	CI	Min	Max
Marbled crayfish					
Eggs					
a	0.70	0.36	0.70	0.008	1.4
h	0.40	0.09	0.17	0.23	0.58
Juveniles					
a	4.26	1.26	2.49	1.78	6.74
h	0.155	0.013	0.03	0.13	0.18

Standard error of mean (SE), confidence interval (CI), minimal (Min) and maximal value (Max)

Table 2 The number of prey eaten, prey killed (attacked but not eaten), and the weight of prey eaten expressed as a percentage of predator biomass in different predator–prey set-ups

Predator	Prey	Prey eaten		Prey killed		Biomass eaten (%)	
		Mean	Range	Mean	Range	Mean	Range
Dv–15 °C	Eggs–S	0.6 ± 0.8^{b}	0–2	1.0 ± 1.1^{b}	0–3	6.6 ± 10.0^{a}	0–32.3
Dv–20 °C	Eggs–M	2.2 ± 1.6^{a}	0–5	7.8 ± 1.7^{a}	3–11	9.4 ± 6.6^{a}	4.8–21.3
Dv–15 °C	Juv.–S	0.3 ± 0.7^{b}	0–3	0.6 ± 0.9^{b}	0–4	6.0 ± 12.9^{b}	0–45.2
Dv–20 °C	Juv.–M	6.3 ± 2.1^{a}	3–10	9.3 ± 2.3^{a}	4–13	27.2 ± 10.0^{a}	5.9–43.8
S–20 °C	Dv	4.7 ± 3.5^{a}	0–15	7.7 ± 5.1^{b}	0–21	6.1 ± 4.7^{b}	0–17.7
M–20 °C	Dv	5.3 ± 2.5^{a}	1–12	13.1 ± 6.4^{a}	3–23	17.0 ± 7.9^{a}	1.9–33.4

killer shrimp able to predate on signal crayfish independent juveniles weighed 47.7 mg.

All killer shrimps were able to kill and eat marbled crayfish independent juveniles (3rd DS). The maximum number of juveniles consumed and killed was 10 and 13, respectively. The minimal size of the killer shrimp that successfully killed and consumed an independent marbled crayfish juvenile was 38 mg (at the density 10), equal to the size of the smallest killer shrimp used. The functional response curve corresponded to the Holling type II (P1 = − 3.71, SE = 0.77, $P<0.001$) (Fig. 1). Calculated attack rate was significantly higher than on marbled crayfish eggs ($P<0.001$) while handling time was significantly lower than on marbled crayfish eggs (see Fig. 2, Table 1).

At the three highest prey densities, killer shrimp ate significantly more marbled crayfish juveniles than signal crayfish juveniles ($Z=-6.00$, $P<10^{-6}$), killed more juveniles ($Z=-6.03$, $P<10^{-6}$) and consumed a greater biomass of marbled crayfish juveniles than signal crayfish juveniles ($Z=-4.79$ $P<10^{-5}$). The average number of eaten prey, attacked prey and the biomass eaten expressed as the percentage of predator body weight is shown in the Table 2.

Killer shrimp (Dv; *Dikerogammarus villosus*), marbled crayfish (M; *Procambarus virginalis*), and signal crayfish (S; *Pacifastacus leniusculus*) were used as predators as well as prey. Juv.–crayfish juveniles in 2nd and 3rd developmental stage of signal (S) and marbled crayfish (M), respectively. Eggs–egg of signal (S) and marbled crayfish (M). Experiments were performed at 15 °C and 20 °C for signal and marbled crayfish eggs and juveniles respectively (prey densities 15, 20, and 25 individuals), according to their temperature specific incubation terminal phases

Different superscripts indicate significant differences within experimental set-ups ($\alpha=0.05$). Data are presented as mean ± standard deviation

Killer shrimp vs. ovigerous crayfish

A highly significant linear relationship was found between the egg number on the 3rd pair of pleopods and overall fecundity in both signal crayfish ($y=35.924+3.019x$, $R^2=0.856$, $P<10^{-6}$) and marbled crayfish ($y=-9.730+3.431x$, $R^2=0.937$, $P<10^{-6}$). This linear relationship was used to estimate fecundity of control and amphipod-exposed females. Egg-carrying signal crayfish females stocked together with killer shrimp individuals had significantly lower final fecundity than estimated ($t=7.39$, $P<10^{-3}$) while control females did not. The difference between estimated and observed fecundity was significantly higher in amphipod-exposed females than in control ones ($Z=2.30$, $P=0.021$), on average 12.6% more juvenile losses than the control. While counting attached juveniles, some were found still attached to pleopods but missing substantial parts of their body (usually whole abdomen and part of the carapace).

Similarly to signal crayfish, marbled crayfish females stocked together with killer shrimp individuals had significantly lower fecundity than estimated ($t=3.79$, $P=0.009$) while control females did not. The difference between estimated and observed fecundity was also significantly higher in amphipod-exposed females than in control ones ($Z=2.68$, $P=0.007$). It is in average 14.6% more juvenile

Table 3 The fecundity estimated (E) and observed (O), and the average difference (%) between estimated and observed values in carrying females of signal crayfish (S; *Pacifastacus leniusculus*) and marbled crayfish (M; *Procambarus virginalis*) when exposed to killer shrimp (Dv; *Dikerogammarus villosus*) and without killer shrimp presence [control (C]

Species	Fecundity E	Fecundity O	Difference between E and O (%)
S			
C	133.4 ± 16.1^{a}	122.1 ± 23.7^{a}	-9.0 ± 9.5^{A}
Dv	151.0 ± 49.3^{a}	119.3 ± 42.0^{b}	-21.6 ± 6.6^{B}
M			
C	186.3 ± 102.6^{a}	191.7 ± 106.2^{a}	4.2 ± 7.9^{A}
Dv	183.4 ± 96.9^{a}	164.9 ± 95.5^{b}	-10.4 ± 6.6^{B}

Significant differences ($\alpha=0.05$) between estimated and observed values in particular rows are highlighted by lower case superscripts

Significant difference in average differences between control and amphipod-exposed females in the last column are highlighted by upper case superscripts. Data are presented as mean ± standard deviation losses compared to control. Estimated and observed fecundity and mean differences are presented in Table 3.

During exposure time females were able to substantially reduce the number of killer shrimps. Signal and marbled crayfish females killed on average 53.6 ± 38.0% and 57.1 ± 6.7% of stocked killer shrimps, respectively.

Crayfish as predators of killer shrimps

Signal crayfish used in the experiments were significantly larger ($t = 16.09$, $P < 10^{-6}$) and heavier ($t = 14.04$, $P < 10^{-6}$) than marbled crayfish. Signal crayfish were able to eat 15 (density 20) or kill 21 (density 25) killer shrimps (killed and uneaten) at maximum while marbled crayfish ate 12 (density 20) and killed 23 (density 25) killer shrimps at maximum. Thus, a ratio between crayfish weight and prey eaten was applied to account for crayfish size.

The functional response curve corresponded to the Holling type II for both signal crayfish ($P1 = -4.99$, $SE = 1.73$, $P < 0.001$) and marbled crayfish ($P1 = -4.29$, $SE = 1.14$, $P < 0.001$) (Fig. 3). Attack rate did not differ significantly between signal and marbled crayfish, while handling time was significantly lower in marbled crayfish than in signal crayfish (Fig. 4).

At the three highest prey densities, the number of eaten killer shrimps did not significantly differ between signal crayfish and marbled crayfish, but the number of killed killer shrimps (i.e. eaten + killed and uneaten) was significantly higher in marbled crayfish ($Z = -3.09$, $P = 0.002$), as well as the biomass eaten ($Z = -4.35$ $P < 10^{-4}$). The average number of eaten prey, attacked prey and the biomass eaten expressed as percentage of predator body weight is shown in Table 2.

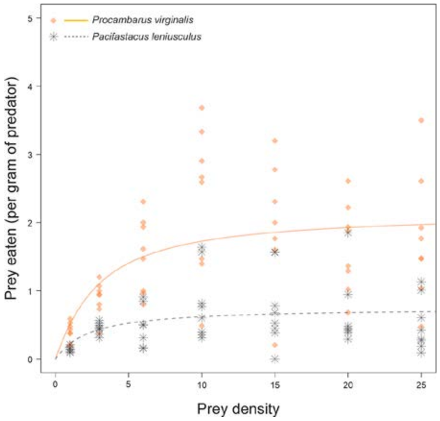

Fig. 3 Curves corresponding to Holling type II calculated for density dependent functional responses from single-consumer (signal crayfish *Pacifastacus leniusculus* and marbled crayfish *Procambarus virginalis*) preying on killer shrimp (*Dikerogammarus villosus*). Individual replicates (dots) overlaid by prediction of the most parsimonious model (dashed line)

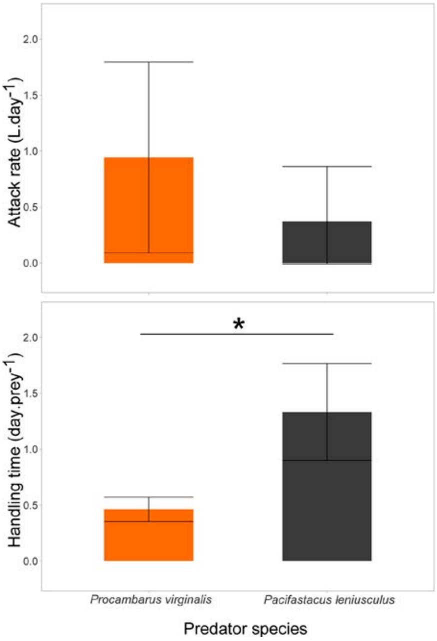

Fig. 4 Comparison between attack rate and handling time of signal crayfish (*Pacifastacus leniusculus*) (n = 56) and marbled crayfish (*Procambarus virginalis*) (n = 56) predating on killer shrimp (*Dikerogammarus villosus*). Data shown as mean ± 95% confidence interval. Significant differences (revealed by comparing the overlap in the 95% confidence intervals (CI) of the parameter estimated values in accordance to Sentis et al. 2012, 2013) are marked by asterisk

Discussion

As a result of multiple invasions, novel and often unexpected species compositions are reported with many effects on native biodiversity. Except the strong effect on native species, non-native invaders can also have an impact on other non-native invaders. These interactions are still not well studied. Killer shrimp co-exist and interact in many European rivers with invasive crayfish species. In the present study the example species included two invasive crayfish species: (1) signal crayfish representing family Astacidae and hence also partially European native crayfish potentially co-existing with killer shrimp, and (2) marbled crayfish representing family Cambaridae which differ from astacids, among others, by the smaller size of eggs and independent juveniles (Kozák et al. 2015). Crayfish, no matter from which family are characterised by intensive brood care and further maternal care even in stages when juveniles are freely moving, fully developed and independent (Aquiloni and Gherardi 2008; Mathews 2011; Vogt 2013).

During embryonal development crayfish clean the clutch and move by pleopods to prepare the best conditions for incubation. After hatching, the female's care continues during the stages of dependent juveniles—1st developmental stage in astacids and 1st and 2nd stage in cambarids (Vogt 2013; Kozák et al. 2015). In the following stages, juveniles become independent, actively feeding and moving in the proximity of the mother, but still use the mother's abdomen as a safe port in danger (Aquiloni and Gherardi 2008; Kubec et al. 2019). Egg clutches and early juveniles therefore seem to be well protected, but are they protected when exposed to strong killer shrimp pressure? As it known, killer shrimp is able to predate on independent cambarid juveniles till 4th or even 5th developmental stage with a potential to reduce the crayfish offspring recruitment (Buřič et al. 2009) but can they predate on larger astacid juveniles or even predate on protected eggs and juveniles directly at the female's abdomen? The present study elaborated the influence of killer shrimp on incubated egg clutches and juveniles from the theoretical (predation on detached eggs/juveniles without guarding females) as well as practical point of view when carrying females were faced with the group of killer shrimps at a realistic field density more than 20 times lower than maximal reported values (Van Riel et al. 2006; Gallardo et al. 2012).

We found that killer shrimp can predate on both eggs and juveniles of both tested crayfish species, although the larger size of astacid eggs and juveniles as well as lower incubation temperature of eggs and thus lower metabolic activity of predators, may partially protect them. But the smaller sized cambarid eggs and first developmental stages were very good prey for killer shrimps, and were consumed in high quantities. The estimated attack rates and handling times indicated that killer shrimps are more efficient at (or prefer) catching juveniles than eggs. Also, shorter handling times indicate that they can consume more juveniles than eggs when their densities are not limited. Overall, it seems that killer shrimps feed more on juveniles than eggs which is a bit surprising. It may be that the nutritional quality of eggs is poor compared to juveniles, or killer shrimp may prefer moving prey. However, we suggest it is more likely that killer shrimps simply struggle to open the egg shell, increasing the handling time. Moreover, crayfish juveniles are more common, so amphipods are more "experienced" in handling them throughout evolution, while crayfish eggs are something rare in the environment because of their attachment to mother during incubation.

The biomass consumed by killer shrimp illustrates its high impact on invertebrate communities (Hellmann et al. 2017). We confirmed that killer shrimp kill more prey than they can eat (only 28% of and 68% of killed marbled crayfish eggs and juveniles were also consumed) as described also Dick and Platvoet (2000). In real conditions, where the clutches of eggs/juveniles are actively guarded and cared by females (Vogt 2013) this rate could differ. We decided to use ovigerous females because the vast majority of the time when females carry a clutch (eggs and first developmental stages of juveniles) consists of egg incubation (Reynolds 2002). But the limited ability of killer shrimp to open and eat big eggs of signal crayfish lead us to use females with fresh hatchlings i.e. juveniles in the 1st DS, to see a possible effect of killer shrimps on the carried clutch. Carrying females of both species guarded their clutches which resulted in more than a 50% decrease of killer shrimp quantity during the experiment. However, clutches were not guarded effectively because females lost a significant proportion of their offspring (12% of hatchlings in signal crayfish and 14% of eggs in marbled crayfish). In the case of signal crayfish, several halves of juveniles were observed among the surviving juveniles, further confirming the destructive impact of killer shrimp on the clutch. The effect of crayfish cannibalism can be ruled out here, because crayfish in the 1st developmental stage of crayfish do not feed yet (Reynolds 2002; Kozák et al. 2009). Some post-manipulation egg losses were observed in marbled crayfish, but these also occurred in the control group which was handled in the same way. Experiment was carried out in limited space which can in fact limit the effect of killer shrimp due to restricted possibilities to escape from the outreach of the crayfish. In natural conditions we suggest even higher clutch losses are likely in killer shrimp populated areas because of the unrestricted space and availability of sheltering spaces for killer shrimp to escape and hide (e.g. at the bottom of several layers of differently sized particles). In such conditions crayfish females have limited opportunities to catch the intruder or limit their overall quantities.

Killer shrimp also play an important role as prey species in European freshwater ecosystems (Gherardi 2007), and as expected both crayfish species we tested were able to prey on them at a considerable intensity. However, the difference between the two crayfish species predatory impact was significant. Despite the smaller size of marbled crayfish (about 74% and 45% of signal crayfish size and weight, respectively), it was able to eat equal numbers of killer shrimps. However, they killed almost twice the number of prey than signal crayfish did, much like other true predators that kill more than is possible to eat (Kruuk 1972; Oksanen et al. 1985; Veselý et al. 2017a, b). They also consumed almost three times the relative biomass eaten by signal crayfish. The much lower handling time of prey by marbled crayfish can help to explain these findings. In addition, they may have a higher metabolic rate at 20 °C as warm water species compared to cold water signal crayfish. Marbled crayfish have also been found to be more voracious and slower to reach satiation than spiny-cheek crayfish (Linzmaier and Jeschke 2019).

Overall, the results from our experiments confirmed the predatory capabilities of the killer shrimp and highlight their extreme boldness when attacking clutches of crayfish eggs or juveniles actively guarded by females. This bold predatory behavior illustrates the serious threat posed by killer shrimp invasions as well as the interesting foraging behavior of pilfering under the "safety camera". It is surprisingly risky behavior, given that crayfish females were were able to eat around 50% of killer shrimp in the experimental stock. Due to its intensive aggressive foraging on various aquatic organisms (Platvoet et al. 2009; MacNeil et al. 2013; Taylor and Dunn 2017) it is no wonder that killer shrimp trophic position is more similar to that of small benthic fish (Van Riel et al. 2006) or even to predatory fish (MacNeil et al. 2010) than to other gammarids. In addition to these direct trophic impacts, they are also strong competitors for resources such as shelters against predators (Van Riel et al. 2007; Platvoet et al. 2009). High population densities of killer shrimp can magnify these effects (Cuthbert et al. 2019) but also can provide a good food source for other aquatic organisms as fish (Gherardi 2007) or crayfish as confirmed by our study. In their early developmental stages marbled crayfish are vulnerable to being preyed upon by killer shrimp, but as adults the roles are reversed and they voraciously predate upon killer shrimp, killing more than they can eat (only consuming ~ 40% of prey killed). Despite this reciprocal predation pressure these two invaders are probably not able to extinguish each other (e.g. due to other food resources) but they probably can negatively affect each other during long term coexistence.

Our results support the need for more complex investigation of over-invaded freshwater ecosystems, where communities accumulate new invasive species over time with an increasing number of interactions types and strength among them (Collin and Johnson 2014). In many large river systems native species are in the minority and in the case of benthic communities this situation is even worse. The ecosystems functioning therefore become changed as the influence of multiple invaders can be additive or even multiple (Ricciardi and Atkinson 2004; Penk et al. 2017). Our study provides further evidence that novel IAS have not only major ecological impacts (including predation on native species, increased competition for habitat and resources, disease transfer or habitat degradation) but can also theoretically inhibit, substitute or (in extreme cases) even extirpate each other.

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Compliance with ethical standards

Conflicts of interest The authors declare no competing financial interests.

Ethical statement The study did not involve endangered or protected species. All experimental manipulations were conducted according to the principles of the Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, based on the EU harmonized animal welfare act of Czech Republic. The above mentioned Ethical Committee (IACUC) specifically approved this study. The principles of laboratory animal care and the national laws 246/1992 and regulations on animal welfare were followed (Ref. number 22761/2009-17210).

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CHAPTER 3

ROUND GOBY VERSUS MARBLED CRAYFISH: ALIEN INVASIVE PREDATORS AND COMPETITORS

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RESEARCH PAPER

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Round goby *versus* marbled crayfish: alien invasive predators and competitors

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Abstract – Aquatic biodiversity is threatened by spread of invasive alien species. Round goby *Neogobius melanostomus* is an invasive fish in large European rivers as well as in coastal waters near their mouths and marbled crayfish *Procambarus virginalis* is a highly invasive crustacean. Both are small, bottom-dwelling species occupying similar habitat and shelters and utilizing similar food sources. We hypothesized that goby presents a threat to both native and non-native astacofauna in invaded ecosystems. We tested this through laboratory experiments designed to determine aggressiveness and competitiveness of goby against marbled crayfish as a model for other North American cambarid crayfish, assessing goby prey size selection and competition with marbled crayfish for space and shelter. Gobies showed high aggressiveness and dominance over the crayfish. Goby predation on juvenile crayfish was limited by mouth gape size. In goby/crayfish pairs of similar weight, gobies were more aggressive, although each affected the behavior of the other.

Keywords: Biological invasion / freshwater / predation / shelter competition / species interaction

Résumé – **Le gobie à tache noire versus l'écrevisse marbrée: des espèces exotiques envahissantes prédatrices et concurrentes.** La biodiversité aquatique est menacée par la propagation d'espèces exotiques envahissantes. Le gobie à taches noires *Neogobius melanostomus* est un poisson envahissant dans les grands fleuves européens ainsi que dans les eaux côtières près de leur embouchure et l'écrevisse marbrée *Procambarus virginalis* est un crustacé très envahissant. Toutes deux sont de petites espèces vivant sur le fond, occupant des habitats et des abris similaires et utilisant des sources de nourriture similaires. Nous avons émis l'hypothèse que le gobie constitue une menace pour l'astacofaune indigène et non indigène dans les écosystèmes envahis. Nous avons testé cette hypothèse par le biais d'expériences en laboratoire conçues pour déterminer l'agressivité et la compétitivité du gobie contre l'écrevisse marbrée comme modèle pour les autres écrevisses cambarides d'Amérique du Nord, en évaluant la sélection de la taille des proies du gobie et la compétition avec l'écrevisse marbrée pour l'espace et les abris. Les gobies ont montré une grande agressivité et une forte dominance sur les écrevisses. La prédation des gobies sur les écrevisses juvéniles était limitée par la taille de l'ouverture de la bouche. Dans les paires gobie/écrevisse de poids similaire, les gobies étaient plus agressifs, bien que chacun ait affecté le comportement de l'autre.

Mots clés : Invasion biologique / eau douce / prédation / compétition pour l'abri / interaction des espèces

1 Introduction

Fish and crayfish have multiple relationships and portraying them solely as prey or predator can be misleading. In aquatic habitats, both groups often represent keystone species, and their competition for resources can have high impact (Bond, 1994; Crandall and Buhay, 2008). Although the ranges of some non-native fish overlap with those of non-native crayfish, with the exception of information with respect to aggressive encounters, little is known about interactions between invasive benthic fish and native and invasive crayfish, although they co-exist and use similar niches and substrates as shelter (Church *et al.*, 2017). Filling the knowledge gaps is worthwhile, because both fish and decapods may regulate community biodiversity through their longevity and trophic

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Table 1. Biometric data of marbled crayfish size groups used in experiment on round goby predation on crayfish (Experiments 1–3). Data are presented as mean ± standard deviation (SD) and range.

Exp.	Size class	Weight (mg)	Total length (mm)	Carapace length (mm)	Carapace height (mm)
1,2	Small	15.2 ± 4.0 (10–25)	10 ± 1.4 (8–12)	4.7 ± 0.9 (3–6)	2.3 ± 0.5 (2–3)
	Medium	58.6 ± 12.7 (40–80)	15.3 ± 0.5 (15–16)	7.3 ± 0.6 (6–8)	3.7 ± 0.8 (3–5)
	Large	210.6 ± 60.3 (100–300)	22.8 ± 1.7 (21–25)	11 ± 0.6 (10–12)	5.7 ± 0.8 (5–7)
3	Small juveniles	5.7 ± 0.6 (5.1–7)	7.1 ± 0.4 (6.5–8)	3.9 ± 0.4 (3–4.5)	–
	Medium juveniles	13.8 ± 4.8 (9–22)	10 ± 1.4 (8–12)	4.7 ± 0.9 (3–6)	2.3 ± 0.5 (2–3)

specialization (Reynolds, 2011). Alien predatory fishes can negatively impact native crayfishes, while fish populations may be affected by predation and competition from multiple organisms, including exotic crayfish (Degerman *et al.*, 2007).

Round goby *Neogobius melanostomus* (Pallas, 1814) is an alien invasive species from the Eurasian Ponto-Caspian region that presents high potential for competition with native species (Jude, 1997; Borcherding *et al.*, 2011; Brandner *et al.*, 2013). The species has invaded, or expanded its range in, large European rivers including the Danube (Vanderploeg *et al.*, 2002), Rhine (Van Kessel *et al.*, 2009), Vistula (Grabowska *et al.*, 2008), and Volga (Copp *et al.*, 2005) and has established invasive populations in North America (Kornis *et al.*, 2013). A primary characteristic is its formation of vital and dense populations with rapid spread both upstream and downstream (Roche *et al.*, 2015; Verliin *et al.*, 2017). Round goby is an adaptable generalist benthic feeder with a broad diet spectrum including zooplankton, benthic invertebrates, and fish eggs and larvae (Kornis *et al.*, 2012). Small benthic fish like round goby can feed on small juvenile crayfish as well as exploit the same food sources and be subject to the same predators as larger crayfish (Dorn and Mittelbach, 1999).

The marbled crayfish *Procambarus virginalis* (Lyko, 2017) is a unique invasive crayfish that reproduces parthenogenetically and has been included in the list of European Union invasive species of concern since August 2016 (EU regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141). The marbled crayfish has been reported established in many European countries and on other continents (Chucholl and Pfeiffer, 2010; Lipták *et al.*, 2016; Hossain *et al.*, 2018; Andriantsoa *et al.*, 2019). It is omnivorous, feeding on algae, detritus, zoobenthos, and macrophytes. It can become abundant and form high-density populations in a short time (Lipták *et al.*, 2019). As *Procambarus fallax* (Hagen, 1870) is a crayfish endemic to Florida and closest relative to the parthenogenetic *P. virginalis*, we used marbled crayfish as a representative of other successful invasive members of the Cambaridae originally from North America (Kouba *et al.*, 2014; Patoka *et al.*, 2016).

Direct interactions between fish and crayfish include predation and competition for shelter. When co-existing fish and decapods are omnivores, there will be competition and mutual predation, depending on relative size and vulnerability (Reynolds, 2011). Bottom-dwelling fish such as round goby can potentially exert negative effects on crayfish in addition to predation, as they use similar food sources and compete for shelter (Gebauer *et al.*, 2019). Limited shelter availability can increase the vulnerability to predation of the weaker opponent (Church *et al.*, 2017).

We hypothesized that (a) round goby represents a predatory threat to smaller crayfish (tested in experiments 1, 2 and 3 in multiple scenarios), (b) this predatory impact and food selection is depending on marbled crayfish sizes available, and (c) round goby is more aggressive and dominates over larger crayfish in competition for shelter (experiments 4 and 5). The goal of the present study was to determine the effect of round goby predation, aggressiveness, and shelter dominance on crayfish under laboratory conditions, using the marbled crayfish as a model for other invasive species.

2 Materials and methods

Round goby (TL 63.54 ± 7.6 mm) were collected from the River Elbe in September 2018 (Ústí and Labem, north of Czech Republic) using a battery powered backpack electrofishing unit (FEG 1500, EFKO, Leutkirch, Germany) while experiments were carried out during November and December 2018. Fish were transferred to the experimental facility of the Research Institute of Fish Culture and Hydrobiology in Vodňany, University of South Bohemia in České Budějovice and held in troughs embedded in a small recirculating system for acclimatization to laboratory conditions. Troughs were filled with aged tap water and cleaned every second day. Fish were fed *ad libitum* with frozen chironomid larvae daily.

Marbled crayfish were obtained from our own culture and fed *ad libitum* on chironomid larvae and carrot daily. Continuous culture enabled the use of all developmental stages in this research.

Animals were weighed using a digital precision balance (Kern 572-35, Kern and Sohn, Germany) to the nearest 0.5 mg. Crayfish total length (TL, from tip of the rostrum to the posterior median edge of telson), carapace length (CL, from tip of the rostrum to the posterior median edge of the cephalothorax) and carapace height (CH) were measured with Vernier calipers, and fish TL (from the tip of the snout to the tip of the tail) was measured with a ruler to the nearest 1 mm. All crayfish individuals were measured (TL, CL, CH) before performing the following experiments (1, 2, and 3) thus separated according to their weight (small, medium, large, small juveniles, medium juveniles) in five different aquariums that later could be used for the experiments (Tab. 1), same as for following experiments (4 and 5) weight-matched pairs of

Table 2. Biometric data of marbled crayfish and round goby used in experiments on competition for shelter (Experiments 4–5). Data are presented as mean ± standard deviation (SD) and range.

Exp.	Animal	Weight (mg)	Total length (mm)
4	Marbled crayfish	116.9 ± 27.5 (60–180)	32.52 ± 14.8 (31–44)
	Round goby	123.5 ± 32.1 (60–190)	44.7 ± 11.29 (38–53)
5	Marbled crayfish	255 ± 10.4 (200–500)	20.61 ± 2.72 (16–25)
	Round goby	272 ± 95.8 (200–500)	57.27 ± 4.02 (52–65)

round goby and crayfish for each experiment were selected and separated before (Tab. 2).

Animals appeared healthy and active and were used only once for each experiment to avoid any learning effect during the experiments. Crayfish with missing or regenerating chelae or showing signs of approaching molt or not fully hardened following molting were omitted from experiments. No specific permissions were required for the location in this study. All facilities used for housing of experimental animals and for experimental procedures were located indoors in separate units with no direct connection to surface waters and were protected against the escape of any organisms used.

2.1 Goby predation on crayfish

2.1.1 Experiment 1, single size predation

Thirty round gobies (TL 74.2 ± 7.25 mm) were placed in separate 27L × 19W × 7.5H cm plastic boxes containing 2 l aged tap water and 150 cm^3 of fine sand for acclimatization and starvation. Water temperature was set at 21 °C and the light regime to 12:12 h light: dark. After 24 h, water was exchanged, and one crayfish was randomly added to each of 30 boxes containing one round goby.

We selected three size classes of crayfish: small (10–25 mg), medium (40–80 mg) and large (100–300 mg) (Tab. 1) with 10 replicates of each size class to assess round goby ability to swallow crayfish of various sizes.

After 24 h we evaluated crayfish type of response with paired goby and we noted if crayfish was consumed, killed and partially consumed or still alive. Following the experiment, we measured round goby total length (TL) and the smallest internal dimension of the fish mouth (here referred as mouth gape) was measured using a set of conical plastic tips consisting of a plastic body with different measurement head attachments. The plastic tip was inserted into the fish mouth until a marked resistance was reached (Supplement 2). In this position, mouth gape size could be determined from fine gradients of the plastic tip to the nearest 0.01 mm.

2.1.2 Experiment 2, choice-size predation

Following exp. 1., we increased crayfish number, so we stocked one round goby (TL 78.0 ± 7.85 mm) and three juvenile crayfish from each size class in 65L × 50W cm elliptical arenas with 650 cm^3 of sand and 10 l water (water level 5 cm). One half of each arena was shaded by an opaque cover to reduce stress during the light period (12:12 h light: dark). The experiment was conducted at 17 °C and at 21 °C (the higher temperature according to prevailing summer water temperatures of River Elbe and the lower one to its slightly colder tributaries). At each temperature, we carried out 18 replicates plus 7 control replicates in which crayfish were stocked without round goby to assess possible cannibalism. At 24 hours post-stocking, we counted the number of crayfish of each size class consumed by round goby and analyzed the association of round goby mouth gape and water temperature on consumption rate and size selection.

2.1.3 Experiment 3, biomass consumption

In the third experiment, single round goby (TL 76.3 ± 3.06 mm) were placed in the experimental elliptical arenas under the same light regime as in the size-choice experiment along with 50 small juveniles or 50 medium juvenile crayfish (Tab. 1). Water temperature was 17 °C. Four replicates were conducted with each class size. After 24 hours, we counted remaining crayfish and measured the weight of surviving and unconsumed crayfish to calculate the biomass consumed per 24 hours.

2.2 Competition for shelter

2.2.1 Experiment 4, daily observations of competition for shelter over 8 days

The experiment was conducted in aquaria (40L × 20W × 25H cm) with a layer of sand (1500 cm^3) and 7 l aged tap water aerated by a single air stone placed in a corner and equipped with a single shelter situated in the middle of the shorter side of the aquarium (half of a ceramic flowerpot, entry diameter: 4.7 cm, height: 4 cm, length 4.5 cm). Round goby and marbled crayfish were weighed to form weight-matched pairs with wet weight difference $<5\%$ (Tab. 2). Prior to the experiment, goby and marbled crayfish were placed separately in 27L × 19W × 7.5H cm plastic boxes with 150 cm^3 sand and 2 l water for 24 hours to standardize the starvation level. The light regime was 12:12 h light: dark and water temperature ~20 °C. The weight-matched pairs were placed simultaneously in each aquarium (20 replications) and observed for the following 8 days. Visual observations of all twenty weight-matched pairs were made only during daylight hours at 08.00, 11.00, 14.00, and 17.00 o'clock for total of 8 days. Animals did not receive supplemental food during the course of the experiment. The position of all individuals was described to record whether an animal was in the shelter, in the proximity of the shelter (near to entry or beside the shelter), hidden in the sand, in a corner, in the corner with the air stone, or active in other areas of the bottom. Mortality and molting events in crayfish were recorded.

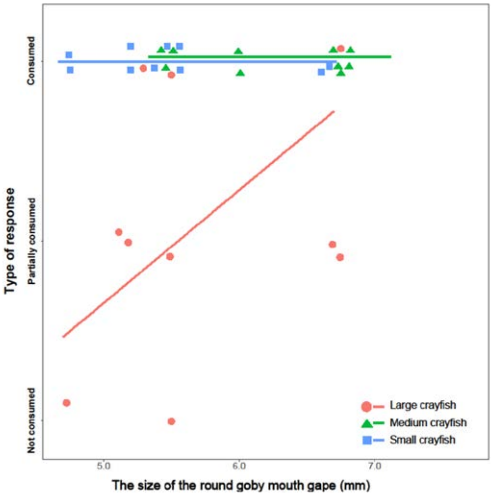

Fig. 1. Relationship of round goby *Neogobius melanostomus* mouth gape and marbled crayfish *Procambarus virginalis* size classes consumed over 24 h.

2.2.2 Experiment 5, twenty-four-hour observation of competition for shelter

We used similar conditions as for the eight-day experiment but with the air stone removed to omit recording disturbance in the experiment of continuous observation for 24 hours. Another 16 pairs of round goby and marbled crayfish were weight-matched (Tab. 2) and acclimated as described in previous experiment. Inter-specific pairs were stocked in aquaria and video-recorded. We conducted 16 replicates. Animal activity was recorded as aggressive interaction (attack, biting, pursuit), time spent in shelter, and avoidance (retreat from opponent prior to attack) (Tabs. 4 and 5), detailed and carefully analyzed by eye. Duration of attack was recorded as the time from the first aggressive act to cessation of interaction (Tab. 5). At the conclusion of the experiment, we measured round goby/marbled crayfish length and weight and assessed injuries.

2.3 Data analysis

Since many data sets did not meet the assumptions for parametric tests, even after transformation, nonparametric tests were used. For experiment 1, we used Firth's bias-reduced penalized-likelihood logistic regression to analyze the type of response between marbled crayfish (which was taken as a factor and assessed as: 0 = not consumed, 0.5 = killed and partially consumed, 1 = consumed) and relationship with round goby mouth gape and total length. The simple linear regression between round goby mouth gape and round goby total length was evaluated. In experiment 2, a generalized linear model (hereafter, GLM) with an assumed quasi-binomial distribution, that accounts for data underdispersion, was used to analyze the quantity of three crayfish size categories consumed and their relationship with round goby mouth gape under two different temperatures. Also, linear relationship between round goby mouth gape and crayfish size class under two temperatures was calculated. For experiment 3, we performed a test using GLM with quasi-binomial distribution, that accounts for data overdispersion, to assess the difference in wet mass of two offered crayfish size groups consumed by round goby. For experiment 4, we used GLM with Poisson distribution. As post-hoc testing was not possible for Poisson distribution errors, results are based on predictions and estimations. We used detailed data from visual observations to better understand interactions of round goby and marbled crayfish over the course of 24 hours. For experiment 5 we performed GLM with Gaussian distribution to analyze the difference in time spent in shelters and to test the number of attacks per animal during the light and dark periods. Analysis was conducted with R software and package ggplot 2 was used for data visualization (R Development Core Team, v. 4.0.3., 2020). In the case of Figures 1-3 points are dispersed within 3 or more lines of the y-axis because of a jitter-like function enabling to visualize individual points without their coverage.

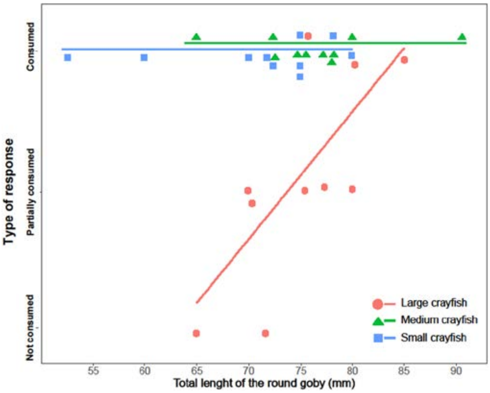

Fig. 2. Relationship of round goby *Neogobius melanostomus* total length and marbled crayfish *Procambarus virginalis* size classes consumed over 24 h.

3 Results

3.1 Round goby predation on crayfish

3.1.1 Experiment 1, single size predation

Mean round goby mouth gape (diameter) was 5.7 ± 0.74 (4.7–7.7) mm. The type of response of crayfish due to round goby predation (which was taken as a factor and assessed as: 0 = not consumed, 0.5 = killed and partially consumed, 1 = consumed) did differ significantly between size groups (likelihood ratio test = 16.49; df = 5; $p = 0.005$). The type of response of all size classes of marbled crayfish was correlated with mouth gape and total length of round goby individuals ($p = 0.005$), indicating that round goby was not able to

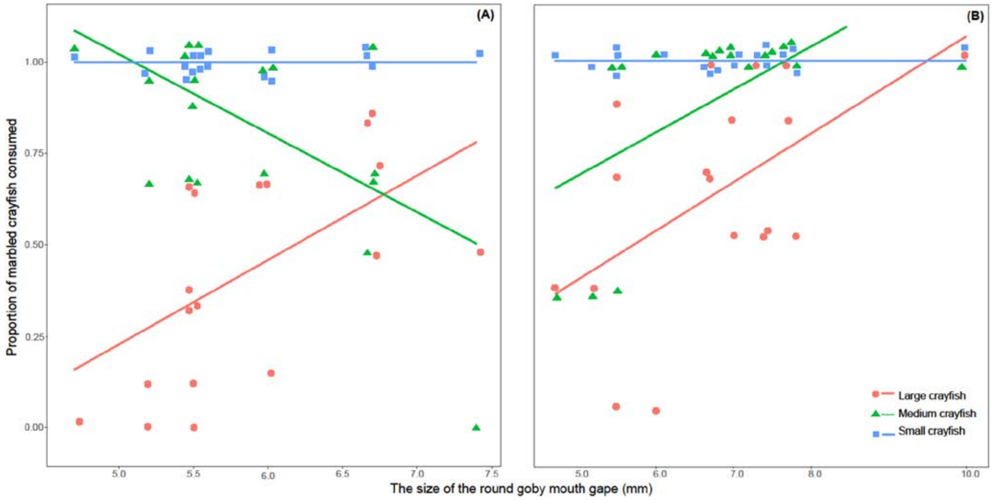

Fig. 3. Relationship of mouth gape of round goby *Neogobius melanostomus* and the proportion of consumed marbled crayfish *Procambarus virginalis* of 3 size classes at 17°C (A) and 21°C (B).

Table 3. Linear relationships between round goby mouth gape and crayfish class size under two temperatures. Negative effect (−), positive effect (+), no effect (0), n = 18.

Crayfish size class	Temperature 17 °C		Temperature 21 °C	
	p-value	R^2	*p*-value	R^2
Small	0.764 (0)	0.480	0.621 (0)	0.472
Medium	0.003 (−)	0.308	0.008 (+)	0.322
Large	0.005 (+)	0.281	0.018 (+)	0.257

consume all provided prey size classes. Due to the significant linear relationship between round goby mouth gape and total length (Supplement 1) we used mouth gape size in further analysis as confounding factor as it is more directly causing the limitation of predation than total length.

Generally, all small and all medium crayfish were consumed (Figs. 1 and 2), while 70% of large crayfish were killed and partially consumed with parts of crayfish found in the experimental arena.

3.1.2 Experiment 2, choice-size predation

Mean mouth gape (diameter) of round goby was 6.2 ± 1.1 (4.7–10) mm. We found a significant relationship among size of the round goby mouth gape and crayfish size class consumption under two different water temperatures ($F_{[2,\ 111]}$ = 17.54, $p < 0.0001$). At 17 °C all small crayfish were eaten in 24 h, with a slightly less number of medium crayfish consumed and few large-sized eaten (Fig. 3). With increasing gape size, round gobies consumed more crayfish of the larger size class, while the number of medium-sized crayfish eaten was negatively correlated with mouth gape (Tab. 3). At 21 °C, all small crayfish were eaten, and the number of consumed crayfish increased compared to 17 °C (Fig. 3). At 21 °C, a positive correlation of mouth gape with the quantity of crayfish of all size classes eaten was observed (Fig. 3, Tab. 3). In general, with an increase of temperature and gape size, predation on crayfish increased. As all small crayfish were consumed at both temperatures, total biomass consumed was 45.6 mg. Average biomass of medium eaten crayfish at 17 °C was 89.29 mg, and at 21 °C was much higher at 156.2 mg. Average biomass of large crayfish eaten was 258.6 mg at 17 °C and 391.95 mg at 21 °C.

3.1.3 Experiment 3, biomass consumption

Mean mouth gape (diameter) of round goby was 5.1 ± 0.34 (4.5–5.5) mm. We found a significant relationship in the number of the two juvenile crayfish classes preyed by round goby ($F_{[1,6]}$ = 11.73, p = 0.01). There was a significant relationship in the amount of consumption of small

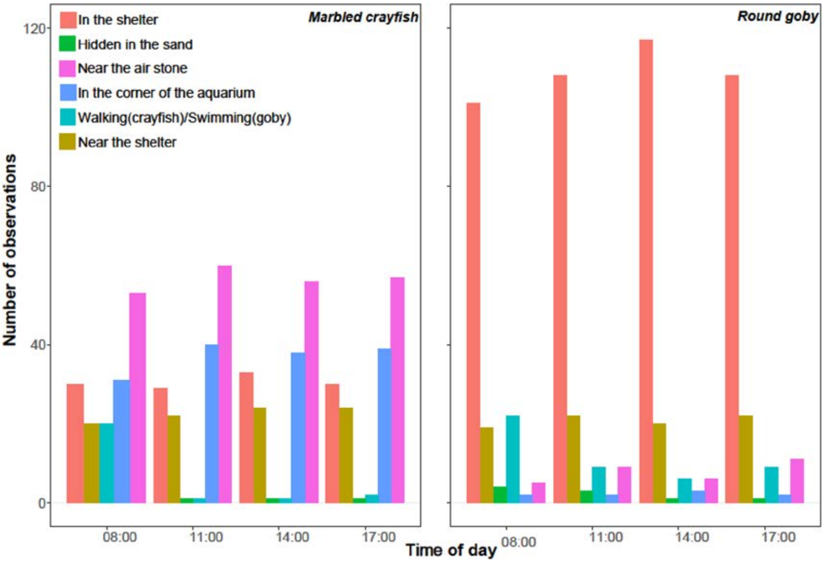

Fig. 4. Number of observations of round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* at specific locations in the experimental arena with respect to time (08:00, 11:00, 14:00, and 17:00).

(5.7 ± 0.63 mg, n = 10) and medium (13.8 ± 4.82 mg, *n* = 10) crayfish biomass by round goby. Round goby consumed a mean of 285 mg small crayfish juveniles (all 50) and ~593.4 mg of medium juvenile crayfish in 24 h. They consumed fewer of the larger group, in average 43 ± 6.48 medium juveniles. The mean biomass of juvenile crayfish consumed was 45.7% of round goby wet weight.

3.2 Competition for shelter

3.2.1 Experiment 4, daily observations of competition for shelter over 8 days

We made 1225 observations. Round goby spent the majority of time in or near the shelter, while marbled crayfish took alternative shelter near the air stone or in the corner of the aquarium (Fig. 4). By day six, 40% of the crayfish had molted and were subsequently killed and partially eaten by round gobies. A single crayfish (weight: 130 mg/TL: 38 mm) was observed (day two) to capture, kill, and consume a round goby (weight: 120 mg/TL: 50 mm).

A GLM analysis with Poisson distribution (dispersion parameter set at 1) showed the number of observations at a given position to differ significantly ($\chi^2 < 806.89$, df = 5, $p < 0.001$). Individuals were observed leaving positions between recorded time points. The number of observations at specific locations at 08:00, 11:00, 14:00, 17:00 h differed significantly ($\chi^2 = 17.36$, df = 18, $p < 0.01$) as well as the number of observations at the locations at 08:00, 11:00, 14:00, 17:00 h between species ($\chi^2 < 526.03$, df = 6, $p < 0.001$) (see Fig. 4).

Table 4. Mean time spent in shelter by round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* during light and dark periods. The data analyzed by GLM with Gaussian distribution. Different superscripts indicate significant differences ($p < 0.05$) between species. Data are presented as mean ± standard deviation.

Animal	Photoperiod	Average time spent in shelter (s)
Round goby	Light	15851 ± 11420^{a}
	Dark	12378 ± 13640^{a}
Marbled crayfish	Light	22365 ± 18435^{b}
	Dark	15723 ± 11114^{a}

3.2.2 Experiment 5, twenty-four-hour observation of competition for shelter

Individuals of both species were always observed to leave the occupied shelter when a specimen of the other species entered. Crayfish pursued goby with open chelae, one capture was observed during the experiment.

We found a significant difference between species in the use of shelter during light and dark periods ($t = 0.51$, $p < 0.05$), as the species alternated in shelter occupancy (Tab. 4). Marbled crayfish spent more time in shelters during light hours. Using GLM analysis, we found a significantly greater number of attacks during the light period than during the dark period by both species ($t = 1.78$, $p < 0.05$) (Tab. 5), with round goby

Table 5. The total number and percentage of attacks observed by round goby *Neogobius melanostomus* and marbled crayfish *Procambarus virginalis* during light and dark periods and the total time spent (s) in aggressive behavior. The data analyzed by GLM with Gaussian distribution. Different superscripts indicate significant differences ($p < 0.05$).

Animal	Photoperiod	Number of attacks (%)	Duration of attacks (s)
Round goby	Light	237 (60.61)	2140^b
	Dark	127 (54.04)	324^a
Marbled crayfish	Light	154 (39.39)	926^b
	Dark	108 (45.96)	274^a

showing higher aggression. Round goby attacked crayfish more during day, while it was less active during night but still performing attacks more than crayfish (Tab. 5).

4 Discussion

Round goby has colonized major European river systems and coastal waters as well as North American freshwater ecosystems and has the potential to cause ecological regime shifts (Borcherding *et al.*, 2011; Hempel and Thiel, 2013; Roche *et al.*, 2015). Many European lake and stream communities also harbor non-native crayfish species that have considerable impact on native species, such as the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), the spiny cheek crayfish *Faxonius limosus* (Rafinesque, 1817), and, relatively recently, the marbled crayfish *P. virginalis* (Kouba *et al.*, 2014; Kubec *et al.*, 2019). We focused on interaction of round goby, recently spread to upper stretches of the River Elbe (Roche *et al.*, 2015; Buřič *et al.*, 2015), with marbled crayfish as a model species representing the North American crayfish family Cambaridae (Hossain *et al.*, 2019).

Round goby usually preys upon small bottom-dwelling vertebrates and invertebrates (Mikl *et al.*, 2017), but is adaptable to feeding on a wide array of available prey types, including scavenging of carcasses, and particle sizes (Polačik *et al.*, 2009, 2015; Perello *et al.*, 2015). Crustaceans and mollusks represent the most important food items (Brandner *et al.*, 2013). Crayfish can serve as an important food source for predatory fish, as demonstrated by a study of marbled crayfish (Lipták *et al.*, 2019). Due to the results of experiment 1, it is hard to interpret if round goby was size-selective or not, but mouth gape and total length were significant limiting factors for consumption of larger crayfish class. Gobies were able to swallow all small and medium crayfish, but also to kill and partially consume the larger prey as larger crayfish were rather too big for smaller goby individuals. Non-selectivity of round goby was confirmed with mussels as prey (Perello *et al.*, 2015). Ray and Corkum (1997) observed that round gobies spit out the entire mussel shell as well as pieces, similar to our observations of crayfish cuticula in aquaria. Higher temperature induced higher consumption of marbled crayfish (especially medium size class) by round goby. The positive temperature effect on round goby food consumption confirmed results of Lee and Johnson (2005) who showed increased consumption to 23–26 °C. But, results of experiment 2 suggest an interaction in proportion of marbled crayfish consumed between medium and large crayfish size with increasing mouth gape (Fig. 3) at 17 °C but not at 21 °C. This indicates that larger gobies might prefer larger prey at lower temperatures while showing no preference at higher temperatures. As the consumption of larger prey items is more energy-efficient (Sih, 1980), this feeding behavior might be advantageous especially at low temperatures because energy loss is more important for a predator (Rall *et al.*, 2010). In contrast, in exp. 1. bigger crayfish were less consumed but here no free prey size selection was possible as we used pairs of single goby and single crayfish and crayfish were randomly put in aquarium, thus it could be that smaller gobies were combined with bigger crayfish and could not predate on them. Round goby was able to consume a large number of crayfish at higher temperatures without showing signs of size-selectivity. This is contrary to crayfish size selection of smallmouth bass *Micropterus dolomieu* (Lacépède, 1802), which chose the smallest crayfish *Faxonius propinquus* (Girard, 1852) first and then consumed animals in ascending order of size (Stein, 1977), but also noted by other predatory fishes. All in all, round goby in our study consumed from 200 to 500 mg of crayfish biomass per day, which was almost half of their own weight.

Generally, crayfish species share habitats with small bottom-dwelling fish species, such as darters (Mayden *et al.*, 1992) and gobies (Church *et al.*, 2017). Benthic fish can affect crayfish abundance through predation, and crayfish may compete with them for food resources (Thomas and Taylor, 2013). Benthic fish and crayfish naturally overlap in habitat use, with both primarily utilizing natural shelter beneath stones (Kubec *et al.*, 2019), and exhibit a strong temporal overlap in their requirement for shelter (Cooper *et al.*, 2009). In addition, both tested species are mainly nocturnal (Savino *et al.*, 2007; Kornis *et al.*, 2012; Kubec *et al.*, 2019), and competition for shelter is likely to be strongest during the daylight hours (Hill and Lodge, 1994). Despite the fact that round gobies feed more during daylight hours, nocturnal feeding is facilitated by well-developed sensory systems enabling rapid and precise localization and capture of prey (Jude, 1997). With the adaptability to utilize food sources and space upon which crayfish depend (Kornis *et al.*, 2012, 2013), it can be a strong competitor.

The previously reported decreased shelter occupancy by round goby during the night (Dubs and Corkum, 1996; Savino *et al.*, 2007) was confirmed in our study. Crayfish, which show highest activity at dusk and during the night, were also reported to seek shelter during daylight (Bubb *et al.*, 2009). An experiment confronting non-native signal crayfish with bottom-dwelling European bullhead *Cottus gobio* (Linnaeus, 1758), resulted in reduced shelter use by the fish (Bubb *et al.*, 2009). However, our results from the experiment 4 with daily observations showed that round goby spent more time in shelters than marbled crayfish, which used alternative hiding places. Round goby and marbled crayfish spent roughly equal amounts of time in the shelter as well as equal time in avoidance. This could be the result of avoidance after dominance establishment subsequent to aggressive interactions, as well as the dominant status of round goby, as crayfish remained outside shelter more than expected. Bilateral avoidance confirms reciprocal alteration of behavior of the tested species.

Due to the results of experiment 5, observations of aggressive encounters showed that crayfish were usually able to resist round goby attacks, except after molting. Their size itself was not an obstacle to consumption by round goby as long as the carapax was intact. Following molt, even a large crayfish can be extremely vulnerable to predation (Stein, 1977), and it is reported that small crayfish in particular may be vulnerable to predation by round goby when they are soft-shelled immediately following a molt (Ray and Corkum, 1997). Our results showed that even crayfish of similar weight to the fish could be preyed upon by round goby after molting, which is remarkable when the mouth gape size of round goby is taken into account.

Both species showed intense aggressive behavior in experiment 5. Round goby displayed more aggressive behavior than crayfish in our study, as reported by Church *et al.* (2017), but we observed a single instance of a crayfish attacking, killing, and feeding on a round goby. Round goby can hide in the sand and attack crayfish from behind, but were more likely to occupy shelters also used by crayfish. Round goby presence altered the shelter use by crayfish, but round goby behavior is also affected by crayfish presence, demonstrating a mutual interaction of two unrelated invasive species that occupy similar habitats.

Further investigation is necessary to elucidate interactions of round goby and larger crayfish in more complex conditions, as well as to estimate an effect on crayfish population structure. Altered behavior in both species can lead to higher vulnerability to predators (Blake and Hart, 1993; Kubec *et al.*, 2019). Competition for resources can induce increased pressure on resources and accelerate migration to habitats with lower pressure as well as to shifts in ecological niche (Gherardi, 2007). Findings of this study can be useful in the management and conservation of the native North American Cambarid species, but also round goby could exert a control on other species juvenile crayfishes, so eradication of round goby would be considered.

As many other factors are responsible for invader success in natural conditions (Gebauer *et al.*, 2018), the present study provides a baseline for ongoing study of particular scenarios and factors that influence the success and spread of alien aquatic organisms. Our results are in accordance with the known predatory capability of round goby and broaden it from the point of interactions with crayfish representative. Early juvenile crayfish are most vulnerable due to round goby predation. Round goby can possibly cause considerable declines in invasive crayfish species of genus Cambaridae in Europe. Naturally, they can have similar effects on other cambarid species in their native range in North American freshwaters. Hence our results can be easily transferred also to localities in North America inhabited by round goby.

Supplementary Material

Figure S1. The simple linear relationship between round goby mouth gape and round goby total length were undertaken using the default lm function in the R statistical program (R Core Team, 2020). Correlations which had an adjusted $R^2 > 0.5$ and $p < 0.05$ were considered to represent significant relationship. Significant relationships between mouth gape and total length would represent a potential confounding factor in further analysis.

Figure S2. Tools used for mouth gape measurement.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2021019/olm.

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CHAPTER 4

COMPARISON OF BEHAVIOR AND SPACE USE OF THE EUROPEAN BULLHEAD *COTTUS GOBIO* AND THE ROUND GOBY *NEOGOBIUS MELANOSTOMUS* IN A SIMULATED NATURAL HABITAT

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Comparison of Behavior and Space Use of the European Bullhead *Cottus gobio* and the Round Goby *Neogobius melanostomus* in a Simulated Natural Habitat

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Simple Summary: Invasive round goby and native European bullhead are bottom-dwelling species that occupy a similar niche and are expected to compete for similar resources. Knowledge of how species behave in novel conditions without competition has implications for how they will interact. Our objective was to investigate the space use, flow velocity preferences and tolerance, shelter use, feeding behavior, and activity patterns of specimens of both species to better understand their basic behavior characteristics. Space in a habitat simulator system was divided for purposes of analysis into seven zones among which fish could move freely. We observed individual fish during acclimatization periods and normal behavior periods of darkness and light. The results showed few differences between species. Round goby showed a preference for areas with slow running water, whereas the bullhead gravitated to higher velocity waters. The species exhibited similar patterns in time spent in zones in a given period, spending most of the time in the shelter and the mid-stream zone. Despite the low differences observed, we can conclude that the fish needed a long acclimatization period for behavior stabilization, which should be considered in future studies.

Abstract: The round goby is an invasive fish in Europe and North America that threatens native species by predation and competition. Its habitat preferences are similar to those of the European bullhead, which it displaces from shelters and out-competes for available resources. We assessed the microhabitat preferences, shelter use, and activity of the round goby and European bullhead in single-species experiments in habitat simulator systems to investigate their behavior in a novel environment. Fish were video-recorded for 28 h in the presence of shelter and feed with water velocity ranging from 0.00 to 0.96 m s^{-1}. The two species showed similar behavior under given conditions. A primary difference was in stress-induced behavior in the initial phases of observation. The round goby spent more time in movement when outside the shelter and a longer time in the escape zone in the exploration period during light. Our results confirmed a significant preference of round goby for low velocity areas and a preference for higher velocities in the European bullhead. Both species were able to cope with velocities > 0.7 m s^{-1}. Therefore, the reported invasion success of round goby is probably not driven by space use or activity patterns, but rather by higher adaptability.

Keywords: habitat overlap; cottiid; gobiid; biological invasion; shelter occupancy; water velocity

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1. Introduction

Freshwater ecosystems increasingly face native species decline and extinctions and biodiversity loss as a consequence of bioinvasions [1,2]. Direct competition between native and non-native species is a main driver of species replacement, with the latter often more active, aggressive, and prolific, and hence more successful in acquiring and holding resources [3,4]. The differences in ecological preferences and tolerances of interacting species can allow abiotic conditions to modify their competition [5]. In freshwater systems, factors such as hydrodynamics, temperature, slope, habitat complexity, and type of substrate may influence the outcome of direct interactions [6–8] and affect the displacement of one species by another, their co-existence in a location, or their separation in space [9]. Consequently, it is necessary to examine the potential results of interactions of invasive and native organisms under the varied abiotic conditions, beginning with obtaining information with respect to species basic behavior patterns, space use, and habitat preferences.

Monitoring of the Ponto-Caspian round goby *Neogobius melanostomus* (Pallas 1814) invasion success [10–12] suggests problematic contacts with populations of the vulnerable and endangered European bullhead *Cottus gobio* (L.). The round goby has been one of the most successful non-native fish invaders in European and North American inland and coastal waters in recent decades [13,14]. The species has invaded and expanded its range in large European rivers, including the Danube (possibly natural dispersal), Rhine, Vistula, Volga, and Elbe [15–20], expanding into their upper stretches and larger tributaries [12,21–23].

The round goby is a small bottom-dwelling species usually associated with crevice habitats [24] and is generally territorial and aggressive [25,26]. Competition with native European and North American species of the family Cottiidae (sculpins) of similar size, environmental requirements, and biology is reported, as both species have the same spawning ground preferences, feeding areas and food types [3,27,28].

The European bullhead is protected under the European Habitat Directive (HD; Annex II, 92/43/EEC) and is an important target species for conservation according to the European Water Framework Directive (WFD; 200/60/EC). It is representative of species potentially affected by round goby invasion [29–31]. The European bullhead occurs in freshwater streams, rivers, and lakes with hard stony substrate and shows a preference for shallow fast-flowing water bodies [9]. Its populations are reported in large rivers living in syntropy with the round goby [23,32], implying that they share an ecological niche and directly compete for resources and space in co-inhabited ecosystems. Natural or artificial stones such as ceramic tiles in rivers are vital to assure viable sculpin populations [33]. The nocturnal habits of European bullhead are confirmed [34,35] with solitary bullheads spending the majority of time in shelter during daylight hours [28]. The round goby is also considered nocturnal, with less shelter occupancy in periods of darkness [36,37].

Reported declines in the European bullhead populations [38] coinciding with round goby invasions of the Danube [39] and Rhine [40], along with similar reports for related species from North America [4,41], suggest that Ponto-Caspian gobies can have an adverse impact on sculpins. The round goby and European bullhead were recently reported to co-occur in the Elbe River near the border of the Czech Republic and Germany and in the lower stretches of its tributaries [12], and moreover in at least three Saxon tributaries (Worischka personal observation).

How the interaction of these species is impacted by such habitat conditions as water velocity, shelter, availability of food, and space for surviving is unknown. In case of increased habitat complexity higher abundances of both species are expected, but on the other hand high complexity habitat could reduce predation and competition, allowing more shelter space for the species to occupy. Observations of basic behavior patterns under simulated natural conditions in single-species trials may reveal species space use and preferences in a novel environment unrestricted by the presence of a competitor species.

The present study aimed to characterize round goby and European bullhead light/dark behavior with respect to water flow velocity, shelter availability, and feeding. We hypothesized (1) a higher activity level of round goby than of European bullhead during all 28 h duration of the experiment, (2) more rapid adaptation of the round goby to the novel environment, (3) species-specific flow velocity preferences; European bullhead would use faster flowing areas and round goby would use low velocity areas, (4) difference in activity modes of species depending on light conditions, and (5) greater food intake of the round goby.

2. Materials and Methods

2.1. Animal Acquisition and Maintenance

Round goby specimens were collected using a backpack pulsed-DC electrofishing unit (FEG 1500, EFKO, Leutkirch, Germany) in April 2019 from recently colonized sites in the Elbe River near Děčín, Czech Republic. The site was electrofished during the day based on accessibility for a set amount of time (15 min), working upstream and sampling from bank to bank covering all microhabitats (total 100 m) and targeting smaller fish species. Each site was finished once by four people, one using the anode and the others assisting in the collecting the stunned fish with a dip-net and transporting them to a big box in which fish were later transported to the Institute. The collecting site had a rocky bankside, bottom covered by stones, sand and aquatic vegetation. The European bullheads were collected by electrofishing based on a permission of the Regional Authority of the South Bohemia Region (No. OZZL 104213/2018/pedo SO 2) in Vyšší Brod (Vltava River, Czech Republic, GPS coordinates: 48°37′11.4″ N, 14°18′51.8″ E). All bullheads originated from stable, long-term existing populations that represent naive populations unaffected by *N. melanostomus* presence. The capture locality represents riffle with water depth 0.1–0.5 m and riverbed formed by a mixture of stones (particle diameter = 0.1–0.5 m) and gravel (particle diameter = 0.03–0.1 m). All bullheads were released at the capture locality after experiments termination.

Fifty round gobies and thirty European bullheads were transferred to the experimental facility of the Research Institute of Fish Culture and Hydrobiology in Vodňany, the University of South Bohemia in České Budějovice, Czech Republic. The species were held separately in two identical recirculation aquaculture systems. Each species was stocked in two rectangular trays each filled with 240 L aged tap water and equipped with an excessive number of shelters (>3 per individual). A retention tank (filled with approx. 1000 L aged tap water) with a filtration unit and pump supplied the water for the trays and enabling constant water flow. Every three days, 1/3 of the water was exchanged for fresh aged tap water and checked for pH (7.8–8.0). Fish were acclimatized to the temperature of 16 °C for 30 days using cooling system (JDK Dixell XR20CX) with a light regime of 12:12 L:D prior to the beginning of the experiment. Acclimation tanks were structured to prevent fish escape and placed indoors with no direct connection to surface waters to prevent unwanted escape of round goby to natural environment. During the acclimation period, fish were fed ad libitum with common flesh-fly *Sarcophaga carinaria* (L.) larvae daily. Uneaten food was removed by siphoning.

2.2. Experimental Setup

Thirty trials (n = 15 fish per species) were conducted in May and June 2019. The experimental setup comprised three independent recirculation systems, each consisting of a channel 2000 mm × 310 mm × 390 mm (L × W × H), a 2000 mm × 510 mm × 400 mm (L × W × H) water retention tank containing aged tap water, and a recirculation pump (WILO IPL 80/1, WILO SE, Dortmund, Germany). Adjustable valves and bypasses enabled regulating the flow velocity.

Experiments were conducted at 16 °C water temperature. Before each trial, flow velocity was measured at 20 points in the channels by a Flowmeter (MiniController MC20 with the Flowprobe for MiniWater20, Schiltknecht Messtechnik AG, Schaffhausen, Switzerland) to characterize water flow variations throughout the channel and ensure similar conditions in all channels (Figure 1, Table S1).

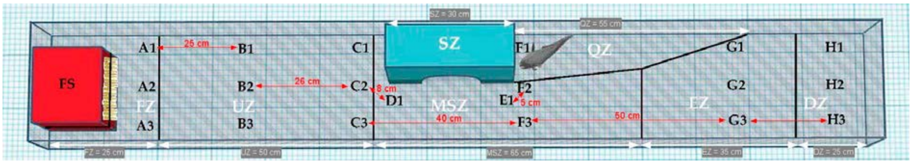


Figure 1. Schematic of the experimental channel showing monitored zones (white letters) FZ = feeding zone where FS = feeding station was situated, UZ = upstream zone, MSZ = mid-stream zone, SZ = shelter zone, QZ = quiet zone, DZ = downstream zone, and EZ = escape zone; points for flow velocity measurement (black numbers A1–H3). Zones are delineated by black lines. Distance between points for flow velocity measurement is noted by red color and distance between monitored zones is marked with white letters on grey background.

Each experimental system was equipped with an 8 cm concrete cube located in the upstream area of the system that served to regulate, buffer, and dissipate the strong turbulent flow (water height: 18 cm) at the channel inlet as well as being a feeding station that formed the beginning of the feeding zone (Figure 1). Fifteen *S. carinaria* larvae were attached to fishing line wrapped around the cube, requiring fish to actively pluck the prey from the line. A second concrete block (8 cm × 16 cm), placed approximately in the middle of the tank blocked the flow to provide a quiet zone, and a 10 cm × 8 cm × 4 cm (L × W × H) cavity cut into the lower side of the block furnished shelter. Each experimental channel was divided into: FZ—feeding zone where FS—feeding station was situated, UZ—upstream zone, MSZ—mid-stream zone, SZ—shelter zone, QZ—quiet zone, DZ—downstream zone, and EZ—escape zone (Figure 1).

Fish were approximately measured before each trial and separated for the starving, to have comparable sizes of both species. Specimens with a wet-weight difference of <5% were used in the experiment (Table 1). Fish were unfed for 24 h prior to stocking in the channel. The channels were monitored by a camera system (iGET Homeguard HGDKV-87704, 1080P 3 Channel Digital Video Recorder) attached above experimental channels and connected to a computer. Photoperiod was simulated in order to keep light/dark conditions without the changes during the experimental trials. Permanent indirect illumination was provided by fluorescent tubes (daylight, 2310 lm). Fish were video-recorded for 28 h, from 10.00 h on day one to 14.00 h on day two. We design acclimatization periods (stress response, exploration in light and dark) separated from normal behavior periods in dark and light. Hence, five time periods within the 28 h were analyzed: 1—stress response period (SR), 10:00–14:00 h; 2—exploration period in light (EPL), 14:00–18:00 h; 3—exploration period in dark (EPD), 18:00–22:00 h; 4—normal behavior in dark conditions (NPD) for 8 h, 22:00–06:00 h; and 5—normal behavior in light conditions (NPL) for 8 h (06:00–14:00 h). Each fish was used only once, and after use, all European bullhead specimens were acclimatized to outside ambient temperature for seven days and released into their place of origin.

Table 1. Biometric data of the round goby (RG) *Neogobius melanostomus* and European bullhead (EB) *Cottus gobio*. Total length (TL), standard length (SL), weight (W), and the average number of *Sarcophaga carinaria* larva consumed during the 28 h experiment. Data are mean ± SD. Same letter in the superscripts within the rows indicate no significant differences between species in measured parameters.

Species	n	TL (mm)	SL (mm)	W (g)	Eaten Larvae
RG	15 (12F-3M)	85.3 ± 11.5	70.7 ± 9.7	8.5 ± 5.9	4.7 ± 5.0^{a}
EB	15	93.3 ± 13.3	78.7 ± 12.5	8.3 ± 3.9	4.7 ± 5.1^{a}

Fish were weighed using a precision digital balance (Kern 572-35, Kern and Sohn, Germany) to the nearest 0.1 g, and total (TL) and standard length (SL) were measured with a ruler to the nearest 1 mm. Sex of the round gobies was determined based on anal papilla shape. We were unable to determine sex of *Cottus gobio* because of absence of a reliable sex distinguishing method (based on external morphological appearance) without the need of fish sacrifice and gonads inspection. The number of uneaten larvae was counted after each trial, and a new line with fresh larvae was prepared for the subsequent trial.

Video-recordings were analyzed using the automatic ethological software EthoVision® XT software 13.0 (Noldus Information Technology, Wageningen, The Netherlands) that recognizes, tracks, and analyzes the behavior, movement, and activity of fishes. Video-recordings were subsequently checked visually and adjusted/trimmed if errors interfered with detection of fish movement. This is a crucial step at the beginning of data collection, since tracking errors affecting multiple sample points can indicate a problem with the experimental set-up, camera set-up, arena settings, trial control settings, and/or detection settings. The active movement during the trial, distance moved, time spent outside the shelter, time spent in motion outside of the shelter, and time spent in a specific channel zone (Figure 1) were recorded in all time periods.

2.3. Statistical Analyses

Data obtained from EthoVision XT 13.0 software were exported to Excel files and analyzed by R software (R Development Core Team, v. 4.0.3., 2020), with the package ggplot2 used for data visualization. Video-tracked behavior patterns included shelter occupancy and space preference, preferred and avoided channel zones, preferred and avoided flow velocities, time spent in motion, distance moved, and number of larvae consumed.

Data were checked for normality and homoscedasticity with Shapiro–Wilks and Bartlett's tests, respectively. When criteria were met, one-way ANOVA was employed to compare water velocity at each of the 20 points among the three experimental channels. Because data showed non-normal distribution even after transformation, the Mann–Whitney U test (Wilcoxon test) was used to determine differences in the number of larvae eaten by the European bullhead and round goby. The ANCOVA with Tukey's post hoc test was used to compare the total number of larvae consumed by each species relative to time spent in the feeding zone. A simple linear relationship was used between number of fly larvae consumed and total time spent in feeding zone by round goby and European bullhead. Kruskal–Wallis tests were used to compare the time that an individual fish spent in each zone during a given time period. Wilcoxon test with Bonferroni correction applied to the significance level was used for species comparison of the time spent in each of the seven zones. Mean distance moved (cm), time spent outside the shelter (in seconds and %), time spent in motion outside the shelter (in seconds and %), and total time of active movement during the trial were calculated separately for each species. Results were considered significant at $p \leq 0.05$.

3. Results

3.1. Water Velocity

Water velocity in the channel ranged from no velocity 0.00 to 0.96 m s^{-1} (min-max) with no significant difference among channels at any velocity measurement point (ANOVA $_{(2, 57)}$, F = 0.33, p = 0.968) (Table S1).

3.2. Food Intake

There was no significant difference in the number of larvae consumed with respect to species (Wilcoxin test = 116.5, p = 0.8833) (Table 1). A significantly higher number of larvae consumed relative to the time spent in the feeding zone was observed in the round goby compared to the European bullhead (ANOVA $_{(1, 26)}$, F = 4.230, p = 0.0499). The time spent in the feeding zone was not significantly related to the number of larvae consumed in either species (European bullhead: Y = 3.47x + 0.00060, R^2 = 0.1704, p = 0.07; round goby: Y = 4.20x + 0.00046, $R^2 = -0.017$, p = 0.398) (Figure S1). The mean water velocity in the feeding zone was 0.71 m s^{-1}, demonstrating that both species successfully coped with high velocities.

3.3. Spatial Pattern in Fish Diurnal/Nocturnal Activity

The activity of the European bullhead and the round goby was expressed as duration of movement during the entire 28 h trial, or per hour in cases of different length of time periods in the seven channel zones.

The species differed significantly with respect to time in a given zone (Figures 2 and 3). During acclimatization periods (stress response: round goby $\chi^2_{(6)} = 51.21$, European bullhead $\chi^2_{(6)} = 54.071$; exploration period light: round goby $\chi^2_{(6)} = 35.42$, European bullhead $\chi^2_{(6)} = 48.32$, and exploration period dark: round goby $\chi^2_{(6)} = 42.56$, European bullhead $\chi^2_{(6)} = 47.03$ ($p < 0.001$, n = 15)) the species showed a similar pattern of movement, with the greatest difference being in time spent in each zone followed by more or less lively movements (Figure 2). Both species spent most of their time in the shelter and mid-stream zone with average water velocity of ~0.29 m s^{-1}, then upstream and downstream zones with water velocity ~0.30 m s^{-1}, with the least time spent in the quiet zone with velocity ~0.02 m s^{-1}, escape zone at ~0.39 m s^{-1}, feeding zone at 0.7 m s^{-1} (Figure 2). The only significant difference between species was during the exploration period light when the round goby was more significantly active in the escape zone (European bullhead 42.4 ± 66.3, round goby 289.5 ± 437.9) ($p = 0.046$) (Figure 2).

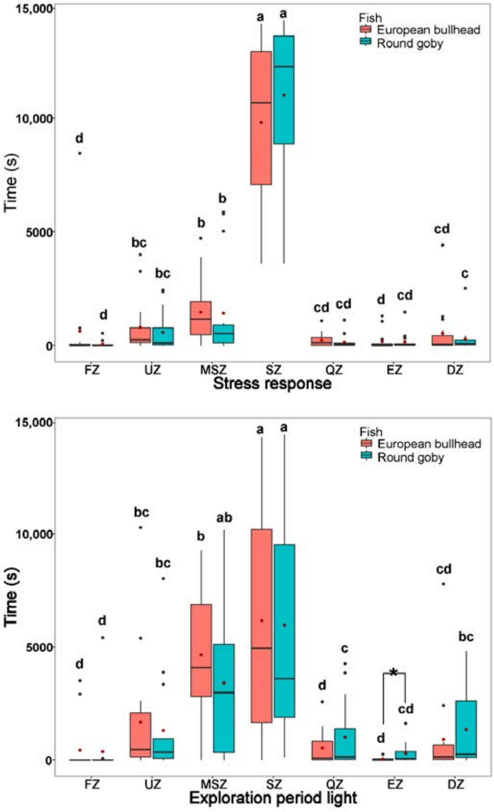


Figure 2. *Cont.*

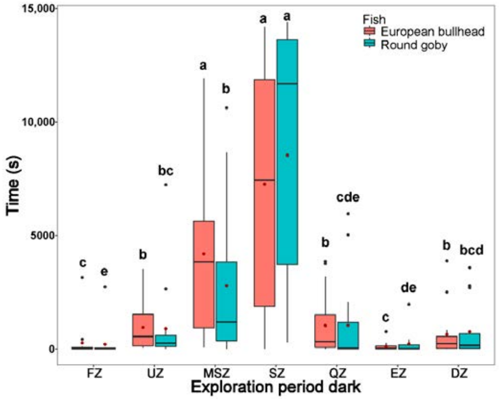
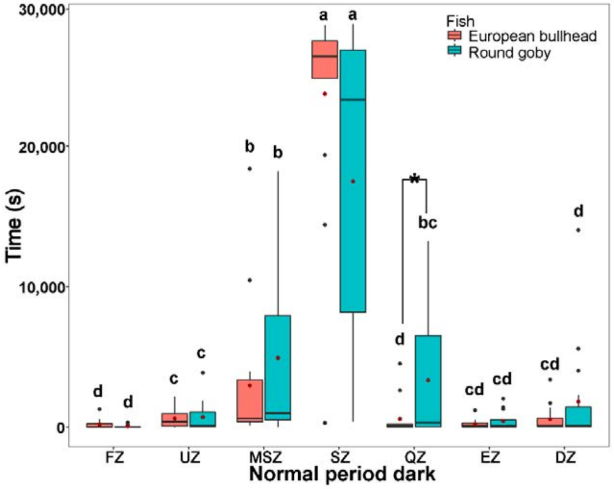


Figure 2. Time spent by *Cottus gobio* (red) and *Neogobius melanostomus* (turquoise) in channel zones during acclimatization periods: stress-response period (upper box plot), exploration period in light (middle box plot), and exploration period in dark (lower box plot). Box limits correspond to upper (Q3—75%) and lower (Q1—25%) quartiles, horizontal bar to the median, and red dot to the mean. Outliers are indicated by points (min = Q1 − 1.5 × IQR; max = Q3 + 1.5 × IQR). FZ = feeding zone, UZ = upstream zone, MSZ = mid-stream zone, SZ = shelter zone, QZ = quiet zone, EZ = escape zone, DZ = downstream zone. Values with different letters indicate significant differences (α = 0.05); asterisk indicates significant interspecific differences.



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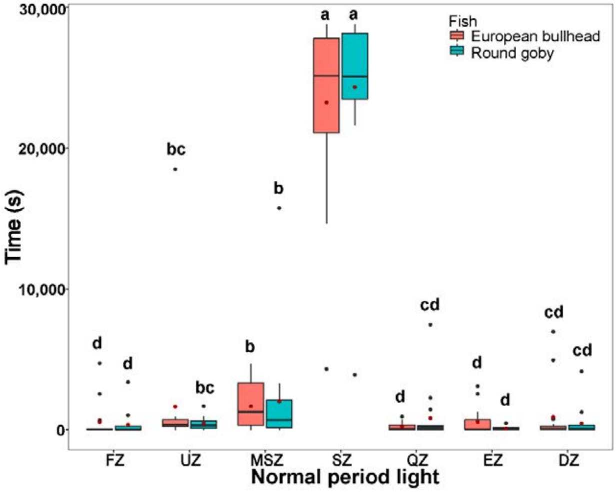

Figure 3. The time spent by *Cottus gobio* (red) and *Neogobius melanostomus* (turquois) in channel zones during two periods of tracking normal behavior in darkness (upper box plot), and light (lower box plot). Box limits correspond to upper (Q3—75%) and lower (Q1—25%) quartiles, horizontal bar to the median, red dot to the mean, outliers are indicated by points (min = Q1 − 1.5 × IQR; max = Q3 + 1.5 × IQR). FZ = feeding zone, UZ = upstream zone, MSZ = mid-stream zone, SZ = shelter zone, QZ = quiet zone, EZ = escape zone, DZ = downstream zone. Values with different letters indicate significant intraspecific differences ($\alpha = 0.05$); asterisks indicate significant interspecific differences.

During the normal behavior periods in dark and light (normal period dark: round goby $\chi^2_{(6)} = 44.66$, European bullhead $\chi^2_{(6)} = 51.51$; normal period light: round goby $\chi^2_{(6)} = 47.83$, European bullhead $\chi^2_{(6)} = 50.41$ ($p < 0.001$, n = 15)) patterns of movement in zones were similar to those in the acclimatization periods, with the round goby showing more activity in moving but not statistically significant through the shelter zone during the normal behavior period in darkness than did the European bullhead, which showed more targeted movement in this zone (Figure 3). In the normal behavior period in light, the European bullhead was more active than the round goby, but both species moved less than in other time periods (Figure 3, Table 2). The primary difference between species in normal period dark was significantly more time spent in the quiet zone by the round goby (European bullhead 569.4 ± 1235.6, round goby 3344.9 ± 4644.5) ($p = 0.033$).

During acclimatization periods, the European bullhead moved longer distances than the round goby, which moved greater distances in the normal behavior period in darkness (Table 2). The round goby showed more time in movement during the time periods, with the exception of stress response, than the European bullhead, but differences were not significant (Table 2). During acclimatization, the European bullhead spent more time outside the shelter in the stress response and exploration period in light compared to the round goby, which spent more time outside shelter during the exploration period in dark (Table 2). During the normal behavior periods, the round goby spent more time outside the shelter in light compared to the bullhead, while time spent outside the shelter during darkness was similar in both species (Table 2).

Table 2. The distance moved (cm), time spent outside shelter (sec and %), time spent in motion outside the shelter (sec and %), and proportion of time spent in movement (%) during the trial observed in *Neogobius melanostomus* (RG) and *Cottus gobio* (EB). SR = stress response period, EPL = exploration period in light, EPD = exploration period in darkness; NPD = normal behavior period in darkness, NPL = normal behavior period in light. Values with different letters within rows indicate significant intraspecific differences ($\alpha = 0.05$). Data are mean ± SD.

Species	Time Period	Distance Moved (cm)	Outside the Shelter (s)	Outside the Shelter (%)	Motion outside the Shelter (sec)	Motion outside the Shelter (%)	Active Movement during the Trial (%)
RG	SR	1129.5 ± 1456.1^{a}	2671.5 ± 2693.0^{a}	18.6 ± 18.7^{c}	1058.0 ± 881.6^{a}	53.6 ± 27.4^{b}	7.3 ± 6.1^{c}
EB	SR	1551.4 ± 1624.4^{a}	3864.8 ± 3077.1^{a}	$26.8 \pm 21.4^{b,c}$	1091.9 ± 975.5^{a}	35.5 ± 23.4^{b}	7.6 ± 6.8^{c}
RG	EPL	3194.2 ± 2871.4^{a}	7720.0 ± 5265.8^{a}	53.6 ± 36.6^{b}	2556.5 ± 2603.6^{a}	35.5 ± 28.5^{b}	17.8 ± 18.1^{b}
EB	EPL	3773.8 ± 3342.2^{b}	8242.5 ± 5117.0^{a}	57.2 ± 35.5^{c}	2415.2 ± 1716.5^{b}	39.8 ± 28.1^{c}	16.8 ± 11.9^{d}
RG	EPD	2478.8 ± 2410.7^{a}	3217.1 ± 4501.2^{a}	$22.3 \pm 31.3^{c,d}$	$1119.2 \pm 1600.3^{a,b}$	$46.7 \pm 27.5^{b,c}$	7.8 ± 11.1^{d}
EB	EPD	2951.4 ± 2579.4^{a}	2030.4 ± 2217.1^{b}	14.1 ± 15.4^{d}	659.7 ± 579.3^{b}	47.2 ± 32.1^{c}	4.6 ± 4.0^{d}
RG	NPD	2294.0 ± 3761.2^{b}	10622.4 ± 8817.1^{a}	36.8 ± 30.7^{c}	4234.0 ± 5488.9^{b}	44.5 ± 27.1^{c}	14.6 ± 18.9^{d}
EB	NPD	1207.1 ± 1234.3^{c}	10411.7 ± 10275.1^{a}	36.1 ± 35.6^{d}	3640.2 ± 4753.0^{b}	45.4 ± 25.1^{d}	12.6 ± 16.5^{e}
RG	NPL	1772.6 ± 2797.5^{b}	9796.1 ± 8455.6^{a}	$34.1 \pm 29.4^{c,d}$	4933.0 ± 4637.1^{a}	52.5 ± 26.1^{c}	17.1 ± 16.2^{d}
EB	NPL	1828.3 ± 2117.4^{b}	6828.0 ± 7682.5^{a}	23.7 ± 26.7^{d}	$2864.3 \pm 3243.2^{a,b}$	54.8 ± 22.9^{c}	9.9 ± 11.3^{d}

3.4. The Temporal Pattern in Fish Diurnal/Nocturnal Activity

There were no significant differences in time feeding (round goby $\chi^2_{(4)} = 0.4$; European bullhead $\chi^2_{(4)} = 5.93$) and in upstream (round goby $\chi^2_{(4)} = 0.9$; European bullhead $\chi^2_{(4)} = 1.58$), mid-stream (round goby $\chi^2_{(4)} = 4.7$; European bullhead $\chi^2_{(4)} = 9.5$), quiet (European bullhead $\chi^2_{(4)} = 4.4$), downstream (round goby $\chi^2_{(4)} = 3.1$; European bullhead $\chi^2_{(4)} = 1.6$), and escape zones (round goby $\chi^2_{(4)} = 3.4$, n = 15; European bullhead $\chi^2_{(4)} = 5.2$) between species with respect to time period ($p > 0.05$, n = 15).

Both the round goby and European bullhead spent a significantly longer time in shelter during all time periods than in other zones (round goby $\chi^2_{(4)} = 23.397$; European bullhead $\chi^2_{(4)} = 44.148$ ($p < 0.001$, n = 15)) (Figure 4). The round goby spent significantly more time in the quiet zone (round goby $\chi^2_{(4)} = 5.6$, $p = 0.049$, n = 15) than did the European bullhead during the normal period in dark.

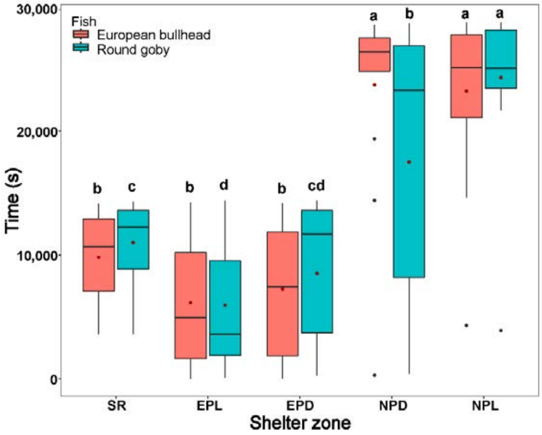

Figure 4. *Cont.*

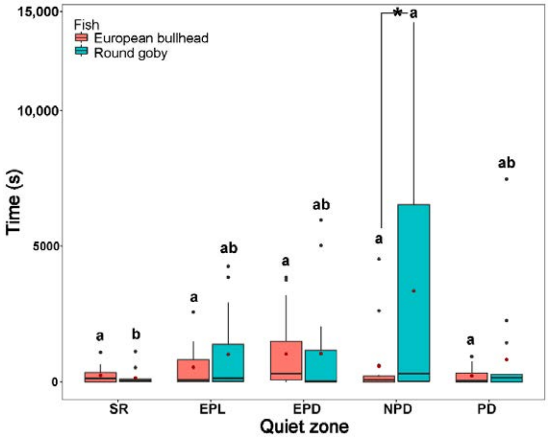

Figure 4. The time in shelter and quiet zones during the stress response period (SR), exploration period in light conditions (EPL), exploration period in dark conditions (EPD), normal behavior period in darkness (NPD), and normal behavior period in light (NPL) by the European bullhead and the round goby in a 28 h trial. Box limits correspond to upper (Q3—75%) and lower (Q1—25%) quartiles, horizontal bar to the median, and red dot to the mean. Outliers are indicated by points (min = Q1 − 1.5 × IQR; max = Q3 + 1.5 × IQR). Different letters indicate significant intraspecific differences ($\alpha = 0.05$). Asterisks indicate significant interspecific differences.

4. Discussion

This study presents evidence of a similar behavior pattern in a native (European bullhead) and an invasive (round goby) benthic fish species in single-species laboratory experiments. The Ponto-Caspian invader, the round goby, continues to spread in European and North American freshwater and coastal ecosystems and invades vulnerable tributaries of main waterways where it threatens native fish species, competing with them for habitat and prey [3,36,42]. Examples of vulnerable native fish species negatively affected by the round goby are the log-perch *Percina caprodes* (Rafinesque 1818) [43,44] and the mottled sculpin *Cottus bairdii* (Girard 1850) [4,36]. The round goby has been described as out-competing native mottled sculpin for preferred habitats and disrupting its reproduction [4,36]. Rapid decline of the river bullhead *Cottus perifretum* [45] was observed by van Kessel et al. [46] following round goby colonization in the river Meuse in the Netherlands. Field and laboratory studies have revealed that cottiids, representative of small benthic fishes, might be especially vulnerable to gobiid impact [3,29]. Hence, we assume adverse effects on other cottiids similar to that on the European bullhead used in our study. These reports may not be conclusive: Janáč et al. [32], in long-term monitoring of rip-rap habitats along the middle Danube, observed that the European bullhead maintained relatively strong reproducing populations despite the long-term presence of invasive gobiids. However, information concerning the European bullhead populations prior to the gobiid invasion is not available.

Similarities between the round goby and European bullheads include size, bottom-dwelling habits, spawning grounds preferences, habitat use, feeding areas, and food type [32,47,48]. Similar to the round goby, the European bullhead is a solitary and territorial fish [49], and both species are reported to be nocturnal [50,51]. During the normal behavior period, we found both species to spend significantly less time outside shelter in daylight than in darkness, while, during the acclimation/exploration period, both were more active in light due to stress.

The European bullhead is believed to primarily inhabit small streams with a strong current [26,52] and to not actively migrate [52], while the round goby is associated with deep, lentic, slowly flowing waters and shows migratory behavior [53,54]. Some evidence suggests that the species can live in syntropy and share habitats [2,32,55–57]. In most cases, the round goby is a more successful species in natural conditions than the European bullhead and other cottiids [39,40]. It is generally assumed that the main drivers of the successful competition of the round goby have higher adaptability to alternative food sources as showing non-selectivity when consuming various size preys (e.g., macrozoobenthos crayfish) [58,59], fast growth and early sexual maturation, leading to rapid formation of dense populations [9,35]. Little is known about the effects of flow velocity and shelter availability on competition between the European bullhead and round goby, and these factors may be crucial for their potential co-occurrence in freshwaters and even for the persistence of the European bullhead in European waters. Our hypothesis that the round goby would exhibit a higher activity level than the European bullhead was confirmed by results showing greater time spent in movement during all investigated time periods and especially during the normal behavior period in light.

Kessel et al. [29], in separate-species experiments, reported *C. perifretum* to show strong preference for shelter, whereas the round goby displayed a more generalist pattern, exploring and moving while occupying various habitat types.

We also initially assumed that the round goby would adapt more readily during the acclimatization period. This was not confirmed: both species needed more time to display stabilized and consistent movement patterns under the novel conditions of the trial.

Although most ethological studies have been performed in still water conditions, Jermacz et al. [9] assessed the effect of flow velocity on interactions between the non-native racer goby *Babka gymnotrachelus* (Kessler 1857) and the European bullhead. The racer goby could displace the native European bullhead from a shelter in water velocities to 0.3 m s^{-1}. In our study, both species coped with the higher water velocities that occurred in the middle and upstream zones, as they spent the most time in the shelter.

Reported habitat use and preferences of the European bullhead and round goby differ among sites and studies. In England, the European bullhead is reported to prefer depths of 0.10–0.30 m and velocities 0.0–0.2 m s^{-1} [57,60,61], whereas Knaepkens et al. [61] in Belgium observed a preference for depths of 0.23–0.44 m and velocities 0.0–0.6 m s^{-1}. Our results also confirmed the preference of the European bullhead for more rapidly running water compared to the round goby. The European bullhead spent more time in the escape zone (water velocity ~ 0.4 m s^{-1}) during normal behavior tracking in the light period than did the round goby, while the round goby spent more time in the quiet zone (~0.02 m s^{-1}) during the normal behavior tracking in both dark and light compared to the European bullhead.

The hypothesis of higher food intake of the round goby is rejected, since the species ate an equal number of larvae. This finding also indicates that both species are able to cope with high water velocities, as flow velocity in the feeding zone exceeded 0.7 m s^{-1}. However, less time spent in the feeding area while ingesting the same quantity of feed in the round goby implies more targeted movement toward the feeding zone.

The round goby showed more consistent behavior during the normal behavior period in light, while the European bullhead demonstrated more consistent behavior during the normal behavior period in the dark.

Comparing the behavior of the species under laboratory conditions simulating a natural habitat, the present study revealed no significant interspecific differences in behavior without competitor presence. However, some specific differences were observed, as the round goby exhibited more consistent behavior than bullhead, which tended to show more or less lively activity behavior. The slight preference for the quiet zone in the round goby and for zones of more rapid flow in the European bullhead was initially expected to occur to a greater extent than was observed.

5. Conclusions

Our results provided evidence that the fish need a relatively long acclimatization period for behavior stabilization. This finding should be taken into account in future ethological studies conducted under laboratory conditions. Fish behavior depends on the habitat structure and integrity, water physical and chemical properties, and the presence of other fauna, which can trigger changes in preferences and factors in adaptation to a new environment, dramatically altering direct interaction between species. The interaction of the round goby and European bullhead could possibly impact native species and increase their vulnerability to other environmental threats. In addition to suggesting future research for further evaluation of behavior and interactions of the round goby and European bullhead, we can conclude that the success of the round goby over the European bullhead will most likely not be driven by the basic behavior patterns investigated in this study. The major factors are likely to be the round goby's higher reproduction rate and greater adaptability to often-changing environmental conditions. Our study shed light on the basic behavior of the studied species, showing very similar preferences of the native and invasive benthic fish. In case of the already widely spread invasive round goby, the implications are that European bullhead habitats are potentially susceptible to round goby invasions. Therefore, the main focus of adequate management actions should be to prevent spreading of the round goby as to ban manipulation with *N. melanostomus* and its release back into the water.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biology10090821/s1, Table S1: The mean values measured at 20 water velocity points (WV in m sec^{-1} measurement points MP (A1-H1) in all three (1–3) experimental channels (EC). Data are mean ± SD., Figure S1: Simple linear relationship between number of fly larvae consumed and total time spent in feeding zone by round goby and European bullhead using the default *lm* function in the R statistical program (R Core Team, 2020).

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Institutional Review Board Statement: The experimental protocols of the study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic, based on the EU harmonized animal welfare act of Czech Republic following the principles of laboratory animal care and national laws Act. no. 246/1992 and following the regulations of animal welfare (Ref. number 22761/2009-17210).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Supplementary data 1.

Table 1. *The mean values measured at 20 water velocity points (WV in m.sec^{-1} measurement points MP (A1-H1) in all three (1-3) experimental channels (EC). Data are presented as mean ± SD.*

EC	MP	WV	MP	WV	MP	WV	MP	WV	MP	WV	MP	WV	MP	WV	MP	WV
1	A1	0.78±0.05	B1	0.20±0.08	C1	0.23±0.06					F1	0.03±0.03	G1	0.11±0.04	H1	0.21±0.04
	A2	0.10±0.03	B2	0.32±0.05	C2	0.31±0.06	D1	0.53±0.04	E1	0.56±0.04	F2	0.33±0.10	G2	0.32±0.03	H2	0.36±0.04
	A3	0.82±0.06	B3	0.34±0.06	C3	0.30±0.04					F3	0.56±0.03	G3	0.46±0.04	H3	0.35±0.06
2	A1	0.79±0.04	B1	0.34±0.04	C1	0.28±0.03					F1	0.02±0.02	G1	0.12±0.04	H1	0.18±0.04
	A2	0.15±0.05	B2	0.25±0.04	C2	0.31±0.02	D1	0.55±0.04	E1	0.59±0.02	F2	0.42±0.11	G2	0.36±0.07	H2	0.33±0.04
	A3	0.89±0.06	B3	0.40±0.08	C3	0.30±0.05					F3	0.56±0.04	G3	0.40±0.02	H3	0.31±0.03
3	A1	0.81±0.05	B1	0.26±0.04	C1	0.26±0.03					F1	0.01±0.01	G1	0.14±0.04	H1	0.20±0.04
	A2	0.08±0.03	B2	0.32±0.03	C2	0.33±0.04	D1	0.52±0.04	E1	0.58±0.02	F2	0.41±0.11	G2	0.39±0.05	H2	0.35±0.03
	A3	0.86±0.05	B3	0.35±0.04	C3	0.29±0.06					F3	0.58±0.02	G3	0.43±0.08	H3	0.33±0.04

Supplementary data 2.

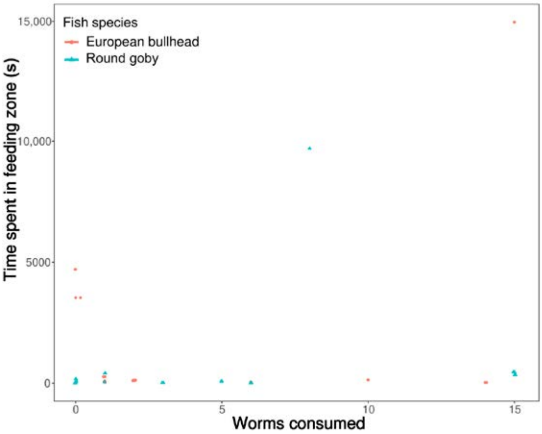

Figure 1. *Simple linear relationship between number of fly larvae consumed and total time spent in feeding zone by round goby and European bullhead using the default lm function in the R statistical program (R Core Team, 2020).*

CHAPTER 5

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

General discussion

Humans are responsible for 1,000 of species being transported from their area of origin around the globe and with accelerated trade and transport, the rate of new introductions has exponentially increased over time. As biological invasions are increasing worldwide, innumerable damage is being caused to ecosystems, biodiversity, socio-economic sectors and the human need for water resources. The significant effect of aquatic invasive species on native species populations and ecosystems has become one of the most critical issues worldwide, alongside other risks such as climate change, pollution and habitat modification.

In recent years, research and policies have focused on identifying and classifying introduction pathways and prioritizing the best management schemes to prevent further biological invasions and invasive species spreading. The Strategic Plan for Biodiversity 2011–2020 (Target 9; Convention on Biological Diversity 2012; https://www.cbd.int/sp/targets/rationale/target-9/) reported that "by 2020", invasive alien species and pathways will be identified and prioritized; however, although there has been a significant improvement in this field, there is still much more to do.

In this thesis, prominent groups of aquatic invaders are analyzed in a series of experimental work and field surveys. Due to the reasons mentioned under 'Objectives' in the General Introduction, only three of the objectives were achieved through the publication of a manuscript or have a prepared manuscript to-date. Other manuscripts will be ready for submission soon, but this thesis accomplished only three objectives. These objectives as well as the methodology and experimental work were chosen in compliance with the observations from field surveys and with the cooperation between German and the Czech scientists on the basis of the project "MoBi-aqua – cross-border monitoring of biological invasions for conservation of native aquatic biodiversity".

The river Elbe has the fourth-largest catchment area in Western and Central Europe, including rivers and streams from the Czech Republic, Germany, Austria, and Poland. The river Elbe is a federal river for its waterways and an important pathway for invasive organisms endangering native species. It is the primary source of invaders and their migratory corridor and, in Germany and the Czech Republic, of great importance for water management and shipping. The river and its numerous tributaries are also the habitats of many aquatic organisms, including endangered native species such as, e.g., noble crayfish, stone crayfish, and European bullhead. Twenty-five sites along the main River Elbe (7 sections), Eder (4 sections) and its tributaries (14 sections) were monitored for aquatic species determination in the Czech Republic, as well as an additional twenty-seven sites of the main river Elbe (7 sections) and its tributaries (20 sections) in Saxony, Germany during the project MoBI-aqua. The stream morphology, macrozoobenthos communities, crayfish population and fish populations (natives and non-natives) were explored and stored in a database. Except for native biota, the main invasive species monitored were: the spiny-cheek crayfish, the zebra mussel, the Asian clam, the New-Zealand mud snail, the three-spined stickleback, the brown bullhead, the black bullhead, the Prussian carp, the pumpkinseed, the round goby and even the alien Demanson's cichlid, endemic to the African lake Malawi and, although it is not a threat to Central European waterways, it is a prime example of an introduced species (probably from aquarium release) (Buřič et al., 2020). Several objectives of the presented thesis arose from these monitoring outputs and field experiences, as well as from co-operation with German colleagues. The temperatures used in the experiments were also in accordance with the temperatures in the Elbe River and its tributaries at a given time (crayfish terminal phase of reproduction and summer season).

In Chapter 2, we assessed the potential of an invasive amphipod, the killer shrimp *Dikerogammarus villosus*, and its ability to cope with other established invaders in European waters – the North American signal crayfish *Pacifastacus leniusculus* as a representative of family Astacidae (Kouba et al., 2014) and the marbled crayfish *Procambarus virginalis* as a model for other North American cambarid crayfish (Hossain et al., 2018). Representatives of these two families in Europe differ in the size of their eggs and the size of early juveniles, as well as the time (stage) when they become independent of their mothers (Holdich, 2002).

Killer shrimp is abundant in large European rivers, where it is reported as a serious threat to native biota (Dick and Platvoet, 2000, 2001). During the crayfish and macrozoobenthos sampling in the Elbe River, we could not find ovigerous spiny-cheek crayfish at places where the highest density of killer shrimps was detected. It was not proven by deep monitoring, but it was an initial idea to start the objective targeted on the ability of the killer shrimp to affect crayfish egg clutches and the early developmental stages of crayfish. Hence, if they can significantly influence their population by reducing the recruitment of new crayfish generations. The particular aims were to investigate if killer shrimp are able to open and eat the eggs of two selected crayfish species, if it is able to kill and eat their free-moving juveniles, and if yes, how the egg (hatchlings) clutches are affected when attached on female pleopods and actively guarded. Besides this predatory role, we also compare to what extent both crayfish species chosen can predate on killer shrimps and which species is more effective at that role.

Our results demonstrated that the killer shrimp is able to open potentially lost eggs and eat them and is also effective in predating on free-living juveniles of cambarid crayfish. The situation was different for signal crayfish, where the egg size and rigid eggshell limited the predation by the killer shrimp. Similarly, signal crayfish juveniles were less affected due to their size. However, it was remarkable that killer shrimp affected the clutches directly guarded by their mothers. Due to the results above, we used ovigerous marbled crayfish females and signal crayfish females with hatchlings (1st developmental stage) that are smaller and more vulnerable than the free-living juveniles in astacids (2nd developmental stage) (Kozák et al., 2015). In both species, we detected significant losses on clutches incubated and actively guarded by their mother. The physical evidence was also made by finding halved (half-eaten) hatchlings on female pleopods. They cannot be eaten either by their mother (physically impossible to reach and cut them) nor conspecifics in the clutch (they still do not feed externally) (Holdich, 2002).

Our findings illustrate the high voracity and competitiveness of killer shrimp and the unexpected boldness of this tiny creature, affecting even much bigger taxa by its pressure on their early developmental stages. Due to this aggressiveness, boldness, and voracity, killer shrimps are also often detected in unexpected trophic positions that correspond more to benthic or even predatory fish than bottom-dwelling invertebrates (Van Riel et al., 2006; MacNeil et al., 2010). Killer shrimps are therefore not only potential prey for fish and crayfish but also their competitor and even a predator on their developmental stages. Species with smaller eggs and early developmental stages are more prone to killer shrimp predation as presented by the higher predation on marbled crayfish eggs and juveniles than those of signal crayfish.

Direct predation by killer shrimp on juveniles of the spiny-cheek crayfish was also observed in a previous study (Buřič et al., 2009) where the first and second independent stage of cambarids (3rd and 4th developmental stage) were prone to predation by killer shrimp, but larger stages of crayfish could resist. A recent study from Warren et al. (2021) showed that killer shrimp could also prey upon early-stage (embryos and larvae) of native common frog *Rana temporaria* Linnaeus, 1758, as well as invasive amphibians and the African clawed frog *Xenopus* *laevis* Daudin, 1802 in the UK. It also exhibited a potentially destabilizing Type II functional response, as having a larger body size translated into the superior functional response through significantly lower handling time and subsequently higher maximum feeding rates. Similarly, as in the crayfish in our study, killer shrimp appeared incapable of consuming larger embryos and predation was observed only when embryos were damaged before exposure. Therefore, the mentioned study suggests that killer shrimp presents a serious threat to animals much larger than itself, especially those with relatively small embryos (Warren et al., 2021). Special consumption rates by large amphipods may be facilitated by more prominent mouthparts and gnathopods, allowing individuals to capture and consume a wider range of prey (Mayer et al., 2008) as well as having a large gut capacity, necessary for digesting high amount of food items efficiently (Vucic-Pestic et al., 2011). It was also presented in our research by the ability of killer shrimp to consume a mean prey biomass of more than 27% (maximum 43%) of its own biomass in 24 hours (marbled crayfish juveniles as a prey). Killer shrimp's ability to be carnivorous and to use a broad spectrum of other food may be a fundamental reason for the invasion's success, being an advantage over other gammarids that often eliminated in places where killer shrimp are present (Mayer et al., 2008).

To see the killer shrimp's role from the other side, it was also highly predated by both species in our study, signal and marbled crayfish. Especially marbled crayfish were successful at killing and foraging on killer shrimps despite their smaller size compared to signal crayfish. This is well illustrated by the significantly higher mean prey biomass eaten by marbled crayfish (17%) than by signal crayfish (6.1%). Marbled crayfish showed that in given conditions they can be more voracious than signal crayfish, a well-established invader in European freshwaters (Kouba et al., 2014).

To summarise, the killer shrimp is a dangerous invader for its benthic counterparts and can also affect much larger animals by preying on their early developmental stages. Due to its ecological advantages such as rapid reproduction, predatory behavior and wider temperature and salinity tolerance the effects it has on freshwater ecosystem are expected to become more pronounced as its range will continue to increase (Bij de Vaate, 2001, 2002). Killer shrimp can sustain extended periods in ballast water tanks and be dispersed over considerable distances (Mayer et al., 2008), so it could invade North America soon (Devin et al., 2001; Müller et al., 2002; Warren et al., 2021).

Chapter 3 represents the next level of invader interactions where crayfish are considered as both prey and competitors of the chosen representative of invasive gobiids, the round goby. The round goby often co-exists with the spiny-cheek crayfish (Buřič et al., 2020). Therefore, we investigated round goby behavior towards crayfish of different sizes mimicking different situations that are common in natural conditions. The marbled crayfish was used as a model representative of those cambarid crayfish already living in syntropy with the round goby in both Europe and North America (Church et al., 2017; Ericsson et al., 2021). They both co-exist in the same habitat, sharing shelters and using similar food sources (Gebauer et al., 2019). We pursued several different goals as a basis for further studies. Firstly, we investigated round goby voracity when paired with juvenile crayfish of different sizes and amounts.

Furthermore, we investigated the behavior of both species when paired with similarly sized individuals during 24 h and during eight consecutive days. In the first round of experiments, the round goby showed increased predation with increased mouth gape, no matter what number and sizes of crayfish juveniles were available. Also, when we compared consumption under two different temperatures (17 °C and 21 °C), the round goby showed increased consumption with increased temperature. Mouth gape and total length were the most limiting factors in predation on crayfish. In the second round of experiments, the observation of weight-matched couples of round goby and marbled crayfish was conducted. Round goby exhibited enormous displays of aggressiveness and competitiveness for space and shelters with crayfish.

To sum up, the round goby showed high aggressiveness and dominance over crayfish. Round goby predation was mostly limited by mouth gape. However, when moulted, even similarly sized crayfish were eaten by round goby because helpless crayfish were not able to defend themselves against goby attacks. However, in preying on smaller crayfish, the bigger the mouth gape, the bigger crayfish they consumed. As both tested species are mainly nocturnal (Savino et al., 2007; Kornis et al., 2012), competition for shelter was most crucial during the day where the round goby was the more successful species.

Meanwhile, during the observations of the eight days experiment, marbled crayfish spent significantly more time in the shelter during the day. Most of the attacks happened during the day, with round goby being more aggressive and initiating fights and trying to enter the shelter. In general, the presence of fish predators can have a negative but sublethal effect because it induces a change in crayfish behavior by reducing their feeding activity and increasing the time spent in the shelter (Aquiloni and Gherardi, 2010). In the study by Anastácio et al. (2011), two invaders, also sharing typical habitats in shallow pools, the red swamp crayfish and eastern mosquitofish *Gambusia holbrooki* Girard, 1859 also predated each other: crayfish consumed fish and fish consumed recently hatched crayfish. Naive predacious fishes such as the invasive largemouth bass *Micropterus salmoides* Lacépède, 1802 showed increased crayfish consumption after four days of feeding on the same prey. This shows that largemouth bass can quickly adapt to new preys in recently invaded habitats (Ramalho and Anastácio, 2010). The general conclusion of this study is that early juvenile crayfish are the most vulnerable to predation by round goby that cause considerable declines in the invasive crayfish species of genus Cambaridae in Europe and North America. The round goby is well adapted to consume crayfish and to compete for available shelters with them. It can be expected that round goby will easily prey on any crayfish species available, both native and alien.

During the field sampling in the Elbe and Eger River, mentioned at the beginning of this discussion, the co-occurrence of the invasive round goby and the native European bullhead was found on the Czech and German sides of the border (Buřič et al., 2020). The success of the round goby and the lack of studies in water flow conditions led to the idea of using a flow simulator to compare the basic behavioral patterns, space usage, and interactions between the round goby and European bullhead. The results of the basic behavioral patterns and space usage under different flow velocities are presented in the form of a manuscript in **Chapter 4**. At the same time, the data from other studies are now ready to be written into a manuscript.

The round goby and European bullhead are known as bottom-dwelling species that occupy a similar niche (Roche et al., 2015; Jurajda et al., 2005), so it is expected that they compete for similar resources. There are many reports about the negative impacts on native benthic fish species (or even fast depletion of them) from the family Cottidae (Janssen and Jude, 2001; Corkum et al., 2004) but there are also reports about populations of the European bullhead and the round goby in syntopy without any negative impacts on native species (Janáč et al., 2018). There are many possible drivers of species success and many possible limiting factors that can support or suppress different species. Our objective was to investigate the space usage, flow velocity preferences or tolerance, shelter use, feeding behavior and activity patterns in a flow simulator in individuals from both species. This information was needed to better understand their basic behavioral characteristics prior to the interaction experiments. The questions of how observed species react in novel conditions and how they cope with initial stress were also worth investigating.

Surprisingly, there were not so many differences between target species with only a slight preference of round goby for areas with slow running water whereas bullhead preferred faster running waters. However, both species were able to cope with high water velocity levels above 0.7 $m.sec^{-1}$, similar to the results by Knaepkens et al. (2002) with a maximum of 0.6 $m.sec^{-1}$. We observed individual fish in acclimatization periods (stress response, exploration period light, exploration period dark) and normal periods of tracking in dark and light. Space was divided for the needs of analysis into seven zones in which species could freely move: feeding zone, upstream zone, mid-stream zone, shelter zone, quiet zone, escape zone, and downstream zone. Both species had similar patterns in the time spent in all zones in particular periods, spending most of the time in the shelter and the mid-stream zone. The only difference was that round goby spent more time in the quiet zone during the exploration period in light conditions. Despite the low differences observed, we can conclude that experimental fish need relatively long acclimatization periods for behavioral stabilization which should be taken into account in future studies. In general, round goby seems to be faster in that regard. The main drivers that determine invasion success by round goby do not lay in the individual, basic behavioral patterns and basic space usage. Rather, their higher reproduction effort, higher adaptability and more aggressive nature should be considered to be the main advantages.

Overall, the presented research in this thesis, including the work on the MoBI-aqua project and its results (Buřič et al., 2020), shows that co-occurring of the alien invasive species can limit each other by competition and predation but also high probability that invasive species will continue to spread further (the round goby population has moved 12 km in the last three years). The movement of aquatic invaders is also influenced by gradients of temperature and elevation, which are therefore fundamental for predicting the autonomous overland dispersal and colonization patterns (Marques et al., 2015). Capinha et al. (2013) predicted that signal and red swamp crayfish will increasingly occupy the many climatically suitable areas still available in Europe. In general, invasive species react faster and more adaptively than native ones, including adaptation to novel conditions, stress and novel predators (Hazlett et al., 2002; Siebeck et al., 2009).

Although invasive species have successfully established viable populations under European climatic conditions, data on all of them in the wilderness or urbanized areas of Europe remains scarce. There is still missing information about interactions and incidence of invasive species in multiply invaded ecosystems where one can limit the other, or instead cause the other to find an alternative niche and therefore increase the pressure on the ecosystem.

Conclusions

As evidenced by the present thesis, killer shrimp can negatively affect larger animals (crayfish, fish, and amphibians) but can also serve as prey that can be consumed in large amounts, as shown in marbled crayfish. Crayfish can also be under pressure from other invaders, such as round goby that showed the potential to swallow relatively large amounts of crayfish of different size classes. They also actively compete for other resources like space and shelter and theoretically can negatively affect each other, e.g., by higher exposure to diurnal predators. Round goby was also found to be very similar in basic behavior and space usage to the native European bullhead, although it had better adaptability to stress conditions and a lower preference for fast-flowing areas. Round goby was confirmed as an aggressive bottom-dwelling predator, sharing a habitat with a lot of other benthic animals like amphipods, crayfish and other fish – natives and aliens.

To sum up, all alien species considered in this thesis are a threat to any native species that share similar characteristics in habitat, shelter preference, or are vulnerable to being attacked and/or predated on. One species can limit the other, or conversely, the outcompeted species will find an alternative niche and therefore increase the overall pressure on the ecosystem.

This thesis represents a baseline for future experimental work that focuses on more complex setups so that we may better understand invasive species and the mechanisms behind their success and interactions.

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English summary

Cocktail of invaders in European inland waters – ecological characteristics, interactions and consequences

Sara Roje

Innumerable alien species have been invading European freshwaters for centuries and they are currently still increasing. Human activity causes the planned or unexpected transport of alien invaders outside of their historical biogeographic boundaries. Consequently, dispersal pathways play a pivotal role in the success of invasive species that can cause devastating environmental and socio-economic impacts. These pathways affect the number, frequency, and geographic range of species dispersed.

Studying invasive species in their native and invaded ranges offers new opportunities for addressing theoretical challenges associated with niche-based models and predictions. However, the effects of introduced species differ according to key characteristics such as size, abundance, invaded habitat type, and community composition. While some introductions are considered beneficial in some cases, some of them have the opposite effect.

This thesis is composed by four main chapters: 1) a general summary of the current knowledge of the impact of aquatic invasive species in Europe, ecological characteristics, main representatives, their interaction and the consequences of spread; 2–3) two studies addressing mutual species impacts between invaders co-occurring together in the same habitat; and 4) one study investigating the differences between the behavior of native and alien fish species. Chapter 1 looks at the theoretical concept and impacts of aquatic invasive species and in particular, four chosen groups of invaders with a review on specifically chosen high-profile aquatic invasive species in freshwaters, their associated impacts and detailed descriptions of the species used in experiments. Chapter 2 focuses on the predatory impact of killer shrimp on the early developmental stages of marbled and signal crayfish, concluding that killer shrimps can represent a threat for them. They were able to feed on different developmental stages of larger freshwater crustaceans even if they were protected by their mothers. Chapter 3 reports the interactions of two invaders: round goby and marbled crayfish. Round goby showed dominant aggressiveness consuming all the juvenile crayfish sizes offered. The study also confirmed that round goby was able to predate on, attack and take shelters from crayfishes. The only limiting factor in this interaction was the larger size of the crayfish. When interacting, both tested species negatively influenced each other, which can mean mutual consequences for both species. Chapter 4 deals with the individual behavior of round goby and native European bullhead during day and night activity under laboratory conditions in a flow simulator. Both species showed very similar behavior, with only slight differences towards adaptability and stress-related activity.

Despite the similarities, round goby was confirmed to be an aggressive, bottom-dwelling predator sharing a habitat with a lot of other benthic animals like amphipods, crayfish, and other fish - native or other aliens. Overall, all alien species are a threat to all populations sharing similar characteristics in their habitat, taking their shelters, attacking them, and predating on them. One species can limit the other, or the outcompeted species can be displaced forcing to find an alternative niche and therefore increase the overall pressure on the ecosystem. The presented thesis clearly shows some new findings mainly about the interactions between invaders and also represents a baseline for future experimental work focused on more complex setups that will enable us to increase our understanding of invasive species.

Czech summary

Koktejl invazivních druhů ve vnitrozemských evropských vodách – ekologická charakteristika, vzájemné působení a následky

Sara Roje

V evropských vodách se již před lety, dokonce i před staletími, rozšířil nespočet invazivních druhů a tento trend nadále pokračuje i v dnešní době. Především lidská činnost způsobuje tyto ať již plánované nebo neplánované přesuny invazivních druhů mimo oblasti jejich původního rozšíření. Cesty šíření invazivních druhů hrají klíčovou roli v jejich následném úspěchu a ovlivňují jejich negativní enviromentální a socioekonomické dopady. Ovlivňují především počet, frekvenci a geografické rozšíření těchto druhů.

Studium invazivních druhů v jejich původních a nepůvodních místech výskytu, nabízí nové příležitosti pro řešení výzev založených na modelování a predikci vývoje ekosystémů. Dopady introdukovaných druhů závisí především na klíčových faktorech, jako je velikost, abundance, typ a stav napadeného habitatu a složení společenstva. I když mohou být některé introdukce považovány za prospěšné, většina případů má spíše opačný účinek.

Tato práce je rozdělena do čtyř hlavních kapitol: 1) obecné shrnutí současných poznatků o vlivu vodních invazních druhů v Evropě, jejich ekologické charakteristiky, hlavní představitele, jejich interakce a důsledky šíření; 2–3) dvě studie zabývající se vzájemnými interakcemi invazivních druhů vyskytujících se společně v jednom habitatu; a 4) porovnání rozdílů mezi chováním původního a nepůvodního druhu ryb. Kapitola 1 se zabývá teoretickým konceptem dopadů vodních invazivních druhů skládajících se ze čtyř vybraných skupin, s přehledem konkrétně vybraných druhů a jejich souvisejícím dopadem a popisem druhů použitých v experimentální práci. Kapitola 2 se věnuje dopadu blešivců ježatých na raná vývojová stadia raků mramorovaných a signálních. Tato studie potvrzuje, že blešivci ježatí jsou jako invazivní druh téměř nezastavitelní, protože byli schopni se živit různými vývojovými stadii větších sladkovodních korýšů, i když byli chráněni svými matkami. Kapitola 3 sledovala interakce dvou invazivních druhů: hlaváče černoústého a raka mramorovaného. Hlaváč vykazoval vyšší agresivitu a dokázal přijímat všechny nabízené velikosti juvenilních raků jako svou potravu. Studie také potvrdila, že hlaváč se dokáže skrýt před rakem zahrabán v písku a poté na něj zaútočit. Jediným omezujícím faktorem může být větší velikost raků. Rozhodně oba druhy navzájem ovlivňují své chování, což může mít oboustranné negativní důsledky. Kapitola 4 se zabývá individuálním chováním hlaváče černoústého a vranky obecné během dne i noci v laboratorních podmínkách v nádržích se simulovaným prouděním. Oba druhy vykazovaly velmi podobné chování, pouze s mírnými rozdíly vůči přizpůsobivosti a činnosti související se stresem.

Celkově byla potvrzena role hlaváče jako agresivního predátora vázaného na dno vodních ekosystémů. Díky tomu hlaváč černoústý sdílí stanoviště s mnoha dalšími bentickými druhy, jako jsou různonožci, raci a další ryby, ať již původní nebo nepůvodní. Obecně platí, že všechny invazivní druhy představují hrozbu pro všechny původní populace, v případě podobných preferencí na habitat, kde dochází ke konkurenci o úkryty a následně i útokům a predaci. Podobně mohou různonožci a raci negativně ovlivňovat invadované ekosystémy. Jeden druh může omezit druhý nebo slabší druh hledá alternativní niku, což může v důsledku vést ke změně v ekosystému. Tato práce překládá některé nové informace především o interakcích invazivních druhů, ale také formuje základy pro budoucí experimenty, které se mohou zaměřit na složitější nastavení jednotlivých experimentů.

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A short time ago, in the Vodňany – České Benátky far, far away…

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Well, I am a thrilled alien that finally succeeded in completed this book, that even world global pandemic *SARS COVID-19* didn't stop. Děkuji moc! 😊

[This Ph.D. thesis is dedicated to my parents Srđana & Nenad, who are no longer in this world, but who will always be in my memory and heart.]

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List of publications

Peer-reviewed journals with IF

Hossain, M.S., Kubec, J., Guo, W., **Roje, S.**, Ložek, F., Grabicová, K., Randák, T., Kouba, A., Buřič, M., 2021. A combination of six psychoactive pharmaceuticals at environmental concentrations alter the locomotory behavior of clonal marbled crayfish. Science of the Total Environment 751: 141383. (IF 2020 = 7.963; Q1)

Roje, S., Drozd, B., Richter, L., Kubec, J., Polívka, Z., Worischka, S., Buřič, M., 2021. Comparison of behavior and space Use of the European bullhead *Cottus gobio* and the round goby *Neogobius melanostomus* in a simulated natural habitat. Biology 10, 821. DOI: https://doi.org/10.3390/biology10090821 (IF 2020 = 5.079; Q1)

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Červený, D., **Roje, S.**, Turek, J., Randák, T., 2016. Fish fin-clips as a non-lethal approach for biomonitoring of mercury contamination in aquatic environments and human health risk assessment. Chemosphere 163: 290–295. (IF 2016 = 4.208): Q1

Manuscripts

Buřič, M., **Roje, S.,** Müllerova, L., Kubec, J., Sentis, A., Vesély, L., Kouba, A., 2021. Reciprocal predation between non-native crayfish and salmonids – Who eats whom? (in preparation)

Musil, M., **Roje, S.**, Let, M., Kouba, A., 2021. Feeding under pressure – food consumption in crayfish as influenced by cues of conspecifics and/or predators. (in preparation)

Richter, L., **Roje, S.**, Buřič, M., Berendonk, T., Worischka, S., 2021. Predation risk assessment by *Dikerogammarus villosus* – interacting effects of shelter distance, food movability and potential predation on food consumption. (in preparation)

Richter, L., **Roje, S.**, Worischka, S., Drozd, B., Buřič, M., 2021. Inter – and intraspecific competition for the shelter of European bullheads (*Cottus gobio*) and round goby (*Neogobius melanostomus*). (in preparation)

Roje, S., Musil, M., Sandler, D., Mayerhofer, G., Kouba, A., Buřič, M., 2021. Signal crayfish behavior and sediment transport in the stream conditions simulator. (in preparation)

Abstracts and conference proceedings

Buřič, M., **Roje, S.**, Veselý, L., Švagrová, K., Fořt, M., Kouba, A., 2019. Possible roles of killer shrimp (*Dikerogammarus villosus*) in relation to invasive crayfish. In: 11th Symposium for European Freshwater Sciences (SEFS 11), June 30 – July 5, 2019, Zagreb, Croatia.

Hossain, S., Guo, W., Kubec, J., **Roje, S.**, Grabicová, K., Randák, T., Kouba, A., Buřič, M., 2019. Cocktail of pharmaceutically active compounds alter the behavior of clonal marbled crayfish. SETAC Latin America 13th Biennial Meeting, 15–18 September 2019, Cartagena, Colombia.

Roje, S., Richter, L., Drozd, B., Franta, P., Kubec, J., Worischka, S., Buřič, M., 2019. Round goby vs. marbled crayfish – some notes to interactions between two important invaders. In: 11th Symposium for European Freshwater Sciences (SEFS 11), June 30–July 5, 2019, Zagreb, Croatia.

Training and supervision plan during study

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Ph.D. courses	**Year**
Applied hydrobiology	2018
Pond aquaculture	2018
Ichthyology and fish systematics	2019
Basics of scientific communication	2019
Biostatistics	2019
Czech language	2019
English language	2019

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2018–2021

International conferences	**Year**
Roje, S., Richter, L., Drozd, B., Franta, P., Kubec, J., Worischka, S., Buřič, M., 2019. Round goby vs. marbled crayfish – some notes to interactions between two important invaders. In: 11th Symposium for European Freshwater Sciences (SEFS 11), June 30 – July 5, 2019, Zagreb, Croatia.	2019
Buřič, M., **Roje, S.**, Veselý, L., Švagrová, K., Fořt, M., Kouba, A., 2019. Possible roles of killer shrimp (*Dikerogammarus villosus*) in relation to invasive crayfish. In: 11th Symposium for European Freshwater Sciences (SEFS 11), June 30 – July 5, 2019, Zagreb, Croatia.	2019
Hossain, S., Guo, W., Kubec, Jan., **Roje, S.**, Grabicová, K., Randák, T., Kouba, A., Buřič, M., 2019. Cocktail of pharmaceutically active compounds alter the behavior of clonal marbled crayfish. SETAC Latin America 13th Biennial Meeting, 15–18 September 2019 in Cartagena, Columbia.	2019

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Prof. Dr. rer. Nat. habil. Thomas Berendonk, technical University of Dresden, Institute for Hydrobiology, Germany (2 months, Daily rhythm/ feeding of *Dikerogammarus villosus* depending on combined factors)	2019
Pedro M. Anastácio, Universidade de Évora, Portugal (Cancelled twice due to the world pandemic situation caused by COVID-19 [SARS-CoV-2] during 2019–2021)	~~2020–2021~~

Pedagogical activities	**Year**
Laboratory tutoring of the students (Bc., Mgr.) in range of 50 hours	2018–2021
Consultant of master thesis "Společenstvo bentický organism na dolním toku řeky Labe, Ohře a jejich pritocich" (Student: Martin Musil)	2018
Consultant of bachelors thesis "Denní a noční aktivita hlaváče černoústého a vranky obecné" (Student: Zdeněk Polívka)	2019
Consultant of master thesis "Pach vítězství: role feromonů v interakcích raků" (Student: Josef Mareš)	2020
Leading of the project entitled "Parameters of co-occurring noble crayfish and signal crayfish populations in small stream" (Student: David Sandler)	2020
Leading of the project entitled «Signal crayfish behavior and sediment transport in the stream conditions simulator» (Student: Gabriel Mayerhofer)	2020

Curriculum vitae

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EDUCATION

2017–present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic
2013–2016	M.Sc. in Mariculture, University of Dubrovnik, Croatia
2010–2013	B.Sc. in Aquaculture, University of Dubrovnik, Croatia
2007–2010	II Gymnasium, Language high school in Split, Croatia

TRAINING

08–09–/09 2020	Biomark – PIT Tag Methodologies and Monitoring Systems; PIT Tag Use Training

RESEARCH STAY AND COLLABORATIONS

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MEMBERSHIPS

European Aquaculture Society -EAS -Student Group – National coordinator for Czech Republic (2019–2021)

International Association of Astacology – member since 2020