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## CONTENT

# CHAPTER 1

## GENERAL INTRODUCTION

## 1.1. General introduction

Biological invasions and human impacts (e.g., habitat destruction and pollution) are the major factors negatively influencing global biodiversity (Sala et al., 2000; Dudgeon et al., 2006). Introduction of species outside of their native distribution often leads to negative environmental and socioeconomic consequences. This can negatively affect the indigenous biodiversity and/or in some cases, also cause particular veterinary and human health problems. Despite this knowledge, introduction of species is globally widely practised, mainly through bio-control programs (Simberloff and Stiling, 1996), aquaculture (Pérez et al., 2003) and forestry (Hughes, 1994). Mobility, transportation, and commercial sectors open new passages for species introductions as well (Strecker et al., 2011; Hulme, 2015; Loureiro et al., 2015; Faulkner et al., 2016; Saul et al., 2017). Aquarist trade has recently been identified as an important pathway for potential intentional and non-intentional introductions (Padilla and Williams, 2004; Patoka et al., 2014; Lipták and Vitázková, 2015). The problem of aquarium fish releases in both freshwater and marine environments has been recognized for a long time (Courtenay and Stauffer, 1990; Semmens et al., 2004), but scantly addressed. Crustaceans represent a significant part of the aquarist trade. In fact, many of the species incorporated in this business are r-strategists, with high dispersal abilities, high genetic variability, phenotypic plasticity, large native range, often being eurytopic and polyphagic (Vogler et al., 2012). Because of these characteristics, they are easily handled by the breeders and holders, but more importantly, they possess valuable tools to become established in a non-native area and become invasive. Rising density of human population and increasing socio-economic conditions favour the chances of non-indigenous species releases (Perdikaris et al., 2012; Chucholl, 2014, Patoka et al., 2016) and some of the species kept in aquaria already became established in the wild (Holdich et al., 2009; Kouba et al., 2014). Indigenous European crayfish species are challenged by the ever increasing number of newly introduced non-indigenous crayfish (Weiperth et al., 2017) and the spread of a crayfish plague (Svoboda et al., 2017), as pointed out in detail below. This matter substantially complicates their population recovery and conservation (Peay and Füreder, 2011; Capinha et al., 2013).

Freshwater ecosystems are one of the Earth's major natural components. Even though fresh water occupies only 0.8% of the Earth's surface area and represents only 0.01% of all water on Earth, it is still a major natural element providing habitat for more than 100 000 species (Dudgeon et al., 2006). This unique environment is seriously endangered due to overexploitation, water pollution, flow modification, destruction of habitats, invasion of non-indigenous species, and climate change (Dungeon et al., 2006; Gallardo and Aldridge, 2013; Capinha et al., 2013). European freshwater crayfish species are among the taxa that have undergone major turbulences due to water quality modifications and pollution as well as overexploitation for commercial purposes (Souty-Grosset et al., 2006; Policar et al., 2018). Due to this reasons, North American crayfish species were introduced to Europe to substitute the declining indigenous crayfish populations and to open new possibilities for aquaculture to cover up the commercial crayfish demands (Souty-Grosset et al., 2006; Holdich et al., 2009). Several non-indigenous crayfish species were introduced in Europe in this way (often termed old non-indigenous crayfish species in the literature). Later on, further species were introduced to the continent particularily via the aquarist pet trade (termed new non-indigenous crayfish species) (Holdich et al., 2009; Kouba et al., 2014). Natural spread and secondary introductions of the old non-indigenous crayfish species progressed through the 20$^{th}$ century. Introducing the non-indigenous species intensively from one water body to another was believed to rebuild the declining native crayfish population, with non-indigenous crayfish resistant to the crayfish plague (Souty-Grosset et al., 2006). However, direct interactions, competition for resources, differing reproductive strategies, and crayfish plague outbreaks in indigenous crayfish populations accelerated their decline (Stebbing et al., 2006; Bubb et al., 2006), affecting the indigenous crayfish populations, the noble crayfish *Astacus astacus* (Linneaus, 1758), the stone crayfish *Austropotamobius torrentium* (Schrank, 1803), the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) and the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) (Holdich et al., 2009).

The crayfish plague is caused by the oomycete *Aphanomyces astaci* Schikora, 1906 and represents the most dangerous disease in crayfish (Evans and Edgerton, 2002; Svoboda et al., 2016). This water mold of North American origin is characterised by the motile flagellate asexual spores called zoospores (Evans and Edgerton, 2002). Infection of the host occurs through encystment of zoospores in host tissue and subsequent germination of the spore and hyphae formation (Kozubíková-Balcarová and Horká, 2015). Both susceptible and resistant crayfish are infected in this way. Whether the pathogen causes the disease or not is determined by the defence response of the infected crayfish (Evans and Edgerton, 2002). The soft cuticle between the segments, or in the limb joints and fresh wounds are targets to pathogen encystment (Nyhlén and Unestman, 1980). Species that are stressed, wounded, or freshly moulted crayfish individuals are thus more susceptible to infection than healthy individuals (Unestman, 1973). The final stage of the infection process is sporulation and release of zoospores. The process primarily occurs prior to, or soon after, death or moulting of crayfish when extra-matrical hyphae grow outwards and give rise to sporangia (Evans and Edgerton, 2002; Svoboda et al., 2013). Primary spores develop to zoospores with flagella allowing swimming and thus infection of further individuals.

Crayfish plague is a fatal disease to all crayfish of non-North American origin, causing rapid mortality of susceptible crayfish and often leading to losses of entire populations. The first epizootic of crayfish plague occurred in the Po Valley in Italy in the 1860s (Alderman and Polglase, 1988). By the early 1990s, the disease has spread and affected freshwater crayfish populations in most of Central, Northern and Eastern Europe. Only isolated populations of freshwater crayfish remained unharmed (Edgerton et al., 2004). Continued spread of the disease resulted in epizootics in freshwater crayfish in Norway, England, Ireland, Spain and Turkey (Edgerton et al., 2004). North American crayfish are the natural hosts of the crayfish plague pathogen being partially resistant to the disease (Unestam, 1972). Hence, North American crayfish species introduced to Europe (the old non-indigenous crayfish species), mainly the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), the red swamp crayfish *Procambarus clarkii* (Girard, 1852) and the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) serve as vectors and reservoirs of the crayfish plague in introduced areas across Europe (Huang et al., 1994; Holdich et al., 2009). Other plausible vectors of the crayfish plague are also fish scales and cuticle in fish guts, where the crayfish plague pathogen *A. astaci* remains viable and can be released in new areas with potential contact of indigenous susceptible crayfish populations (Oidtmann et al., 2002). Recent research postulated evidence that freshwater shrimp (Svoboda et al., 2014a) and crab (Svoboda et al., 2014b; Schrimpf et al., 2014; Putra et al., 2018) species can also become crayfish plague carriers, which suggest a possible new transmission pathway of the zoospore of this disease. Five genotype groups of *A. astaci* have been identified so far (Svoboda et al., 2016). Group A comprises the first genotype group identified in Europe, isolated from the infected noble crayfish individuals (Huang et al., 1994). B and C group are transmitted through the signal crayfish, the red swamp crayfish is the host of the D group and the spiny-cheek crayfish being the vector of the E genotype of *A. astaci* (for details see Svoboda et al., 2016). Strains of different genotype groups differ in their virulence (Makkonen et al., 2014; Viljamaa-Dirks et al., 2013; Viljamaa-Dirks et al., 2016) and environmental preferences (Diéguez-Uribeondo et al., 1995). Hence, the scenario of the crayfish plague outbreaks can vary. Recently, also chronically infected European crayfish wild populations have been observed in noble crayfish in Finland (Viljamaa-Dirks et al., 2013), narrow clawed crayfish in Romania (Schrimpf et al., 2012) and Turkey (Svoboda et al., 2012), the stone crayfish in Slovenia (Kušar et al., 2013) and the white-clawed crayfish in Italy (Cammà et al., 2010). Populations of the indigenous European crayfish species can react differently when challenged to the crayfish plague pathogen. For example, one out of eight populations of the white-clawed crayfish from the Pyrenees exhibited 100% survival, while other populations exhibited high mortality rates when exposed to AP03 crayfish plague strain isolated from the red swamp crayfish (Martín-Torrijos et al., 2017). Despite this, documented crayfish plague outbreaks occur in the Scandinavian countries with tens to hundreds of these outbreaks recorded every year (Bohman et al., 2006; Viljamaa-Dirks et al., 2013). In the course of the past years, crayfish plague outbreaks were also recorded in Great Britain, France, Austria, Germany, Spain, Italy and in the Baltic countries (Kozubíková-Balcarová and Horká, 2015), thus remaining a threat for indigenous crayfish species. The spiny-cheek crayfish, the signal crayfish and the red swamp crayfish are the three most prominent species, with high geographical distribution, that are chronic reservoirs of the crayfish plague pathogen (Westman, 2002; Holdich et al., 2009; Kouba et al., 2014; Ruokonen et al., 2018).

Slovakia is an important harbour of indigenous European crayfish species having a strong population of the stone crayfish restricted in the Malé Karpaty Mountians (Stloukal and Harváneková, 2005), which represents the only region with a confirmed occurrence in the country. The stone crayfish occurs in 17 brooks in this region, with the most abundant population in the Vydrica brook (Stloukal et al., 2013a). The noble crayfish has a much wider distribution in the country, occurring at many sites across the entire country (Stloukal et al., 2013b). Slovakia has thus been a shelter for the noble crayfish during the crayfish plague outbreaks, human overexploitation, habitat destruction and pollution and remained safe until recent emergence of the non-indigenous crayfish species (discussed below), with potential threat to noble crayfish refuges in the country. A third crayfish species, the narrow-clawed crayfish, also occurs in Slovakia, but its distribution remains restricted in the southern parts (Lipták, 2014). The narrow clawed crayfish is the rarest crayfish species in the country, with an unknown population status. It is endangered due to the progression of the spiny-cheek crayfish in the region and research assessing its status is urgently needed.

## 1.2. Spiny-cheek crayfish

The spiny-cheek crayfish was first introduced to Europe to a fish pond near a tributary (Mysla River) of the Oder River in Germany at the end of the 19$^{th}$ century (1894) (Smolian, 1926). A second release of the species was carried out in a tributary of the Loire River in 1912, France (Pretzmann, 1994). Only the first introduction was successful, and the species is now extensively distributed in the area of its first introduction in North-Western Poland, mainly along the Oder River (Filipová et al., 2011). Introduction of spiny-cheek crayfish in the Danube Basin started in 1959 in Hungary, where several thousand individuals were released in the vicinity of Budapest (Thuránszky and Forró, 1987). The first records of spiny-cheek crayfish in the Danube (in 1985) was confirmed from Hungary and Germany (Bavaria) at the same time. The spiny-cheek crayfish was found in the vicinity of Budapest (Hungary) and Ingolstadt (Germany) respectively (Thuranszky and Forro, 1987; Nesemann et al., 1995). Spiny-cheek crayfish spread fast along the Danube River in Hungary, colonizing the whole Hungarian stretch of the river by now (Puky and Schad, 2006) and continues to expand its territory to new water bodies in the region (Seprös et al., 2018). Approximately at the same time as the species reached Serbia (Lipták et al., 2013), its occurrence was reported from the middle section of the Tisa River in Hungary in 2005 (deliberate introduction). Spiny-cheek crayfish is present in several tributaries of the middle Tisa River and lake Balaton as well, progressively colonizing new areas (Sallai and Puky, 2008; Szepesi and Harka, 2011; Györe et al., 2013; Seprös et al., 2018). This invasive species was found in Serbia in 2002 in the Danube River near Apatin and later in 2004 in the vicinity of Smederevo (Karaman and Machino, 2004; Pavlović et al., 2006). The spiny-cheek crayfish was detected in 2003 in the Park of Nature Kopački Rit in Croatia in the flood zone bordered by the Danube and the Drava Rivers (Maguire and Klobučar, 2003; Maguire and Gottstein-Matočec, 2004), and spread considerably in the region since its first observation, with progressive colonisation toward the Drava River in Croatia (Hudina et al., 2009; Maguire et al., 2011). The species was recorded in the Tamiš River (Serbia) in 2011, indicating its expansion trend to Danube River tributaries in the region (Lipták et al., 2013). In 2008, the spiny-cheek crayfish reached the Romanian Danube (Pârvulescu et al., 2009). Spiny-cheek crayfish spread successfully in the Balkan region; thus the occurrence of the species can be even broader than documented so far (Lipták et al., 2013) and even reached Bulgaria (Trichkova et al., 2015). The species also colonised the North-Eastern Germany, where it expanded to every water body in the Mecklenburg-Vorpommern region (Zettler, 1998). The species expanded drastically in Germany and it occurs in river systems like: Necker, Weser, Mosel, Nahe, Main, Aller, Ems, Elbe, Rhine, Lahn, Midland Canal, Lippe and many others (Geelen, 1978; Kouba et al., 2014). Spiny-cheek crayfish occurs in the upper part of the Vistula River (Poland), but a colony has also been detected in the middle part of the river (Schulz and Smietana, 2001; Grabowski et al., 2005). The species is recorded in the North-Eastern part of Poland, reaching Kaliningradskaya (Russia) and in North-Western Belarus and South-Eastern Lithuania (Burba, 2010; Aklehnovich and Razlutskij, 2013). Several populations of the spiny-cheek crayfish have also been reported from England (Holdich and Black, 2007). The distribution of the species in the Czech Republic was evaluated in detail by Petrusek et al. (2006). The species could have been present in the territory of the Czech Republic already in the 1960s, but the first data were published much later in 1989 (Hajer, 1989). Current occurrence of the species in the Czech Republic includes rivers such as: Ohře, Vltava, Jizera, Mrlina, Cidlina, Doubrava, Metuje and Úpa (tributaries to Elbe) and Otava, Lužnice, Sázava and Malše (triburaties to the Vltava) (Petrusek et al., 2006). The core of spiny-cheek crayfish distribution remains in the Elbe and Vltava Rivers, where the species occurs throughout their navigable parts (Petrusek et al., 2006).

The spiny-cheek crayfish species was not recorded in Slovakia until 2007, when the species was detected in the lower stretch of Váh and Ipeľ rivers – major tributaries of the Danube in Slovakia (Janský and Kautman, 2007; Puky, 2009). At present, the spiny-cheek crayfish occurs also occurs in the Hron River (Lipták, 2015) and even in the vicinity of Bratislava (Lipták, 2013). The spiny-cheek crayfish occurrence was confirmed on several sites along the Danube in 2013, near the villages Patince and Zlatná na Ostrove (Lipták and Vitázková, 2014). The occurrence of the species in the vicinity of Bratislava is most probably a result of persisting dispersion of spiny-cheek crayfish (Hungarian population) in the upstream of the river Danube. However, there is a second population occurring in Austria (Danube) and in the Morava River. Sustaining populations are identified in the region of Vienna, discovered in 1991, and in the Morava River near Schlosshof in 1990 (although its occurrence in the Morava River has not been confirmed since) (Nesemann et al., 1995; Pöckl and Pekny, 2002). South-Western Slovakia could have been inhabited by spiny-cheek crayfish back in the 1990s, but it remained undetected until recently, most probably remaining at low densities.

Estimated colonization speed of the spiny-cheek crayfish in the Danube is set within a range of 13–16 km $yr^{-1}$ (Puky and Schád, 2006; Pârvulescu et al., 2012). In the Drava River the colonization speed was calculated to be 2.5 km $yr^{-1}$ (Hudina et al., 2009). Furthermore, the data obtained by Hudina et al. (2009) indicates an average colonization speed in the Danube River to incredible 84 km $yr^{-1}$ in Croatia and Serbia, 48 km $yr^{-1}$ in Romania and 13 to 16 km $yr^{-1}$ in Hungary (Puky and Schád, 2006). Through these estimations and the evaluated colonisation trends, the future distribution of spiny-cheek crayfish can be considerable.

## 1.3. Signal crayfish

The first European signal crayfish introduction occurred in Sweden in in 1959 in attempt to substitute the declining noble crayfish populations (Souty-Grosset et al., 2006; Bohman et al., 2011). The species is now common mainly in the southern parts of Sweden, Denmark, Finland (Westman, 1995; Edsman, 2004; Skov et al., 2011; Vralstad et al., 2011), Western Belgium, France, Spain and England (Holdich, 2002, 2009). There are also some populations in Northern Poland, Lithuania, Latvia and Estonia (Souty-Grosset et al., 2006). Signal crayfish also occurs in Greece, where it was deliberately introduced in 1982 (Holdich et al., 2009). It was introduced in the 1970s to Austria and in the 1980s to Czech Republic for commercial purposes (Holdich et al., 2009). The species has spread outside their culturing sites, especially due to anthropogenic introductions to many water bodies, from where it has later spread actively, colonizing new areas outside its breeding sites into the wild. In Central Europe, its main abundance resides in Austria (Pöckl, 1999). Signal crayfish later spread to Slovakia, invading the Morava River (although anthropogenic transport cannot be excluded here as well) (Petrusek and Petrusková, 2007). Signal crayfish was also recently discovered in the Danube River near Bratislava (Lipták, 2013; Lipták and Vitázková, 2014), suggesting an extending trend of the Moravian population. The first record of signal crayfish in the Danube Basin dates back to 1985, when the species was introduced to Carinthia, Austria (Weinländer and Füreder, 2009). The species was introduced to Isel, Drava, Glan, Gurk and Lavant River (Danube Basin rivers in Austria), from where it has spread intensively (Wainländer and Füreder, 2009), causing a dramatic decline of the local populations of the noble crayfish and to a lesser extent the stone crayfish. In 2008, the signal crayfish reached Croatia, spreading through the Mura River, colonizing the whole stretch of the Mura in the country (Maguire et al., 2008). Later, the species reached Slovenia, spreading from the Drava River (from Austria; Maguire et al., 2011). The species was recently discovered in the Korana River (Sava Basin) in Croatia as well, where the species was introduced through human mediated transport (Hudina et al., 2013). In a review of Kouba et al. (2014), the signal crayfish invaded 29 territories, becoming the most widespread non-indigenous crayfish species in Europe. The colonization speed of the signal crayfish was previously calculated to be in a range of 18-24 km $yr^{-1}$ for Mura River (Hudina et al., 2009) and 1.4 km $yr^{-1}$ for Korana River (Hudina et al., 2013). These estimates clearly suggest a considerable expansion in their future distribution in just a few decades.

## 1.4. Marbled crayfish

An emerging crayfish invader in European freshwaters is the marbled crayfish, also known as the Mamorkrebs. It was considered a parthenogenetically reproducing form of *Procambarus fallax* (Hagen, 1870) (Martin et al., 2010a), originally discovered in the aquarist trade (Scholtz et al., 2003). Recently it is identified as an independent species named *Procambarus virginalis* (Lyko, 2017) (Lyko, 2017). Marbled crayfish is a widely available undemanding pet, frequently sold both in brick and mortar shops and online (Chucholl, 2013, 2015; Faulkes, 2013; Mrugała et al., 2015; Lipták and Vitázková, 2015). Due to its asexual mode of reproduction, marbled crayfish can overpopulate a home aquarium in a short time. Such situation often leads to sale or disposal of redundant individuals by aquarium keepers (Patoka et al., 2014). In the wild, marbled crayfish were first recorded in Germany in 2003 (Marten et al., 2004). Since then, their presence was reported from various European countries, including the Netherlands, Italy, Slovakia, Croatia, Hungary and even Ukraine and Sweden, Romania, Austria and Malta (Chucholl et al., 2012; Kouba et al., 2014; Samardžić, 2014; Novitsky and Son, 2016; Lőkkös et al., 2016; Pârvulescu et al., 2017; Latzer and Pekny, 2018; Deidun et al., 2018; Szendőfi et al., 2018). The crayfish got apparently well established in Madagascar (Jones et al., 2009; Kawai et al., 2009; Andriantsoa et al., 2019) and Japan (Faulkes et al., 2012). The first reliable record of an established population in Central Europe had been reported in 2010 from South-Western Germany (Chucholl and Pfeiffer, 2010), and by 2012 at least six established marbled crayfish populations were known in Europe (Chucholl et al., 2012). Moreover, the marbled crayfish has been recently confirmed to be a vector of the crayfish plague pathogen *A. astaci* (Keller et al., 2014; Mrugała et al., 2015; Andriantsoa et al., 2019), which is responsible for substantial population declines and local extinctions of native European crayfish species (Holdich et al., 2009). The presence of the marbled crayfish in natural ecosystems may, therefore, facilitate the spread of this disease and thus affect native European crayfish species if they get into contact with an infected carrier. The marbled crayfish was first detected in Slovak surface waters in 2010, when more than 150 individuals were collected from a small gravel pit near the village Koplotovce (Janský and Mutkovič, 2010). From the time when it was first recorded in the country, the marbled crayfish was recorded several kilometres downstream from the locality of the first recording and may have reached the nearby Váh River (Lipták et al., 2016). A thermally polluted stream in Slovakia was later found to harbour the marbled crayfish, just in the vicinity of the Nitra River (Lipták et al., 2016). Marbled crayfish were also recorded in the sidearm of the Danube River in the Bratislava region in Slovakia (Lipták et al., 2017). The presence and potential spread of the marbled crayfish in Slovak freshwaters represents a threat, not only to the native astacofauna, but potentially also to other aquatic biota. Fast growth, early maturation, high fecundity and parthenogenetic reproduction strategy (Vogt et al., 2004; Vogt, 2011) combined with a capacity for competition with other crayfish species (Jimenez and Faulkes, 2010; Linzmaier et al., 2018; Hossain et al., 2019) and an ability to spread crayfish plague pathogen (Keller et al., 2014; Mrugała et al., 2015; Andriantsoa et al., 2019), characterize a very successful invader. Given the fact that the species is widely available in the aquarist trade and already introduced to several locations in Europe and elsewhere, a management aiming to prevent further expansion is crucial.

## 1.5. Aquarist trade

The most common freshwater crayfish available in the aquarium trade is the marbled crayfish, red swamp crayfish, Florida crayfish *Procambarus alleni* (Faxon, 1884), redclaw *Cherax quadricarinatus* (von Martens, 1868) and yabby *Cherax destructor* Clark, 1936 (Holdich et al., 2009; Chucholl, 2013). This represents only a fraction compared to the general availability of exotic crayfish species in the aquarium trade, for example, counting some 120 different species in Germany in the past (Chucholl, 2013). Recent research of the aquarist trade in Greece revealed availability of eight non-indigenous species, counting red swamp crayfish, Florida crayfish and a few *Cherax* and *Cambarellus* sp. (Papavlasopoulou et al., 2014). Through online monitoring of the Marmorkrebs in the USA, Faulkes (2013) discovered the availability of the species in 28 American states and in 5 Canadian provinces and Ireland (Faulkes, 2015). In total, 27 crayfish species are advertised and marketed in Czech Republic (Patoka et al., 2014) and 28 different crustacean species are marketed in Turkey (Turkmen and Karadal, 2012). In the Ukraine for example, 15 different non-indigenous crayfish species are present in the aquarist trade (Kotovska et al., 2016), while 12 different crayfish species were present in the Lower Volga Region (Russian Federation) (Vodovsky et al., 2017). In total 26 different crustacean taxa were recorded in the aquarist trade in Slovakia (Lipták and Vitázková, 2015). It is important to note that one-third of the 100 worst invasive species list, created by the International Union for the Conservation of Nature, are from the aquarist pet trade (Padilla and Williams, 2004).

There are already several records of releases of the non-indigenous crayfish species associated with the aquarist trade discovered in Europe. The red swamp crayfish is considered an old non-indigenous invasive crayfish species (Holdich et al., 2009), dramatically expanding its territory in western parts of Europe. Spain and Portugal are the most widely colonised (Kouba et al., 2014). Except this distributional concentration, the species occur in France, Italy, Germany, United Kingdom, Belgium, the Netherlands, Austria and Switzerland (Holdich et al., 2009; Kouba et al., 2014). The red swamp crayfish was first introduced in Europe in 1973 in Spain with an attempt to enhance the commercial production. Later on, its attractive coloration led to its popularity in the aquarist trade, which resulted in intentional introductions mediated by aquarium holders. Intentional releases of the species continued further, and reached even the Azores, Canaries and Hawaii (Souty-Grosset et al., 2006; Gherardi, 2006). The species is currently listed in the top 100 worst invasive species in Europe (Delivering Alien Invasive Species Inventories for Europe, 2019). Even though the species is considered to be restricted to warmer waters, red swamp crayfish also flourish in the colder climates at higher altitudes (Chucholl, 2011). Except for these, several additional crayfish species also occur in the wild. Yabby occurred at one site in central Italy, with an established, sustaining population discovered in 2008, which crashed due to red swamp crayfish colonisation (Scalici et al., 2009; Mazza et al., 2018). The species likely persists in Spain (Kouba et al., 2014) and newly appeared in South-Eastern Sicily (Deidun et al., 2018) and Hungary (Szendőfi et al., 2018). Even the Australian redclaw and the Mexican dwarf crayfish *Cambarellus patzcuarensis* Villalobos, 1943 were recently recorded in Hungary (Weiperth et al., 2017, 2018). There are by far three records of the occurrence of the non-indigenous freshwater shrimp species in the European wild, located in Germany, Hungary and Poland. The species was identified as *Neocaridina davidi* (Kemp, 1918) occurring in Gillbach and Erft river and *Macrobrachium dayanum* (Henderson, 1893) found in Gillbach River, located west of Cologne City in North Rhine-Westphalia in 2012 (Klotz et al., 2013), while *Neocaridina denticulata* (Bouvier, 1904) was recorded in Eastern part of Hungary in the Tisa basin (Weiperth et al., 2019). *N. davidi* was also recorded in Poland at a thermally polluted canal of the river Oder (Jabłońska et al., 2018).

## 1.6. Conservation of indigenous European crayfish

Indigenous crayfish species are nowadays endangered on a continental scale in Europe (Kouba et al., 2014). Orientation towards their protection is critical. South-Western Romanian populations of the stone crayfish have recently been classified as endangered, due to further colonization by the non-indigenous spiny-cheek crayfish species both from Hungary (Tisa River; Pârvulescu and Zaharia, 2013) and Serbia (Tamiš River; Lipták et al., 2013). Noble crayfish and white-clawed crayfish are listed as critically endangered and stone crayfish are endangered in Germany (Chucholl, 2013; Chucholl and Schrimpf, 2016). The decrease in native species are also recorded in Austria and Hungary (Wainländer and Füreder, 2009; Györe et al., 2013). The distribution of the invasive species will extensively expand in the next decades in Europe if no efficient conservation management will come into force (Kouba et al., 2014). They will pose an ever increasing danger for the indigenous crayfish populations and impair the whole aquatic assemblages and energy flow in the rivers (Strayer, 2010; Ercoli et al., 2015). Removal of these species from the environment could, however, restore the native assemblage structure (Moorhouse et al., 2014). The declining water quality, extensive and intensive management of landscape, and direct human impact challenges the indigenous crayfish populations even further. The deliberate introduction of non-indigenous species should not be practised, and the public should be aware of this problem, to restrict the deliberate introductions and further opening of the colonisation routs (Patoka et al., 2014; Patoka et al., 2018). Due to persisting invasive attributes on the non-indigenous invasive crayfish species, protective measurements towards the native crayfish population conservation should be a priority among the conservation practices of Europe (Peay, 2009; Patoka et al., 2017; Patoka et al., 2018).

This situation increases the pressure on local public and environmental agencies to promote adequate preventive actions, as the lack of proper education may promote translocations and introduction of the non-indigenous crayfish to new waterbodies. The socioeconomic drivers increase the likelihood of species introductions, particularly in areas with high gross domestic product and high human population density (Chucholl, 2014), such as the Vienna-Bratislava region and nearby Budapest metropolitan area in Hungary. We believe that public education focused on the mechanisms and consequences of crayfish spread along with the development of more intensive regulation of ornamental aquarist trade should constitute a basis of any management action.

## 1.7. Objectives of the Ph.D. thesis

The current study investigated the non-indigenous crayfish species in Slovakia focusing on the aquarist pet trade as a prominent introduction pathway and on the novel invader, the marbled crayfish, discovered with well-established and sustaining populations during this study. The spiny-cheek crayfish and the signal crayfish occurring in Slovakia were already investigated by the author before the start of this study (Lipták, 2013; Lipták et al., 2013; Lipták, 2014; Lipták and Vitázková, 2014; Lipták et al., 2015). Therefore, our primary focus was investigating the marbled crayfish introduction pathways, its distribution in the wild, population ecology and trophic interactions. This thesis investigates the following objectives:

1. To investigate the non-indigenous crayfish species and other relevant crustacean taxa availability and frequency in the Slovak aquarium trade and subsequently to identify the species which could potentially appear in the wild through intentional and unintentional human mediated introductions.
2. To determine the distribution of the marbled crayfish populations occurring in Slovakia and contribute to the population ecology and reproduction of this species as these studies are sparse due to only short introduction history of this species.
3. To describe for the first time the trophic role and characterise the diet of the marbled crayfish occurring in a lentic freshwater habitat in Slovakia by means of carbon $^{13}C$ and nitrogen $^{15}N$ stable isotopes analysis.

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## CHAPTER 2

## BEAUTIFUL, BUT ALSO POTENTIALLY INVASIVE

**Lipták, B.,** Vitázková, B., 2015. Beautiful, but also potentially invasive. Ekológia (Bratislava) 34, 155–162.

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# BEAUTIFUL, BUT ALSO POTENTIALLY INVASIVE

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Abstract

Lipták B., Vitázková B.: Beautiful, but also potentially invasive. Ekológia (Bratislava), Vol. 34, No. 2, p. 155–162, 2015.

Introduction of non-indigenous exotic species to new areas, where they may establish viable populations and become invasive, is a considerable problem in the protection of nature worldwide, as these species may alter the indigenous species population structure and potentially even decrease the biodiversity. The European fauna underwent through major negative changes on the continent and nowadays, it experiences another new treat, represented by the expanding aquarium pet trade, and with it, associated species (and disease) introductions. Exotic freshwater crustaceans are one of the taxa widely incorporated in the business, counting a remarkable number of species. Recent records of the exotic marbled crayfish or Marmorkrebs (*Procambarus fallax* f. *virginalis*) in German in open ecosystems in Slovakia pointed to human-mediated introductions associated with aquarium pet trade in the country. In this regard, a study of the aquarium pet trade both in expositions and shops and online was assessed. Several crustacean taxa are available both in pet trade exhibitions and online through the Internet. Altogether 26 different species were identified in the aquarium trade in Slovakia. These are *Procambarus fallax* f. *virginalis*, *P. clarkii*, *P. alleni*, *Cherax quadricarinatus*, *C. destructor*, *C. holthuisi, C. peknyi*, *Cambarellus patzcuarensis* and *C. diminutus* occurring in the aquarium pet trade in Slovakia (n = 9). *Procambarus fallax* f. *virginalis*, *P. clarkii* and *C. patzuarensis* are the most common in this regard. There is also a quantity of other related taxa in the aquarium pet trade in Slovakia, mainly *Caridina* spp. (n = 5), *Neocaridina* spp. (n = 4), *Atyopsis moluccensis, Atya gabonensis, Arachnochium kulsiense* and several taxa of exotic crabs (n = 5) belonging to three different genera (*Cardiosoma*, *Geosesarma* and *Gecarinus*) present. *Neocaridina davidi* is identified as the most frequent in this regard. As some of the species can become established and form viable populations in natural ecosystems in Europe, we alert the public to handle the animals responsibly and thus maintain and protect indigenous European fauna.

*Key words*: aquarium pet trade, Europe, exotic species, invasions, Slovakia.

## Introduction

Global biodiversity is nowadays extremely challenged. Humankind has become a major factor negatively influencing the species richness, affecting nearly every ecosystem on earth. Negative factors associated with human actions can thus be divided into local (e.g. species introductions) and global (e.g. environmental pollution), although local ecosystem disturbances can dramatically extend. One of the main causes of diversity declines are species introductions (Dudgeon et al., 2006). Holding of aquarium species has become very popular in Europe and nowadays is becoming increasingly attractive. There is a rich availability of species in the aquarium pet trade, with extending tendencies in the species assortment and perfection of the service (e.g. online shopping). Availability of ornamental species in the aquarium trade represents a major introduction pathway. Crustaceans in the aquarium trade are not an exception in this regard. Between the most common of the freshwater crayfish in the aquarium trade are mainly crayfish species as *Procambarus fallax* f. *virginalis* (marbled crayfish, also known as Marmorkrebs), *P. clarkii*, *P. alleni*, *Cherax quadricarinatus* and *C. destructor* (Holdich et al., 2009; Chucholl, 2013). This represents only a fraction compared to the general availability of exotic crayfish species in the aquarium trade, for example, counting some 120 different species in Germany (Chucholl, 2013). Recent research of the aquarium trade in Greece revealed availability of eight non-indigenous species, counting *Procambarus clarkii*, *P. alleni* and a few *Cherax* and *Cambarellus* species (Papavlasopoulou et al., 2014). Through online monitoring of the Marmorkrebs in the USA, Faulkes (2013) discovered the availability of the species in 28 American states and in 5 Canadian provinces and Ireland (Faulkes, 2014). In total, 27 crayfish species are advertised and marketed in Czech Republic (Patoka et al., 2014) and 28 different crustacean species are marketed in Turkey (Turkmen, Karadal, 2012). Although crayfish species become a subject of scientific interest, there is sparse data on the related taxa in the aquarium pet trade. To date, 17 non-indigenous crustacean species occur in the inland waters in Slovakia (Lipták, 2013). These species occur here as a consequence of introductions, shipping, aquaculture and interconnection of the waterways. The other front of introductions represents the aquarium pet trade. It is important to note that one-third of the 100 worst invasive species list created by the International Union for the Conservation of Nature are from aquarium pet trade (Padilla, Williams, 2004).

The current study is the first and to date the only study referring to exotic crustacean species in the aquarium pet trade in Slovakia, with a hint for the regulation and control of introduction.

### Material and methods

Various aquarium expositions orientated to the pet trade in Slovakia were inspected from September 2013 to June 2014. In addition, major pet markets were examined for crustaceans along the same time period. Decapods were identified in the aquarium containers at the exhibitions by visual observation. The same identification procedure was applied in the pet markets. Additional information on the availability of exotic crustaceans in the country was assessed through the Internet. Each exhibitionist, pet shop or insertion on the Internet was classified as presence and frequency was assessed from the total number of subjects containing exotic crustaceans in their assortment. The data were then sectioned to compare the availability, the frequency and the species spectrum found in expositions and shops, with the species availability, the frequency and the species spectrum identified on the Internet. Thirty subjects (market exhibitionists and pet shops) and 117 insertions on the Internet were identified and evaluated.

### Results

Three North American freshwater crayfish species belonging to genus *Procambarus* (Cambaridae), two dwarf crayfish species of the genus *Cambarellus* (Cambaridae) and four species of Australian genus *Cherax* (Parastacidae) were identified in the aquarium trade in Slovakia, counting together nine different species. Five species of exotic crabs belonging to three genera (*Cardisoma*, *Geo-*

T a b l e 1. The exotic species identified in the Slovak aquarium pet trade in 2013–2014.

| Species | Authority | Common name | Trade name in SK | Family | Native origin | Introductions known |
|---|---|---|---|---|---|---|
| **Freshwater crayfish** | | | | | | |
| *Procambarus fallax* f. *virginalis* | Hagen, 1870 | Marbled crayfish | Rak mramorovaný | Cambaridae | South-Eastern United States | Yes |
| *Procambarus clarkii* | Girard, 1852 | Red swamp crayfish | Rak červený | Cambaridae | South-Eastern United States | Yes |
| *Procambarus alleni* | Faxon, 1884 | Florida crayfish | Rak modrý | Cambaridae | South-Eastern United States | Yes |
| *Cherax quadricarinatus* | von Martens, 1868 | Red claw crayfish | Rak modrý | Parastacidae | Northern Australia, Papua New Guinea | Yes |
| *Cherax destructor* | Clark, 1936 | Yabby | Rak modrý | Parastacidae | Eastern Australia | Yes |
| *Cherax holthuisi* | Lukhaup and Pekny, 2006 | New Guinea crayfish | NA | Parastacidae | Papua New Guinea | Unknown |
| *Cherax peknyi* | Lukhaup and Herbert, 2008 | Zebra crayfish | Rak zebra | Parastacidae | Papua New Guinea | Unknown |
| *Cambarellus patzuarensis* | Villalobos, 1943 | Dwarf orange crayfish | Rak mexický | Cambaridae | Central Mexico | Unknown |
| *Cambarellus diminutus* | Hobbs, 1945 | Least crayfish | NA | Cambaridae | Southern United States | Unknown |
| **Freshwater crabs** | | | | | | |
| *Cardisoma armatum* | Herklots, 1851 | Rainbow crab | NA | Gecarcinidae | Western Africa | Unknown |
| *Geosesarma notophorum* | Peter and Cheryl, 1995 | Mandarin crab | NA | Grapsidae | Indonesia | Unknown |
| *Geosesarma bogorensis* | Bott, 1970 | Vamp crab | NA | Grapsidae | Indonesia | Unknown |
| *Gecarcinus quadratus* | de Saussure, 1853 | Halloween crab | NA | Gecarcinidae | Central America | Unknown |
| *Gecarcinus ruricola* | Linnaeus, 1758 | American land crab | NA | Gecarcinidae | Caribbean's | Unknown |
| **Freshwater shrimp** | | | | | | |
| *Atyopsis moluccensis* | De Haan, 1849 | Bamboo shrimp | NA | Atyidae | Malaysia | Unknown |
| *Atya gabonensis* | Giebel, 1875 | Vampire shrimp | NA | Atyidae | West Africa | Unknown |
| *Arachnochium kulsiense* | Jayachandran, Lal Mohan and Raji, 2007 | NA | NA | Palaemonidae | India | Unknown |
| *Caridina babaulti* | Bouvier, 1918 | Green shrimp | NA | Atyidae | Little Asia | Unknown |
| *Caridina breviata* | N.K. Ng and Cai, 2000 | Bumble bee shrimp | NA | Atyidae | Small stream near Zhapu Village | Unknown |
| *Caridina cantonensis* | Yu, 1938 | Crystal red | NA | Atyidae | Southern China | Unknown |
| *Caridina multidentata* | Stimpson, 1860 | Amana shrimp | Krevetka Japonská | Atyidae | East Asia | Unknown |
| *Caridina propinqua* | De Man, 1908 | Mandarin shrimp | Krevetka mandarínková | Atyidae | Peninsular Malaysia, Singapore, Brunei | Unknown |
| *Neocaridina davidi* | Bouvier, 1904 | Red cherry | NA | Atyidae | East and central China | Yes |
| *Neocaridina denticulata* | De Haan, 1844 | Green neon shrimp | NA | Atyidae | East Asia | Unknown |
| *Neocaridina palmata* | Shen, 1948 | NA | NA | Atyidae | Southern China | Unknown |
| *Neocaridina zhangjiajiensis* | Cai, 1996 | White pearl, Blue pearl | NA | Atyidae | Hunan and Guangdong provinces (China) | Unknown |

T a b l e 2. The availability and frequency of the identified species in the Slovak aquarium pet trade in 2013–2014 (0.0–0.25 very rare, 0.25–0.50 rare, 0.50–0.75 common, 0.75–1.0 very common).

| Species | Online availability | Expositions and shops availability | Total availability | Online frequency | Status | Expositions and shops frequency | Status | Total frequency |
|---|---|---|---|---|---|---|---|---|
| **Freshwater crayfish** | | | | | | | | |
| *Procambarus fallax* f. *virginalis* | 26 | 9 | 35 | 0.22 | Very rare | 0.3 | Rare | 0.238 |
| *Procambarus clarkii* | 7 | 9 | 16 | 0.06 | Very rare | 0.3 | Rare | 0.109 |
| *Procambarus alleni* | 1 | 2 | 3 | 0.009 | Very rare | 0.067 | Very rare | 0.02 |
| *Cherax quadricarinatus* | 4 | 1 | 5 | 0.034 | Very rare | 0.033 | Very rare | 0.034 |
| *Cherax destructor* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| *Cherax holthuisi* | 1 | NA | 1 | 0.009 | Very rare | 0 | Not available | 0.007 |
| *Cherax peknyi* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| *Cambarellus patzuarensis* | 8 | 13 | 21 | 0.068 | Very rare | 0.433 | Rare | 0.143 |
| *Cambarellus diminutus* | 3 | 7 | 10 | 0.026 | Very rare | 0.233 | Very rare | 0.068 |
| **Freshwater crabs** | | | | | | | | |
| *Cardisoma armatum* | 1 | 1 | 2 | 0.009 | Very rare | 0.033 | Very rare | 0.014 |
| *Geosesarma notophorum* | 2 | 1 | 3 | 0.017 | Very rare | 0.033 | Very rare | 0.02 |
| *Geosesarma bogorensis* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| *Gecarcinus quadratus* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| *Gecarcinus ruricola* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| **Freshwater shrimp** | | | | | | | | |
| *Atyopsis moluccensis* | 2 | 8 | 10 | 0.017 | Very rare | 0.267 | Rare | 0.068 |
| *Atya gabonensis* | 1 | 2 | 3 | 0.009 | Very rare | 0.067 | Very rare | 0.02 |
| *Arachnochium kulsiense* | 1 | NA | 1 | 0.009 | Very rare | 0 | Not available | 0.007 |
| *Caridina babaulti* | 2 | NA | 2 | 0.017 | Very rare | 0 | Not available | 0.014 |
| *Caridina breviata* | 1 | NA | 1 | 0.009 | Very rare | 0 | Not available | 0.007 |
| *Caridina cantonensis* | 8 | 9 | 17 | 0.068 | Very rare | 0.30 | Rare | 0.116 |
| *Caridina multidentata* | 1 | 8 | 9 | 0. 009 | Very rare | 0.267 | Rare | 0.061 |
| *Caridina propinqua* | NA | 1 | 1 | 0 | Not available | 0.033 | Very rare | 0.007 |
| *Neocaridina davidi* | 36 | 19 | 55 | 0.308 | Rare | 0.633 | Common | 0.374 |
| *Neocaridina denticulata* | 2 | NA | 2 | 0.017 | Very rare | 0 | Not available | 0.014 |
| *Neocaridina palmata* | 3 | 1 | 4 | 0.026 | Very rare | 0.033 | Very rare | 0.027 |
| *Neocaridina zhangjiajiensis* | 1 | 7 | 8 | 0.009 | Very rare | 0.233 | Very rare | 0.054 |

*sesarma* and *Gecarcinus*) were identified. Freshwater shrimp species were represented by 12 different species belonging to five genera (*Atyopsis*, *Atya*, *Arachnochium*, *Caridina* and *Neocaridina*). Altogether 26 species (Table 1) are identified in the aquarium trade in Slovakia, from which *Procambarus fallax* f. *virginalis*, *Cambarellus patzuarensis* and *Neocaridina davidi* are the most common. Altogether, 21 different species were identified in expositions and pet shops together, and 20 species were recorded online on the Internet (Table 2). Except crustaceans, a vast number of other potentially invasive taxa (e.g. molluscs) were observed at each exhibition and pet shop. Although there is a high selection of species both found online or in expositions and shops (Fig. 1), there is much higher frequency of the species available through the expositions and pet shops (Fig. 2). This is mainly because online availability is most commonly oriented to just one species, where one insertion represents a single species.

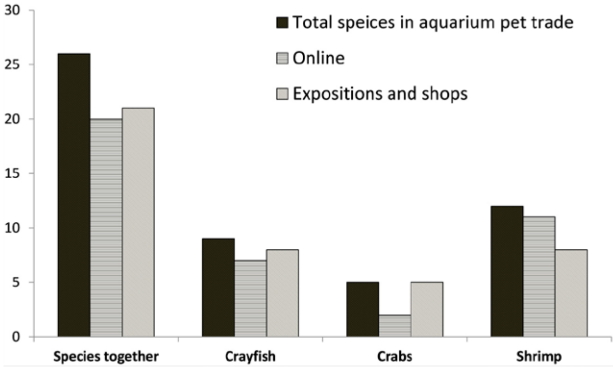


Fig. 1. The overview of species, which have occurred in the aquarium pet trade in Slovakia in 2013–2014.

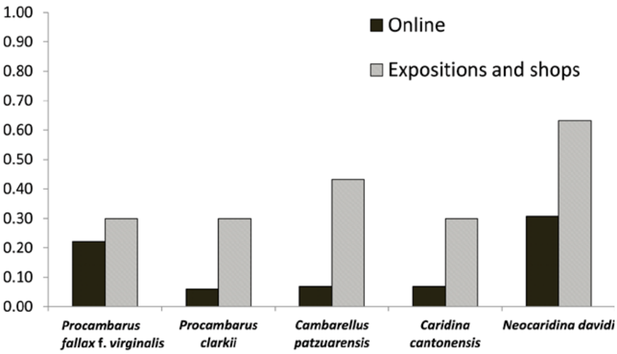


Fig. 2. The frequency of the most available species in the aquarium pet trade in Slovakia in 2013–2014.

## Discussion

There is a considerable number of exotic (and potentially invasive) species that are identified in the aquarium pet trade in Slovakia. This potential species invasiveness and disease introduction highlights the reverence of deliberate introductions mediated through the owners and breeders. Owners of these exotic species must pay special attention as they represent a high likely introduction pathway. Occasional releases of exotic species should not be practiced or encouraged. Managing this problem by dissemination of relevant information should be one of the priorities in order to prevent deliberate introductions of exotics. Regulation through the legislation still remains incomplete. In this regard, education on the matter should be promoted by the legislation binding the provider and the appropriate ministry. A law should be enacted to introduce obligatory educational programmes by specialists to ornamental species providers to further educate the aquarium enthusiast.

There are already several records of releases of the non-indigenous crayfish species associated with the aquarium trade discovered in Europe. The first record of the marbled crayfish originates in Germany, where the specimen was found near the Rhine River in 2003 (Marten et al., 2004). Further records of the species came from Saxony (Germany) at a lowland brook belonging to the Elbe River basin in 2009 (Martin et al., 2010) and the lake Moosweiher near Freiburg (upper Rhine catchment) (Chucholl, Pfeiffer, 2010). Currently, there are 16 records of marbled crayfish in Europe, with the majority of records from Germany (Chucholl et al., 2012; Bohman et al., 2013).

The red swamp crayfish *Procambarus clarkii* is considered an old non-indigenous invasive crayfish species (Holdich et al., 2009). The species is already widely distributed in some regions of Europe, mainly in its western parts. Spain and Portugal are the most widely colonised. Except this distributional concentration, the species occur in France, Italy, Germany, United Kingdom, Belgium, the Netherlands, Austria and Switzerland (Holdich et al., 2009). The red swamp crayfish was first introduced in Europe in 1973 in Spain in an attempt to enhance the commercial production of crayfish. Intentional releases of the species continued further, and reached even the Azores, Canaries and Hawaii (Souty-Grosset et al., 2006; Gherardi, 2006). The species is currently listed in the top 100 worst invasive species in Europe (Delivering Alien Invasive Species Inventories for Europe, 2010). Even though the species is considered to be restricted to warmer waters, *P. clarkii* flourish in the colder climates at higher altitudes equally successfully (Chucholl, 2011).

Except these, several additional crayfish species occur in the wild. *Cherax destructor* occurs at one site in central Italy, with established sustaining population, discovered in 2008 (Scalici et al., 2009) and *Orconectes immunis* occurs in Germany from Strasbourg to Mannheim in the Rhine River, discovered in the mid-1990s (Schrimpf et al., 2013). There are also *Cherax quadricarinatus*, *Orconectes juvenilis*, *O. virilis*, and *Procambarus acutus* populations present in the ecosystems of Europe (Kouba et al., 2014).

North American crayfish species are well known crayfish plague carriers (Söderhall, Cerenius, 1999). Recent studies reported the crayfish plague pathogen in nine different crayfish species in the aquarium pet trade (Mrugała et al., 2014). The crayfish plague pathogen was even detected in wild marbled crayfish populations in Germany (Keller et al., 2014). North American crayfish species thus represent a potential for disease entry pathway into Europe.

Although freshwater crayfish species successfully established viable population under European climatic conditions, still, data on the occurrence of other related crustacean taxa in the wilderness or urbanised areas of Europe remains absent. So far, there is no record of such presence documented, although thermally polluted waters are suggested to support their occurrence. There is only one record of the occurrence of the exotic freshwater shrimp in the European wild, located in Germany. The species was identified as *Neocaridina davidi* occurring in Gillbach and Erft river and *Macrobrachium dayanum* found in Gillbach River, located west of Cologne City in North Rhine-Westphalia in 2012 (Klotz et al., 2013). Due to low water temperature tolerance of *N. davidi*, there is a hypothesis it could extend further in the region and even reach the Rhine River in the future. Recent research postulated evidence that freshwater shrimp (Svoboda et al., 2014a) and crab (Svoboda et al., 2014b) species can also become crayfish plague carriers, which suggest a possible new transmission pathway of the zoospore of this disease. Schrimpf et al. (2014) already confirmed that Chinese mitten crab (*Eriocheir sinensis*), a highly migratory species penetrating freshwaters up to a distance of several hundred kilometres, serves as a vector of crayfish plague pathogen, transmitting the disease to susceptible noble crayfish (*Astacus astacus*). Thus, freshwater shrimps and crabs in the aquarium trade should also be considered as potential crayfish plague reservoirs and handled with care also.

Exotic aquarium pet trade species have a high introduction risk and represents a new risk factor for nature. This new risk is enhanced by the ever increasing assortment of exotics in the aquarium pet trade and absence of its control and regulation. Areas with higher density of the human population, with higher gross domestic product and growing socio-economic aspects are the introduction risk hotspots (Perdikaris et al., 2012; Chucholl, 2014). The aquarium species selected are easy to breed, favour the common conditions of aquariums and thus, are generally more tolerant, increasing the chances of an establishment when introduced. As the species tolerance to general conditions increases, it increases the possibility to establish a viable reproducing population (if introduced to favourable conditions) and potentially expand in its new environment. Introduction of the marbled crayfish in Slovakia is a good example to introductions mediated *via* the aquarium enthusiast. And Slovakia is, we believe, another of the countries in centre of these potentially troubling events of the ever-changing nature and consequences of human impact.

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## CHAPTER 3

# EXPANSION OF THE MARBLED CRAYFISH IN SLOVAKIA: BEGINNING OF AN INVASION IN THE DANUBE CATCHMENT?

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My share on this work is about 40%.

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**ORIGINAL ARTICLE**

# Expansion of the marbled crayfish in Slovakia: beginning of an invasion in the Danube catchment?

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*ABSTRACT*

*The marbled crayfish,* Procambarus fallax *f.* virginalis*, is a taxon widely available in the aquarium pet trade, which has been introduced to open waters in several European countries and in Madagascar. Recent studies confirmed this parthenogenetically reproducing crayfish as a high-risk invasive species, and vector of the crayfish plague pathogen,* Aphanomyces astaci*. It has been first discovered in Slovakia in 2010, but the status of the local population was not studied since then. Due to enlarged sampling area around the first report and one locality, where we presupposed the crayfish occurrence, we identified three new marbled crayfish populations in Slovakia. Two populations are located critically close to the Váh River, a major tributary of the Danube River; one of them being directly connected to the Váh River* via *a side channel during occasional floods. The third established marbled crayfish population was found at the mouth of a thermal stream flowing into the Nitra River, a tributary of the Váh River. In this stream, crayfish coexist with exotic fish and gastropod species of aquarium origin. We presume that the reported localities may serve as a source for further expansion of the marbled crayfish in the mid-part of the Danube catchment. Floods, active dispersal (including overland), passive dispersal by zoochory or anthropogenic translocations are among the major drivers facilitating the marbled crayfish colonization. We have not detected the crayfish plague pathogen in any of the studied populations. However, if spreading further, the marbled crayfish will encounter established populations of crayfish plague carriers in the Danube River, in which case they may acquire the pathogen by horizontal transmission and contribute to spread of this disease to indigenous European crayfish species.*

*Key words: Aquarium pet trade; crayfish plague; freshwater crayfish;* Procambarus fallax *f.* virginalis*; species introductions.*

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## INTRODUCTION

Biological invasions and human impacts (*e.g.*, habitat destruction and pollution) are the major factors negatively influencing global biodiversity (Sala *et al*., 2000; Dudgeon *et al*., 2006). One of the important introduction pathways of potential invaders is the global pet trade, of which aquatic organisms represent a great portion (Padilla and Williams, 2004). The problem of releases of aquarium fish in both freshwater and marine environments has been recognized for a long time (*e.g.*, Courtenay and Stauffer, 1990; Semmens *et al*., 2004), but scantly addressed. More recently, establishment of ornamental crayfish populations received attention, particularly in Europe. Rising density of human population and increasing socio-economic conditions favor the chances of crayfish releases (Perdikaris *et al*., 2012; Chucholl, 2014) and some of the species kept in aquaria become established in the wild (Holdich *et al*., 2009; Kouba *et al*., 2014). Native European crayfish species are challenged by the ever increasing number of newly introduced alien crayfish and the risks associated with them (particularly disease transmission), which substantially complicates their population recovery and conservation (Peay and Füreder, 2011; Capinha *et al*., 2013).

One of the emerging crayfish invaders in European freshwaters is the marbled crayfish, also known as Mamorkrebs, a parthenogenetically reproducing form of *Procambarus fallax* (Hagen, 1870) (Martin *et al*., 2010a) discovered originally in the aquarium trade (Scholtz *et al.*, 2003). Marbled crayfish are widely available undemanding pets, frequently sold both in brick and mortar shops and online (Chucholl, 2013, 2015; Faulkes, 2013; Mrugała *et al.*, 2015; Lipták and Vitázková, 2015). Due to its asexual mode of reproduction, marbled crayfish can overpopulate a home aquarium in a short time. Such situation often leads to sale or disposal of redundant individuals by aquarium holders (Patoka *et al*., 2014a).

In the wild, marbled crayfish were first recorded in

Germany in 2003 (Marten *et al*., 2004). Since then, their presence was reported from various European countries, including the Netherlands, Italy, Slovakia, Croatia, and even Sweden (summarized in Chucholl *et al*., 2012; Kouba *et al*., 2014; Samardžić, 2014), and the crayfish got apparently well established in Madagascar (Jones *et al*., 2009; Kawai *et al*., 2009). The first reliable record of an established population in Central Europe had been reported in 2010 from southwestern Germany (Chucholl and Pfeiffer, 2010), and by 2012 at least six established marbled crayfish populations were known in Europe (Chucholl *et al*., 2012). Moreover, the marbled crayfish has been recently confirmed as a vector of the crayfish plague pathogen, *Aphanomyces astaci* (Keller *et al*., 2014; Mrugała *et al*., 2015), which is responsible for substantial population declines and local extinctions of native European crayfish species (for review, see Holdich *et al*., 2009). The presence of the marbled crayfish in natural ecosystems may, therefore, facilitate the spread of this disease and thus affect native European crayfish species if they get into contact with an infected carrier.

The marbled crayfish had been first detected in Slovak surface waters in 2010, when more than 150 individuals were collected from a small gravel pit near the village Koplotovce (Janský and Mutkovič, 2010). The aim of our study was to evaluate the present status of the marbled crayfish in Slovakia, and its potential for further spread and impact. We report additional sites with the established marbled crayfish populations, in which we assessed the population structure. Furthermore, we tested the collected animals for the potential presence of the crayfish plague pathogen, *Aphanomyces astaci.*

## METHODS

### Study sites

The Slovak Republic is located in the heart of Europe, and its lowland regions are characterized by a continental climate with warm summers and cold winters. All three studied sites with marbled crayfish populations are located in the southwestern part of the country at relatively low elevations (Fig. 1; Tab. 1).

#### *Koplotovce site*

This is the first site from which the marbled crayfish was first reported in Slovakia (Janský and Mutkovič, 2010), it comprises seven adjacent groundwater-fed gravel pits (Fig. 1) ranging in area from 1600 m$^2$ to 21,600 m$^2$. The gravel pits freeze over in winter (with the bottom temperatures not exceeding 4°C), while the epilimnion warms up to 23-25°C in summer. Two of these pits (one being the site of the first marbled crayfish record for Slovakia) are privately owned. The area of the gravel pits is separated from the adjacent Váh River by an embankment that provides protection from the occasional floods. The pits have fluctuating water level and varying depth (up to


**Fig. 1.** The marbled crayfish (*Procambarus fallax* f. *virginalis*) occurrence in Slovakia. Black lines in the central map indicate country borders, blue lines indicate the river network, and the orange rectangle represents privately owned and thus inaccessible sites. Red stars represent newly discovered marbled crayfish populations, while the yellow star represents the original site of the first record in the country.

2-3 m), and are partially overgrown with macrophytes. Although isolated under standard hydrological and meteorological conditions, all gravel pits get periodically interconnected following excessive rainfall (last such events occurred in 2006 and 2010). The pits are frequently visited fishing grounds, seasonally restocked with fish.

### *Leopoldov site*

It is represented by a single large gravel pit (surface area ca. 130,600 $m^2$), connected during floods with a side channel (Drahovský kanál) of the Váh River (last such event occurred in 2010). Depth of the gravel pit varies, reaching 5-7 m at its southern and 4 m in its northern section (Fig. 1). The water temperature regime of this gravel pit is similar to those in Koplotovce. The site is a frequently visited fishing ground, seasonally restocked with fish.

### *Opatovce site*

It is a thermal stream flowing through Opatovce nad Nitrou, a small village next to the popular thermal spa town Bojnice. The water temperature in the stream varies little during the year, ranging from 29 to 31°C; pH values increase from 7.15 in the middle section to 8.30 at the stream mouth (Májsky 2007). The stream (ca. 1 m wide) empties into the Nitra River (ca. 8 m wide), a tributary of the Váh River. The stream bed is formed by concrete blocks, and the stream banks are continuously lined with dense vegetation.

### Field work

We failed to get an access to the privately owned gravel pit in Koplotovce to inspect the original site of the marbled crayfish record. Therefore, the adjoining gravel pits were surveyed. A pilot study at the Koplotovce site was carried out in three gravel pits and one adjoining periodical pool on 15 May 2014. The survey was performed manually with small hand-held net and with 30 fishmeat-baited crayfish traps. The traps were left overnight and collected in the morning. Subsequently, the Koplotovce site was visited on 6 September 2014. Two gravel pits (previously sampled on 15 May 2014) were investigated with electrofishing equipment. In the larger gravel pit (ca. 17,000 $m^2$), the sampling was conducted along ca. 40 m of the shore; the smaller adjoining pit (ca. 1600 $m^2$) was surveyed for 10 min in a 10 m long shore area. Finally, an additional survey with a standardized sampling effort was carried out on 17 October 2014 in one of the pits where crayfish had been recorded during a previous visit. The animals were collected with a small hand-held net for 45 min in a shore area approximately 30 m long.

The Leopoldov site was inspected for three days on 16, 17 and 18 September 2014. On this occasion, crayfish were mainly observed and photographed. The collection of crayfish took place on 18 October 2014, with the same effort as during the last-mentioned samping at the Koplotovce site (*i.e.*, by manual search with a hand-held net for 45 min along an approx. 30 m long shore area).

The thermal stream in Opatovce was visited for the first time on 19 September 2013, when the site was inspected for crayfish by electrofishing. Subsequently, on 17 October 2014, crayfish individuals were sampled as in the gravel pits by a small hand-held net for 45 min along an approx. 30 m long stream section.

Upon capture, the carapace length of crayfish individuals was measured. The numbers of juveniles carried by females obtained at the Koplotovce site were counted; brood sizes of females from the Leopoldov site were roughly estimated from available photographs. Subsequently, all individuals were stored in 96% ethanol for further analyses.

### Molecular analyses

From each locality, up to 16 specimens (as given in Tab. 1) were used for screening for the presence of the crayfish plague pathogen. From each crayfish, we dissected the whole soft abdominal cuticle, the tail fan, and two joints of walking legs (in individuals smaller than 4.5 cm all basal joints with legs). Furthermore, we inspected the crayfish for the presence of melanized spots, potentially indicating an immune response to pathogens; if these were observed, the respective body part was included in the analysis.

**Tab. 1.** Details on the sampled localities with the marbled crayfish (*Procambarus fallax* f. *virginalis*) populations in Slovakia, and on collected crayfish individuals.

| Sampling site | River basin | Type of water body | Coordinates Latitude (N) | Coordinates Longitude (E) | Elevation (m) | Sampling date | Collected crayfish | CL (mm) | Crayfish tested for *A. astaci* |
|---|---|---|---|---|---|---|---|---|---|
| Koplotovce | Váh | Gravel pit | 48°28’11” | 17°48’15” | 141 | 6 Sep 2014 | 11 | 13.3-44.8 | 6 |
| | | | | | | 17 Oct 2014 | 10 | 5.6-24.3 | 10 |
| Leopoldov | Váh | Gravel pit | 48°27’02” | 17°47’11” | 140 | 18 Oct 2014 | 21 | 5.4-30.9 | 12 |
| Opatovce | Nitra | Thermal stream | 48°46’01” | 18°34’39” | 254 | 17 Oct 2014 | 38 | 7.4-35.7 | 12 |

*CL, carapace length.*

The genomic DNA was extracted with the DNeasy tissue kit (Qiagen) from up to 50 mg of the mix-tissue samples ground beforehand in liquid nitrogen (as in Kozubíková *et al*., 2009). The molecular detection of *A. astaci* was performed with the TaqMan minor groove binder quantitative polymerase chain reaction (qPCR; after Vrålstad *et al.*, 2009) as described in Svoboda *et al*. (2014). Additionally, the identity of the crayfish species was investigated by sequencing of a 648 bp long fragment of the mitochondrial gene for the cytochrome c oxidase subunit I (COI) from one crayfish individual per population. We used the universal primer pair LCO1490/ HCO2198 (Folmer *et al*., 1994), following the protocols described in Mrugała *et al*. (2015).

**RESULTS**

Three new established populations of the marbled crayfish have been confirmed in Slovakia. As expected for this parthenogenetically reproducing taxon, all captured individuals were females. At the Koplotovce site, only exuviae of a single crayfish individual was found on 15 May 2014, despite the overnight use of crayfish traps and manual sampling effort. Five adult and six medium-sized (carapace lenght (CL) 10-20 mm) individuals were caught at this site on 6 September 2014 (Fig. 2A). Three mature females, captured on 6 September 2014, carried 372, 412 and 455 juveniles, respectively. The fourth female lost some of the offspring during manipulation, and thus carried only 81 juveniles at the time of counting. Ten more marbled crayfish were collected on 17 October 2014 (Fig. 2B), in the survey with a standardized sampling effort. Two mature marbled crayfish females with eggs were photo-documented at the Leopoldov site on 16 September 2014. Although the egg numbers were not counted, the assessment of photographs suggests that both brood sizes reached at least 300 eggs. Furthermore, young individuals were observed on 17 and 18 September 2014. During the survey on 18 October 2014, 21 medium-sized (CL 5-15 mm) individuals were collected (Fig. 2C). The crayfish were found mainly in the leaf litter accumulated at the banks of the gravel pit.

At the Opatovce site, four crayfish individuals were collected on 19 September 2013 and further 38 crayfish were caught during a standardized sampling on 18 October 2014 (Fig. 2D). Three ornamental fish species, the guppy (*Poecilia reticulata*), the Mozambique tilapia (*Oreochromis mossambicus*) and the convict cichlid (*Amatitlania nigrofasciata*), were observed at the site as well. Moreover, the stream bottom substrate was dominated by a dense population of the red-rimmed melania (*Melanoides tuberculata*), an alien gastropod frequently



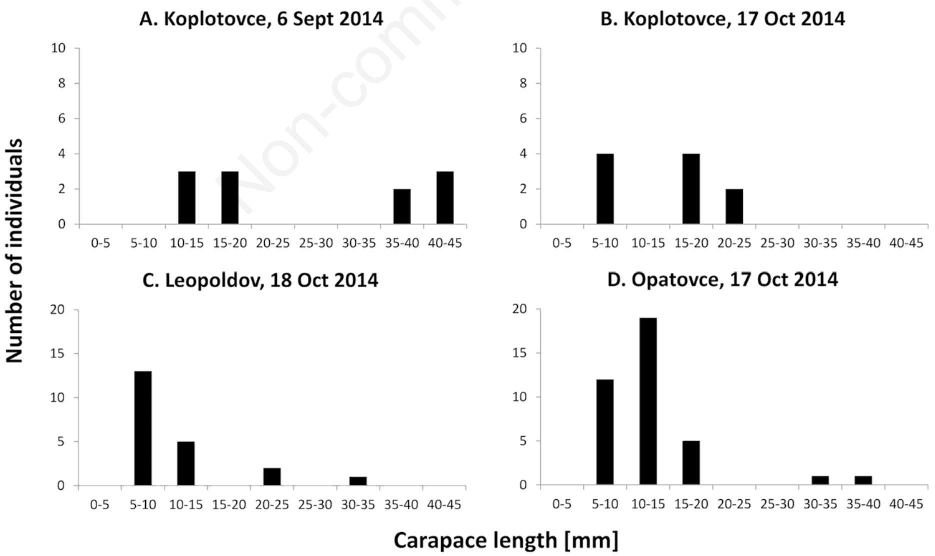


**Fig. 2.** Body size distribution (expressed as carapace length) of the marbled crayfish (*Procambarus fallax* f. *virginalis*) from the inspected Slovak crayfish populations.

kept in aquaria. All four crayfish individuals sampled on 19 September 2013 were transferred alive to laboratory for breeding; two individuals died soon but the two others were still alive in January 2015. By that time, both surviving females had reproduced five times, approximately every three months. First reproduction of their offspring was observed at the age of 6 months, in synchrony with the maternal generation.

The DNA barcoding confirmed the morphological identification of captured crayfish as *P. fallax* f. *virginalis*. All obtained COI fragments matched completely the publicly available reference sequences of the marbled crayfish from GenBank (acc. nos. KC107813, HM358011, JF438007; Martin *et al.*, 2010a; Filipová *et al.*, 2011; Shen *et al.*, 2013). No traces of *Aphanomyses astaci* DNA were detected in any of the analyzed marbled crayfish.

## DISCUSSION

The marbled crayfish presence in Central Europe is an excellent example of successful introductions of an ornamental species. Although original prognoses questioned its survival in the wild, especially in temperate climate (Martin *et al.*, 2010b), it is now recognized as an established invader both in Europe and in Madagascar (Jones *et al*., 2009; Kawai *et al*., 2009; Chucholl *et al*., 2012; Kouba *et al*., 2014). Due to its parthenogenetic reproduction strategy, theoretically no more than one individual is needed to establish a viable population (Scholtz *et al*., 2003). Based on our data and on findings from Germany (Chucholl and Pfeiffer, 2010; Chucholl *et al.*, 2012), it is evident that the species survives and successfully reproduces in Central European climatic conditions. Its overwinteringt ability, with successful survival at 2 to 3°C for three months, was also confirmed experimentally (Veselý *et al*., 2015). As the marbled crayfish is widely available in the aquarium pet trade in Europe, this raises concerns of its further introductions (Chucholl, 2013, 2014; Patoka *et al*., 2014b).

However, even if no new marbled crayfish populations become established in near future in Slovakia or adjoining countries, the already known Slovak populations are an obvious threat, as they may serve as the foothold for the spread of this species in the Danube basin. Several North American invasive crayfish are known for their considerable capacity for active migration and colonization. For example, survival potential of a desiccation for up to several hours has been documented for the congeneric red swamp crayfish *Procambarus clarkii* as well as for the signal crayfish *Pacifastacus leniusculus* (Banha and Anastácio, 2014). This may promote crayfish passive dispersal over long distances, but also allows crossing of terrestrial barriers to new suitable habitats. Active overland dispersal has been recently documented for the spiny-cheek crayfish *Orconectes limosus* (Puky, 2014), and living marbled crayfish have been also observed out of water, over 100 m from a lake (Chucholl *et al*., 2012). Moreover, water birds may possibly serve as the translocation vectors for crayfish. Small juveniles of the red swamp crayfish were reported to climb to mallard feet, remain there for several minutes and survive on air for up to three hours (Águas *et al*., 2014).

If the marbled crayfish manages to successfully colonize rivers in the Danube basin (which seems likely as the population in the thermal stream in Opatovce is not separated from the Nitra River by any barrier, and the other populations are in a close vicinity of the Váh River and its side channel), the species' relatively fast dispersal can be expected unless restricted by environmental factors. The colonization potential of invasive crayfish can be dramatic, spreading downstream and even upstream in a considerable speed (Bubb *et al*., 2004). A good example is the colonization of the Danube River by the spiny-cheek crayfish in Hungary and adjoining countries (Puky and Shád, 2006; Pârvulescu *et al*., 2012; Lipták and Vitázková, 2014). The expansion of marbled crayfish may be further enhanced by passive dispersal along the rivers, in particular downstream by currents and floods. Single individuals of sexually reproducing crayfish invaders, when dispersing over long distances, are highly unlikely to establish a population unless a mated mature female or female with a clutch survives the translocation (note, however, that it remains unclear under which conditions facultative asexual reproduction, reported for spiny-cheek crayfish, takes place; Buřič *et al*., 2011). However, due to the obligate asexual reproduction of the marbled crayfish, this taxon is not limited by the Allee effect at very low population densities, and even dispersal of juvenile individuals may allow their subsequent reproduction in newly colonized sites. Floods (such as those occurring in the Váh basin in 2006 and 2010) may thus not only allow the crayfish to spread from the gravel pits to the river system, but also facilitate their rapid downstream dispersal.

Thermal streams, both fed by natural warm springs and those thermally polluted by human activities (*i.e.*, cooling water from industry), represent a specific category of habitats that may support introductions of ornamental aquatic species in temperate regions (Emde *et al*., 2016). Many of such species are elsewhere limited by the low water temperatures and are unable to proliferate outside the thermal streams; however, some of them tolerate a wide range of temperatures and may disperse successfully out of these sites of introduction. Numerous cases of establishment of aquarium crustaceans, in particular crayfish, have been documented in such habitats across Europe. The red swamp crayfish in a thermal stream in Austria (Petutschnig *et al.*, 2008) and the tropical redclaw crayfish, *Cherax quadricarinatus*, in an oxbow lake in Slovenia (Jaklič and Vrezec, 2011) seem so far restricted to thermal waters. In Germany, establishment of two aquarium shrimp species, one of which may tolerate also lower temperatures, has been documented in a stream fed by cooling water from a coal power plant (Klotz *et al*., 2013). In case of the marbled crayfish in a thermal stream in Slovakia, the temperature does not seem a limiting factor (as apparent from the other established marbled crayfish populations in Central Europe; Chucholl *et al*., 2012). Furthermore, the relatively fast current of the thermal stream can facilitate crayfish movement into the Nitra River.

Juveniles observed in autumn, and the presence of medium-sized individuals in our samples (Fig. 2), indicate at least two seasonal clutches of the marbled crayfish in studied sites. It is estimated that under the laboratory conditions, the marbled crayfish can complete up to seven reproduction cycles during its lifespan of 2 to 3 years, and the generation time is about 6-7 months (Vogt, 2010). The amount of juveniles increases with each cycle in relation to size increase of the maternal individuals (Vogt, 2011), and may reach very high values for large females. Under laboratory conditions, Vogt (2011) reported the maximum number of 427 juveniles in one clutch. Some field-collected individuals were nevertheless even more fecund: one female from Madagascar studied by Jones *et al.* (2009) carried approximately 530 eggs (see Fig. 2 in Jones *et al*., 2009), and Chucholl and Pfeiffer (2000) reported as many as 724 eggs in a single marbled crayfish clutch from a German population. Thus, 455 juveniles carried by one marbled crayfish from the Koplotovce site do not seem to be exceptional, even under Central European conditions, and this number confirms a substantial reproduction potential of this invasive species.

The ability of the marbled crayfish to act as an *A. astaci* vector deserves considerable attention as well. Although no *A. astaci* infection was detected in our study, a complete absence of the pathogen cannot be ascertained. Infected marbled crayfish have been already confirmed in the aquarium pet trade, laboratory cultures, as well as in the wild (Keller *et al*., 2014; Mrugała *et al*., 2015), and genotyping of the pathogen suggested that the species got infected by horizontal transmission from another species (Mrugała *et al*., 2015). If the marbled crayfish successfully colonizes the Danube, it is thus likely that it will acquire the infection from the spiny-cheek crayfish, confirmed to carry the crayfish plague pathogen in this river (Kozubíková *et al*., 2010; Pârvulescu *et al*., 2012). Due to the marbled crayfish potential to rapidly expand its range, it is possible that it might spread the infection also into habitats that the other American species has not reached yet.

## CONCLUSIONS

The presence and potential spread of the marbled crayfish in Slovak freshwaters represents a threat not only to the native astacofauna but potentially also to other aquatic biota. Fast growth, early maturation, high fecundity and parthenogenetic reproduction strategy combined with a capacity for competition with other crayfish species (Jimenez and Faulkes, 2010) and an ability to spread crayfish plague pathogen (Keller *et al*., 2014; Mrugała *et al*., 2015), characterize a very successful invader. Given the fact that the species is widely available in the aquarium trade and already introduced to several locations in Europe, a management aiming to prevent further expansion is crucial.

This situation increases the pressure on local public and environmental agencies to promote adequate preventive actions, as the lack of proper education may promote translocations and introduction of the crayfish to new waterbodies, and thus contribute substantially to the marbled crayfish further colonization of the Danube catchment. The socioeconomic drivers increase the likelihood of species introductions, particularly in areas with high gross domestic product and high human population density (Perdikaris *et al*., 2012; Chucholl, 2014), such as the Vienna-Bratislava region and nearby Budapest metropolitan area in Hungary. Thus, our findings of established marbled crayfish might not be the last from this region. We believe that public education focusing on the mechanisms and consequences of crayfish spread, along with the development of more intensive regulation of ornamental trade, should constitute a basis of any management action. Furthermore, it should be supported by a further research evaluating marbled crayfish impacts on the native communities and habitats, and eventually, a development of the effective elimination means of alien crayfish from the natural environments.

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## CHAPTER 4

# SLOVAK SECTION OF THE DANUBE HAS ITS WELL-ESTABLISHED BREEDING GROUND OF MARBLED CRAYFISH *PROCAMBARUS FALLAX* F. *VIRGINALIS*

**Lipták, B.,** Mojžišová, M., Gruľa, D., Christophoryová, J., Jablonski, D., Bláha, M., Petrusek, A., Kouba, A., 2017. Slovak section of the Danube has its well-established breeding ground of marbled crayfish *Procambarus fallax* f. *virginalis*. Knowledge and Management of Aquatic Ecosystems 418, 40.

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SHORT COMMUNICATION OPEN ACCESS

# Slovak section of the Danube has its well-established breeding ground of marbled crayfish *Procambarus fallax* f. *virginalis*

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**Abstract –** Established populations of the non-indigenous parthenogenetically reproducing marbled crayfish *Procambarus fallax* f. *virginalis* have been recently reported from various European countries. The colonised sites are usually lentic and relatively isolated from major watercourses and in such cases the immediate threat of the spread of this taxon is limited. Here we report on a marbled crayfish population that is likely to become a seed for colonisation of the Danube in Slovakia. It is located in a channel within the Slovak capital Bratislava in the immediate vicinity of a pumping station that occasionally releases significant amounts of water into the side arm of the Danube. The population is well established with a high growth potential: numerous adult marbled crayfish individuals were observed at the site in September and October 2016 and the progeny (eggs or first two developmental stages) of 27 berried females exceeded 11 000 individuals. The maximum observed fecundity per female reached 647 juveniles in the second developmental stage. The Danube side arm downstream of the pumping station harbours a population of spiny-cheek crayfish *Orconectes limosus* infected with the crayfish plague pathogen *Aphanomyces astaci*. We presume that marbled crayfish is already present below the pumping station and it is just a matter of effort and time until it is discovered. The investigated specimens of marbled crayfish were found free of *A. astaci*, but horizontal transmission from infected spiny-cheek crayfish may be expected, as well as further spread of marbled crayfish in the Danube.

**Keywords:** pet trade / aquatic invasion / fecundity / asexual reproduction / Slovakia

**Résumé – Une portion slovaque du Danube est un site de reproduction bien établie d'écrevisse marbrée *Procambarus fallax* f.*virginalis*.** Des populations établies d'écrevisses marbrées non-indigènes à reproduction parthénogénétique *Procambarus fallax* f. *virginalis* ont récemment été signalées dans différents pays européens. Les sites colonisés sont généralement lentiques et relativement isolés des grands cours d'eau et, dans de tels cas, la menace immédiate de propagation de ce taxon est limitée. Nous rapportons ici sur une population d'écrevisses marbrées qui risque de devenir une source pour la colonisation du Danube en Slovaquie. Elle est localisée dans un canal situé dans la capitale slovaque Bratislava, à proximité immédiate d'une station de pompage qui libère occasionnellement d'importantes quantités d'eau dans le bras latéral du Danube. La population est bien établie avec un fort potentiel de croissance: de nombreux adultes d'écrevisse marbrée ont été observés sur le site en septembre et octobre 2016 et la progéniture (œufs ou deux premiers stades de développement) de 27 femelles grainées dépasse 11 000 individus. La fécondité maximale observée par femelle a atteint 647 juvéniles au deuxième stade de développement. Le bras latéral du Danube en aval de la station de pompage abrite une population d'écrevisses américaines *Orconectes limosus* infectées par l'agent de la peste de l'écrevisse *Aphanomyces astaci*. Nous supposons que les écrevisses marbrées sont déjà présentes au-dessous de la station de pompage et c'est juste une question de prospection et de temps jusqu'à ce qu'elles soient découvertes. Les spécimens étudiés d'écrevisses marbrées ont été trouvés exempts d'*A. astaci*, mais on peut s'attendre à une

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transmission horizontale à partir d'écrevisses infectées et à une propagation accrue des écrevisses marbrées dans le Danube.

**Mots-clés :** commerce d'animaux de compagnie / invasion aquatique / fécondité / reproduction asexuée / Slovaquie

## 1 Introduction

Biological invasions have devastating consequences on the native biota, which is particularly apparent in freshwater ecosystems (Richman *et al.*, 2015). Introduced non-indigenous crayfish species affect the invaded biotopes, with negative community-level impacts (Moorhouse *et al.*, 2014; Roukonen *et al.*, 2016). Among alien crayfish, the marbled crayfish *Procambarus fallax* f. *virginalis* is an emerging threat, particularly in Europe. It is the only known crayfish reproducing via obligate apomictic parthenogenesis, producing genetically uniform offspring (Martin *et al.*, 2010). This species is characterised by early maturation (Seitz *et al.*, 2005), reproduces throughout the whole year under favourable conditions (Vogt *et al.*, 2004; Seitz *et al.*, 2005), and its high competitiveness for food and shelters has been documented (Jimenez and Faulkes, 2011). Its survival under low temperatures was proven both in the laboratory and the field (Veselý *et al.*, 2015; Lipták *et al.*, 2016). The marbled crayfish was first discovered in the German aquarium trade in the mid-1990s, from where it further dispersed (Scholtz *et al.*, 2003). Its availability at the pet markets is usually high (*e.g.* Kotovska *et al.*, 2016; Vodovsky *et al.*, 2017). At the beginning of the new millennium, reports on occurrence of single specimens from the wild appeared, followed by confirmation of established populations in Germany and Slovakia in 2010; since then, the number of invaded European countries has steadily increased (see Patoka *et al.*, 2016 and references cited therein), and the ability of marbled crayfish to carry the crayfish plague pathogen has been confirmed both in aquarium trade (Mrugała *et al.*, 2015) and in the field (Keller *et al.*, 2014). Due to all these characteristics, the marbled crayfish became listed among the invasive alien species of European Union concern according to recent legislation (EU Regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141). Here we report an established marbled crayfish population in Bratislava, Slovakia, which has presumably initiated the colonisation of the Danube.

## 2 Material and methods

The marbled crayfish was discovered by a chance during research focused on the ecology of another alien species, the yellow-bellied slider *Trachemys scripta scripta* and the red-eared slider *T. s. elegans*, both native to North America. Two marbled crayfish were caught in turtle traps on August 25, 2016, in front of the pumping station in the Chorvátske rameno in Bratislava. Chorvátske rameno is a dead-end artificial canal within the town district Petržalka, which ends at a pumping station (48.0996 N, 17.1306 E) next to a side arm of the Danube (Jarovecké rameno) directly connected to the river (Fig. 1A, B). The canal is approx. 5 km long and 20 m wide with a depth of 2–3 m in its centre. Submerged macrophytes are present in some sections of the canal, and its banks are usually lined with emergent macrophytes. The canal bed is formed by fine gravel mixed with organic detritus.

Two installed pumps at the station in Chorvátske rameno have a capacity of 260 l·s$^{-1}$ and are activated mainly during elevated flow rates (floods) in the Danube and during extensive rainfalls in the area in order to regulate ground waters in this highly populated town district. They are also occasionally activated when being checked for functionality. The pumping activity will transfer any biota in the immediate vicinity of the station into the side arm of the Danube, with no further barriers to dispersal to the river itself.

After accidental finding of marbled crayfish, two additional field samplings followed, the first on September 11, and the second on October 24, 2016. Both samplings focused on the areas just above and below the pumping station, *i.e.*, places where the presumed chance of successful capture of crayfish was highest. The first survey of the Chorvátske rameno canal was performed by a single researcher, who explored 2 m long stony section of the shore for 30 min. The second survey was performed by three researchers on a 10 m stretch. The sampling lasted for 40 min. Thanks to the high abundance of the marbled crayfish and easy access to the site, no crayfish trapping was needed. The Jarovecké rameno side arm is stabilised by heavy stones forming several layers. Thus manual search (ineffective in such conditions) was combined with trapping, using six baited traps exposed overnight during the first survey and 25 traps in the second survey.

Carapace length of sampled crayfish was measured to the nearest 0.1 mm. The eggs and juveniles in the first two developmental stages were counted if present. Juveniles in the third developmental stage become gradually independent and their quantification would be inaccurate. All captured crayfish individuals were preserved in 96% ethanol. Screening of the presence of the crayfish plague pathogen *Aphanomyces astaci*, using the quantitative PCR-based methods of Vrålstad *et al.* (2009), was conducted on all adult crayfish captured at both investigated sites (Chorvátske rameno and Jarovecké rameno). Details of the laboratory protocols are described in Mrugała *et al.* (2015) and Lipták *et al.* (2016).

## 3 Results

During the two field sampling events, altogether 39 adult marbled crayfish (11 + 28 females) and 9 spiny-cheek crayfish *Orconectes limosus* (7 + 2 individuals of both sexes) were captured. All marbled crayfish were caught above the pumping station in the Chorvátske rameno canal, while all spiny-cheek crayfish individuals were caught into traps below the pumping station in the Jarovecké rameno side arm (Fig. 1B).

The carapace length (totalling *ca.* 50% of the body size) of marbled crayfish specimens ranged from 21.8 to 48.1 mm, with a mean of 39.2 mm (Fig. 1C). In total, 27 marbled crayfish (69% of the catch) carried eggs or juveniles. The quantity of the offspring ranged between 147 and 647 (on average 420) eggs or juveniles per female, with a positive correlation with

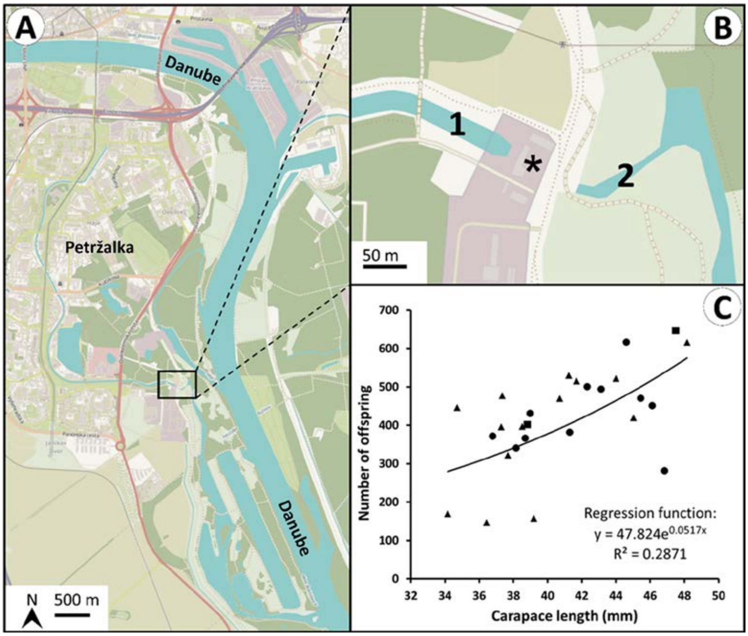


**Fig. 1.** Map showing the marbled crayfish *Procambarus fallax* f. *virginalis* occurrence in Bratislava, Slovakia – general view (A) and detailed location (B). Asterisk refers to the pumping station, while 1 and 2 to the locations in the Chorvátske rameno canal and the Jarovecke rameno side arm where marbled crayfish and spiny-cheek crayfish *Orconectes limosus* were found, respectively. Fecundity of marbled crayfish females (C) expressed as the number of eggs (circles), stage 1 (triangles) and stage 2 juveniles (squares), respectively, and the relationship between female carapace size and the number of offspring (exponential regression, with all three age categories pooled). The basis for the maps is available under the Open Database License (www.openstreetmap.org).

the size of the mother (Fig. 1C). Altogether, the 27 captured berried females carried 11 348 offspring. No trace of *A. astaci* DNA was detected in any analysed marbled crayfish.

Of the spiny-cheek crayfish (6 males, 3 females, carapace length 25.0–52.1 mm, mean 42.6 mm), one specimen was confirmed as being infected with *A. astaci* (agent level A3, according to the method of Vrålstad *et al.*, 2009).

## 4 Discussion

Due to irresponsible or uninformed hobby breeders, marbled crayfish are intentionally released into the wild and become established, as documented across Europe (Chucholl *et al.*, 2012; Kouba *et al.*, 2014). Most of the sites with well-documented established populations are lentic habitats relatively isolated from the main watercourses. However, records from some sizeable rivers (*e.g.* the Rhine in Germany or the Po delta in Italy) were also reported, although their recent population status remains unclear (Chucholl *et al.*, 2012; Vojkovská *et al.*, 2014; Patoka *et al.*, 2016 and literature cited therein). Weiperth *et al.* (2015) refer to several specimens of various sizes detected in thermal ponds and their outflows including adjacent Danube in Budapest, Hungary. Evaluation of the population status in the river is an issue of on going research (Weiperth A., pers. comm., 2017).

The newest discovered site with the marbled crayfish in Bratislava, Slovakia, also occurs in the immediate vicinity of the Danube, separated only by a pumping station that occasionally releases its waters to one of the river arms. This section of the Danube is already colonised by the non-indigenous spiny-cheek crayfish which invaded this river section in the last two decades and, recently, also by signal crayfish *Pacifastacus leniusculus* (Lipták and Vitázková, 2014). We have not confirmed syntopic occurrence of marbled crayfish with these species yet, but we consider that confirmation of marbled crayfish in the side arm of the Danube is just a matter of time and search effort. Water pumping, intentional translocation of marbled crayfish by humans, or active migrations of marbled crayfish, are factors that can transfer (or may have already transferred) the species into the Danube (Chucholl *et al.*, 2012; Lipták *et al.*, 2016).

The conditions in the side arm of the Danube, Jarovecké rameno, are favourable for crayfish, as indicated by the locally present spiny-cheek crayfish population. The documented presence of *A. astaci* in that species corresponds to its infection status elsewhere in the Danube (Kozubíková *et al.*, 2010; Pârvulescu *et al.*, 2012). Upon contact of marbled crayfish with infected spiny-cheek crayfish, we may expect a horizontal transmission of *A. astaci* between the two host species (see James *et al.*, 2017). This means that thereafter the marbled crayfish expansion in the Danube catchment will be very likely accompanied by the expansion of the crayfish plague pathogen, which causes mass mortalities of indigenous crayfish stocks in Europe (Holdich *et al.*, 2009).

Any attempts to eradicate this marbled crayfish population are likely to be ineffective because of its obligate parthenogenetic reproduction mode, when even a single survivor may re-establish the whole population. Its remarkable reproductive capacity and extremely high fecundity, low-temperature tolerance and high competitiveness (Vodovsky *et al.*, 2017 and literature cited therein) all suggest that the marbled crayfish will become a permanent part of the Danube ecosystem, with great potential for an extension of its range, with largely unknown consequences so far. Some of its life history characteristics (*e.g.* higher fecundity, earlier maturation, supposedly faster growth and more reproduction events per year) provide significant advantages, even compared to other non-indigenous crayfish species already present in this section of the Danube, the spiny-cheek crayfish and the signal crayfish (Lipták and Vitázková, 2014).

To conclude, we expect that marbled crayfish might be already present in a side arm of the Danube, where horizontal infection with crayfish plague pathogen originating from spiny-cheek crayfish will occur. We presume that the marbled crayfish will spread actively further (mainly downstream), but its range extension may be accelerated by the occasional floods where successful reproduction of even single dispersed specimens is not limited. A competition with this new invader might have severe consequences for remaining stocks of the indigenous narrow-clawed crayfish *Astacus leptodactylus*, already under pressure of spiny-cheek crayfish (*cf.* Pârvulescu *et al.*, 2012, 2015). Successful competition of marbled crayfish with other non-indigenous crayfish species already present in the Danube may also be expected. However, given the role of crayfish in ecosystems in general and characteristics of marbled crayfish in particular, the spread of marbled crayfish has the potential for significant consequences for much broader range of taxa. This is a serious issue since the Danube possesses habitats for diverse biota, being a unique ecosystem of European importance. Future monitoring of marbled crayfish in the Danube is warranted, but at early phases of establishment may be methodologically challenging in such large river course. Utilisation of eDNA methods might be an useful tool in this regard.

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# CHAPTER 5

## TROPHIC ROLE OF MARBLED CRAYFISH IN A LENTIC FRESHWATER ECOSYSTEM

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**Research Article**

# Trophic role of marbled crayfish in a lentic freshwater ecosystem

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## Abstract

Species' introductions may cause severe adverse effects on freshwater ecosystems and their biota. The marbled crayfish, *Procambarus virginalis* Lyko, 2017, is an invasive parthenogenetically reproducing crayfish with rapid reproduction, maturation and tolerance to a wide range of environmental conditions, which was introduced to many sites across Europe during the last decade. Due to its recent speciation and limited number of field studies, the knowledge of trophic interactions of the marbled crayfish in freshwater food webs is scarce. An invaded area located in Central Europe was studied to identify the marbled crayfish food web interactions using analysis of carbon $^{13}C$ and nitrogen $^{15}N$ isotopes. This study brings the first insight into the trophic ecology of marbled crayfish in lentic freshwater ecosystems. Algae and detritus were identified as the most important food sources for the marbled crayfish, while zoobenthos and macrophytes were less important. Moreover, the marbled crayfish was found to be an important food source for top fish predators, but marginal for omnivorous fish. Being able to utilize energy from the bottom of the trophic food web, the marbled crayfish may have important roles in the ecosystem, transferring energy to higher trophic levels. It processes allochthonous and autochthonous matter in the ecosystem, thus being a competitor to other organisms with similar food preferences and impacting zoobenthos, algae and macrophytes through predation or direct consumption. To sum up, the marbled crayfish has a strong ability to utilize food sources from different trophic levels, and, thanks to its life history, can be a highly adaptable invader.

**Key words:** biological invasion, Central Europe, parthenogenetic species, *Procambarus virginalis*, stable isotope

## Introduction

Crayfish (Decapoda: Astacidea) are a highly diverse taxonomic group of freshwater organisms, containing both critically endangered endemic species and highly invasive species, responsible for numerous cases of successful invasions in Europe and North America (Holdich et al. 2009; Lodge et al. 2012). Many populations of indigenous crayfish species (ICS) in Europe have been lost or substantially reduced, largely due to direct or indirect effects of non-indigenous crayfish species (NICS) (Kouba et al. 2014). Furthermore, the entire functioning of an invaded ecosystem can be irreversibly altered by NICS (Lodge et al. 2000; Rodríguez et al. 2005), as they often exhibit higher population densities, faster life cycles and occupy a wider trophic niche when compared to ICS (Lodge et al. 2000). Additionally, North American NICS are also vectors of crayfish plague, caused by the oomycete *Aphanomyces astaci* (Schikora), which is lethal to other crayfish (Svoboda et al. 2017). Overall, North American NICS have been responsible for local extinctions in Europe, replacing ICS and affecting the food webs and communities of the invaded ecosystems through species-specific interactions (Rodríguez et al. 2005; Matsuzaki et al. 2009). Crayfish are large omnivorous macroinvertebrates often representing an important proportion of the benthos biomass, serving as a prey for a range of predators (Holdich 2002), and mediating nutrient and energy flow in freshwater ecosystems (Correia and Anastácio 2008; Grey and Jackson 2012; Ruokonen et al. 2012). Unsurprisingly, crayfish are considered not only as keystone species, but also strong ecosystem engineers (Reynolds and Souty-Grosset 2012).

Many examples of diverse negative effects of invasive crayfish on invaded ecosystems are known, with the red swamp crayfish *Procambarus clarkii* (Girard, 1852) being the most often studied species (Lodge et al. 2012; Twardochleb et al. 2013). Nyström et al. (1996) compared ponds with presence or absence of signal crayfish *Pacifastacus leniusculus* (Dana, 1852). They found decreases in macrophyte biomass, cover and species richness, reduced benthic invertebrate taxa and biomass, shifts in invertebrate community and lower organic matter content in sediments in invaded localities. Ruokonen et al. (2014) and Ercoli et al. (2015) reported reduced aquatic invertebrate biodiversity (especially mollusc taxa) in boreal lakes as a consequence of signal crayfish introductions. The virile crayfish, *Faxonius virilis* (Hagen, 1870), impacted the phytoplankton abundance and metaphytic algae and had a dramatic effect on American bullfrog *Lithobates catesbeianus* (Shaw, 1802) tadpoles and benthic invertebrate biomass (gastropods) by disturbing breeding adults, destroying nest attachments or by feeding on their eggs, and direct predation, respectively. On the other hand, the virile crayfish also positively affected the zooplankton biomass due to highly reduced abundance of the bluegill larvae *Lepomis macrochirus* (Rafinesque, 1819) caused by egg predation (Dorn and Wojdak 2004).

The marbled crayfish, *Procambarus virginalis* Lyko, 2017, is one of the most invasive crayfish (Kawai et al. 2015; Nentwig et al. 2018), being included among the top invasive species of European Union concern (EU 2016). Even though the first known individuals of this species were found in the German aquarist trade in the mid-90s (Scholtz et al. 2003), its exact native range remains uncertain. Its closest relative is the slough crayfish *Procambarus fallax* (Hagen, 1870), native to Florida and Georgia in the South-Eastern United States (Gutekunst et al. 2018). Unique among all decapods, the marbled crayfish reproduces through apomictic parthenogenesis and its global population likely represents a single clone (Vogt et al. 2015; Gutekunst et al. 2018). Originally kept in captivity, as a popular pet, the marbled crayfish quickly became established in the European wild (Chucholl et al. 2012; Patoka et al. 2016), owing to its fast growth, early maturation, high fecundity, short intervals between reproductive cycles, and competitiveness in behaviour interactions (Vodovsky et al. 2017 and references cited therein). The substantial ability of the marbled crayfish to withstand extreme environmental conditions (Veselý et al. 2015; Kouba et al. 2016; Veselý et al. 2017) may allow the species to spread and establish populations in many habitats, where it may cause water turbidity by disturbing fine sediment particles while burrowing for shelter, searching for food or escaping (Kouba et al. 2016; Pledger et al. 2016). Taken altogether, the marbled crayfish is a highly invasive species with potential to negatively affect ecosystem services and biodiversity.

The majority of the growing body of literature on the marbled crayfish deals primarily with laboratory experiments on its biology, establishment in the wild and use as a model organism (Patoka et al. 2016; Veselý et al. 2015). Other main targets are biogeography and pet trade, with risk assessment of the species (Uderbayev et al. 2017; Weiperth et al. 2018). However, data from the field are particularly scarce (Vogt 2018), leading to gaps in our basic knowledge of the species ecology. In this study, we hypothesized that (i) marbled crayfish utilize sources on multiple trophic levels and macroinvertebrates, macrophytes and detritus are likely the most important food items, (ii) marbled crayfish is an important food source for higher trophic levels, and (iii) it may act as a key species and transfer energy from the detritus to higher trophic levels. This study aims to provide the first insight into the trophic ecology of the marbled crayfish by investigating its trophic role in a recently colonized lentic freshwater ecosystem, through stable isotope analysis of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$).

## Materials and methods

### *Study site*

The flooded gravel pit in Leopoldov (48°27′2″N; 17°47′6″E) is a rather oligotrophic site, located in the south-western part of Slovak Republic. This lowland region (ca. 138 m a.s.l.) is characterized by a continental climate, with warm summers and cold winters. The water at the locality warms up to 23–25 °C in summer and freezes over in winter, with temperatures not exceeding 4 °C. The surface area of the study site is ca. 13 ha, with maximum depth of 7 and 4 m in its southern and northern part, respectively. The shoreline is surrounded by terrestrial vegetation, including trees, and the majority of the bottom is covered by submerged macrophytes and algae. During the floods, the locality can be interconnected with the side channel (Drahovský kanál) of the Váh River that flows into the Danube River; the last such event occurred in 2010. The site is a frequently visited recreational and fishing ground, seasonally restocked with common carp *Cyprinus carpio* (Linnaeus, 1758) and has an established population of marbled crayfish. It was first recorded at the site in 2014, most likely introduced by hobby aquarists or fishermen, possibly from the system of flooded gravel pits near Koplotovce, where the marbled crayfish was first observed in the country in 2010 (Lipták et al. 2016).

*Sample collection*

The locality was sampled for all potential food sources of each proposed ecosystem trophic level in mid-August 2016. Fish were collected by angling during the day and night. Crayfish were caught by manual hand search assisted with handheld nets, as well as with traps baited with fresh fish meat, placed along the shoreline in late afternoon and collected the following morning. Bulk zooplankton samples were collected using a net (mesh size 250 μm) pulled horizontally through the water column. Zoobenthos was collected, up to 1 m depth, using a hand net (mesh size 500 μm). Macrophytes, periphyton and autochthonous and allochthonous detritus were collected from the shoreline. All samples were kept frozen on dry ice after collection at the locality and transferred to the laboratory freezer (−30 °C) until further processing for stable isotope analysis (SIA) of carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N).

*Stable isotopes analysis*

In fish, a piece of white dorsal muscle tissue was taken, while in crayfish, a piece of abdominal muscle tissue was used as recommended by Stenroth et al. (2006). Samples of zoobenthos, terrestrial detritus and macrophytes were mostly separated to species or genus level for analysis. Later, to analyse energy flow in food web, fish and zoobenthos species were divided into functional groups (Supplementary material Table S1, Table S2 and Table S3). We also analysed macrophytes (*Myriophyllum aquaticum* (Vellozo) Verdcourt, *Chara vulgaris* Linnaeus, *Potamogeton obtusifolius* Mertens and W.D.J. Koch), algae, mosses and detritus (Table S4). All samples for SIA were dried at 50 °C for 48 h to constant weight and grounded to a fine homogenous powder. Approximately 0.5 mg of animal samples and 1.5 mg of plant and detritus samples were precisely weighed into tin cups. Stable isotope analyses were performed using a Carlo Erba

Flash EA 1112 elemental analyser connected to Thermo Finnigan DELTAplus and Advantage continuous-flow isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA).

The standards used as reference materials were Vienna Pee Dee belemnite for carbon and atmospheric $N_2$ for nitrogen. Muscle tissue of pike *Esox lucius* Linnaeus, 1758 and potato *Solanum tuberosum* Linnaeus leaves of known isotopic compositions were run as internal working standards for animal and plant samples, respectively, after every 6 samples to control for instrument stability. Results are expressed using the conventional δ notation as parts per thousand difference from the international standards. Analytical precision was < 0.1 ‰ for $\delta^{13}C$ and < 0.3 ‰ for $\delta^{15}N$.

Trophic position of each species/functional group was calculated using the formula of Anderson and Cabana (2007):

$$T_P = ((\delta^{15}N_{sample} - \delta^{15}N_{baseline})/3.23) + E_P \quad (1)$$

where *Tp* is the trophic position of an organism, $\delta^{15}N$ *sample* represents the nitrogen isotope value of the given organism, $\delta^{15}N$ *baseline* is the isotopic ratio from several individuals of grazers (*Anopheles* spp. and *Physa* spp.), *3.23* is the nitrogen isotope fractionation between trophic levels (Vander Zanden and Rasmussen 2001) and $E_P$ 2 is the expected trophic position.

*Stable isotope mixing models*

The common carp was excluded from the analyses, as this species is the most popular game fish in the region and the fishing grounds are repeatedly stocked during fishing season, thus not reflecting the isotopic signal of the study site. To assess the contribution of the different food sources to the isotopic signature of each target organism or functional group, a separate Bayesian mixing model with a specific number of putative sources was run in SIAR-package (Parnell et al. 2010) in R (R Core Team 2016). For predatory fish, a four-source mixing model was produced (zooplankton, zoobenthos, crayfish, and omnivorous fish). For omnivorous fish, a five-source mixing model including autochthonous sources (autochthonous detritus, macrophytes, and algae), allochthonous sources (allochthonous detritus), zooplankton, zoobenthos and crayfish was applied. In crayfish, a five-source mixing model including algae, autochthonous detritus, allochthonous detritus, macrophytes, and zoobenthos was run. However, in final models for omnivorous fish and for crayfish, crayfish were omitted as a putative source in both cases due to their low contribution (2%). Lastly, for zoobenthos a two-source mixing model including autochthonous (autochthonous detritus, macrophytes, algae) and allochthonous sources (allochthonous detritus) was used. As recommended by Vander Zanden and Rasmussen (2001), fractionation factors assumed in the model were 3.23 ± 0.41 ‰ for $\delta^{15}N$ and 0.47 ± 1.23 ‰

for $\delta^{13}C$ for animals, and 2.4 ± 0.42 ‰ for $\delta^{15}N$ and 0.40 ± 0.28 ‰ for $\delta^{13}C$ for detritus and macrophytes (McCutchan et al. 2003).

Recently, several experimental studies have examined crayfish specific isotopic turnover times and fractionation factors (e.g. Rudnick and Resh 2005; Carolan et al. 2012; Jussila et al. 2015; Glon et al. 2016). The results of these studies vary a lot, depending on the food sources (high or low protein, single diet etc.), condition of animals, and experimental conditions. Our unpublished results suggest that fractionation factors in marbled crayfish fed on either single plant or meat diet in both carbon and nitrogen could be twofold higher than some reported fractionation factors in crayfish (Veselý et al., *unpublished data*). Moreover, obtaining accurate values for all possible food source combinations is almost impossible. Thus, in our mixing models we decided to use the fractionation factors mentioned at the end of the previous paragraph (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003), which are probably the most often applied in aquatic ecosystem stable isotope studies. These values fall within the range of those documented for crayfish specific factors. Another caveat to our study is that, due to isotopic turnover, the isotopic signatures may reflect feeding preferences earlier in the season. However, our unpublished studies suggest low isotopic turnover times in marbled crayfish tissue, thus samples taken in August should reflect the high vegetation season.

## Results

The trophic food web of the studied lentic ecosystem consists of three trophic levels, with predatory fish at the apex, primary producers on the bottom and crayfish together with grazers, filter-feeders, predatory zoobenthos, and omnivorous fish occupying an intermediate trophic level (Figure 1, Table S3). Omnivorous fish occupied a higher trophic position than crayfish, while zoobenthos occupied an intermediate trophic position between primary sources and crayfish.

The main food sources for predatory fish (Table 1; Figure S1) were zooplankton (mean 34%) and omnivorous fish (mean 28%), while the contribution of crayfish and zoobenthos amounted only to 19%. Omnivorous fish used most putative sources equally and all sources had a rather similar contribution of 30%, except for zooplankton that was slightly less preferred than other sources (mean 11%) (Table 1; Figure S2).

Crayfish used mostly allochthonous detritus (mean 30%), algae (mean 25%) and autochthonous detritus (mean 21%) as a food sources (Table 1, Figure S3), with zoobenthos and macrophytes on average contributing 9 and 14%, respectively. Zoobenthos showed a slightly higher utilization of allochthonous material (mean 59%) when compared to autochthonous material (mean 41%) (Table 1; Figure S4).

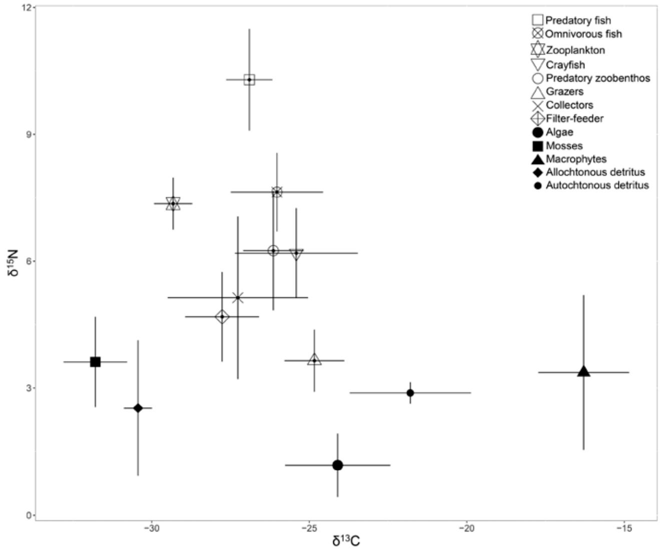


**Figure 1.** Mean values ± SD of carbon and nitrogen stable isotope values (‰) of food web of the flooded gravel pit in Leopoldov, Slovak Republic.

**Table 1.** The relative contributions (means with upper and lower 95% highest density region – hdr) of putative food sources to the diets of marbled crayfish in the flooded gravel pit in Leopoldov, Slovak Republic.

| Consumer | Source | Low 95% hdr | Mean % contribution | High 95% hdr |
|---|---|---|---|---|
| Predatory fish | Zooplankton | 0.21 | 0.33 | 0.46 |
| | Omnivorous fish | 0.04 | 0.28 | 0.49 |
| | Crayfish | 0 | 0.19 | 0.38 |
| | Zoobenthos | 0 | 0.19 | 0.37 |
| Omnivorous fish | Autochthonous sources | 0.15 | 0.34 | 0.52 |
| | Allochthonous sources | 0.03 | 0.28 | 0.48 |
| | Zoobenthos | 0 | 0.28 | 0.53 |
| | Zooplankton | 0 | 0.11 | 0.24 |
| Crayfish | Allochthonous detritus | 0.13 | 0.29 | 0.46 |
| | Algae | 0 | 0.25 | 0.46 |
| | Autochthonous detritus | 0 | 0.21 | 0.42 |
| | Macrophytes | 0 | 0.15 | 0.29 |
| | Zoobenthos | 0.03 | 0.09 | 0.17 |
| Zoobenthos | Autochthonous sources | 0.49 | 0.59 | 0.70 |
| | Allochthonous sources | 0.30 | 0.41 | 0.51 |

## Discussion

This study indicates that the marbled crayfish utilizes a wide range of food sources, likely impacting and modifying the food web structure in the ecosystem. In line with our hypotheses, detritus (both autochthonous and allochthonous) was found to be the most important source in the diet of the marbled crayfish, while other sources such as zoobenthos, algae and macrophytes were utilized to a lesser extent. Moreover the marbled crayfish was also an important food source for predatory fish, which supports our hypothesis of the multi-trophic functional role of this species in the ecosystem. As a result of its dominance in the benthic community, the marbled crayfish has a high potential to negatively impact local species diversity and ecosystem functioning (Creed et al. 2009; Ruokonen et al. 2014; Lipták et al. 2017), as documented for the red swamp crayfish (Gutiérrez-Yurrita et al. 1998; Souty-Grosset et al. 2016).

We revealed that the marbled crayfish serves as an important decomposer, processing allochthonous and autochthonous matter in the ecosystem. This concurs with Usio (2000), stressing the importance of a New Zealand crayfish *Paranephrops zealandicus* (White, 1847) in leaf processing in a temperate stream. Due to its preference for detritus, the marbled crayfish may compete with other organisms (e.g. native crayfish, collectors, shredders) utilizing allochthonous and autochthonous detritus (Dorn and Wojdak 2004; Ercoli et al. 2015). The marbled crayfish also utilizes algae and macrophytes, which is consistent with the behaviour of other NICS introduced to Europe, such as the signal crayfish and the red swamp crayfish (Nyström et al. 1999; Carreira et al. 2014). Although other studies reported especially strong negative effects or a high dependence of NICS on the mollusc taxa (e.g. Nyström et al. 1996, 1999; Glon et al. 2017), this was not confirmed in our research. Zoobenthos contributed little to the diet of the marbled crayfish, possibly due to the generally low population densities of zoobenthos at the locality, which is rather oligotrophic.

The marbled crayfish rapidly became a new element of the European fauna, but this invasive species may have negative effects to native species and communities. The utilization of a wide range of food sources by this highly plastic species may contribute to its successful establishment under a wide range of conditions. Moreover, marbled crayfish can form populations with high density and become very abundant in a short time (Janský and Mutkovič 2010; Lipták et al. 2017; Andriantsoa et al. 2019). As we hypothesized, marble crayfish has the ability to exploit an ecosystem at various trophic levels potentially impacting on a large range of organisms within the community. As a large-bodied macroinvertebrate, the marbled crayfish acts as a predator as well as a prey for organisms at higher trophic levels. Thus, based on our results, it can be considered as a keystone species with ability to transport the energy from the bottom of the chain to top predators, potentially driving important changes in the invaded ecosystems, as demonstrated for the red swamp crayfish (Geiger et al. 2005). The impacts of NICS associated with their trophic role may vary, depending on the plasticity of the invading crayfish (Ruokonen et al. 2014), their life stages or thermal regimes experienced (Carreira et al. 2017). Thus, the negative effects on the invaded ecosystem can be established in many perspectives and fronts. Therefore, more studies on the feeding dynamics and seasonal behaviour of marbled crayfish are needed and we call for further field and experimental studies dealing with marbled crayfish under various biotic and abiotic conditions.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Biometry (mean ± SD) of fish and crayfish used in stable isotope analysis.
**Table S2.** Composition of macroinvertebrate functional groups used in stable isotope analysis.
**Table S3.** The isotopic value, trophic position, and number on analysed samples of functional group of organism (mean ± SD).
**Table S4.** The isotopic value and number of analysed samples of primary producers and detritus (mean ± SD).
**Figure S1.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of predatory fish in the flooded gravel pit in Leopoldov, Slovak Republic.
**Figure S2.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of omnivorous fish in the flooded gravel pit in Leopoldov, Slovak Republic.
**Figure S3.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of marbled crayfish in the flooded gravel pit in Leopoldov, Slovak Republic.
**Figure S4.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of zoobenthos in the flooded gravel pit in Leopoldov, Slovak Republic.

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## Supplementary material

***Table S1.*** *Biometry (mean ± SD) of fish and crayfish used in stable isotope analysis.*

| | | |
|---|---|---|
| **Predatory fish** | | |
| | Standard length (mm) | Weight (g) |
| *Esox lucius* Linnaeus, 1758 | 230.0 ± 94.9 | 222.5 ± 230.8 |
| *Lepomis gibbosus* (Linnaeus, 1758) | 79.8 ± 11.1 | 13.7 ± 5.5 |
| *Perca fluviatilis* Linnaeus, 1758 | 115.0 ± 17.8 | 27.2 ± 11.8 |
| **Omnivorous fish** | | |
| *Rutilus rutilus* (Linnaeus, 1758) | 158.8 ± 69.4 | 63.3 ± 39.1 |
| *Scardinius erythrophtalmus* (Linnaeus, 1758) | 233.0 | 375.0 |
| **Marbled crayfish** | | |
| | Carapace length (mm) | Weight (g) |
| *Procambarus virginalis* Lyko, 2017 | 31.9 ± 12.7 | 10.1 ± 7.0 |

***Table S2.*** *Composition of macroinvertebrate functional groups used in stable isotope analysis.*

| Functional group | Species |
|---|---|
| **Predator** | *Sympetrum* sp. |
| | *Gerris lacustris* (Linnaeus, 1758) |
| | *Sialis lutaria* (Linnaeus, 1758) |
| **Collector** | *Cloeon dipterum* (Linnaeus, 1761) |
| | *Ceanis robusta* Eaton, 1885 |
| | *Micronecta* sp. |
| **Grazer** | *Physa fontinalis* (Linnaeus, 1758) |
| | *Anopheles* sp. |
| **Filter-feeder** | *Dreissena polymorpha* (Pallas, 1771) |

***Table S3.*** *The isotopic value, trophic position, and number of analysed samples of functional group of organism (mean ± SD).*

| Functional group | $\delta^{13}C$ | $\delta^{15}N$ | Trophic position | No. of samples |
|---|---|---|---|---|
| Predatory fish | -26.9 ± 0.73 | 10.29 ± 1.2 | 3.03 ± 0.35 | 12 |
| Omnivorous fish | -26.02 ± 1.41 | 7.63 ± 0.93 | 2.25 ± 0.27 | 6 |
| Zooplankton | -29.32 ± 0.61 | 7.36 ± 0.61 | 2.16 ± 0.18 | 5 |
| Crayfish | -25.41 ± 1.95 | 6.19 ± 1.07 | 1.82 ± 0.31 | 14 |
| Predatory zoobenthos | -26.14 ± 0.96 | 6.25 ± 1.41 | 1.84 ±0.42 | 9 |
| Grazers | -24.84 ± 0.95 | 3.65 ± 0.73 | 1.07 ± 0.22 | 5 |
| Collectors | -27.27 ± 2.23 | 5.14 ± 1.92 | 1.51 ± 0.57 | 8 |
| Filter-feeders | -27.76 ± 1.17 | 4.68 ± 1.06 | 1.38 ± 0.31 | 4 |

***Table S4.*** *The isotopic value and number of analysed samples of primary producers and detritus (mean ± SD).*

| Group | $\delta^{13}C$ | $\delta^{15}N$ | No. of samples |
|---|---|---|---|
| Algae | -24.1 ± 1.67 | 1.17 ± 0.75 | 6 |
| Mosses | -31.79 ± 1.01 | 3.62 ± 1.07 | 3 |
| Macrophytes | -16.29 ± 1.44 | 3.37 ± 1.83 | 14 |
| Allochthonous detritus | -30.43 ± 0.45 | 2.52 ± 1.61 | 3 |
| Autochthonous detritus | -21.79 ± 1.92 | 2.88 ± 0.25 | 3 |

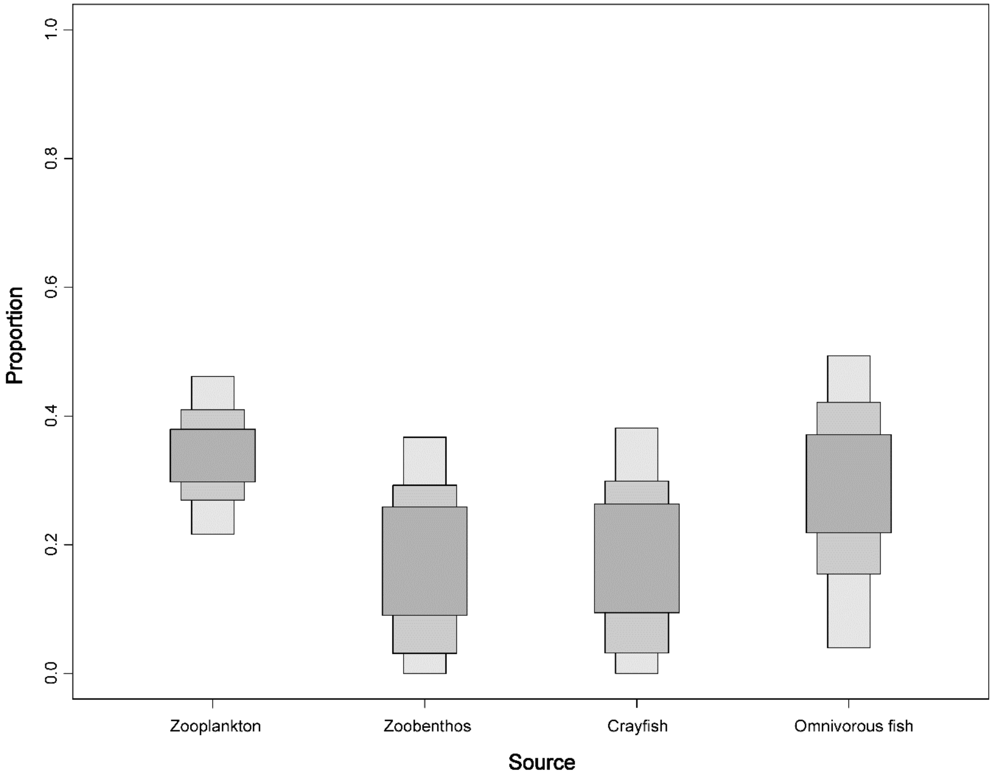


***Figure S1.*** *The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of predatory fish in the flooded gravel pit in Leopoldov, Slovak Republic.*

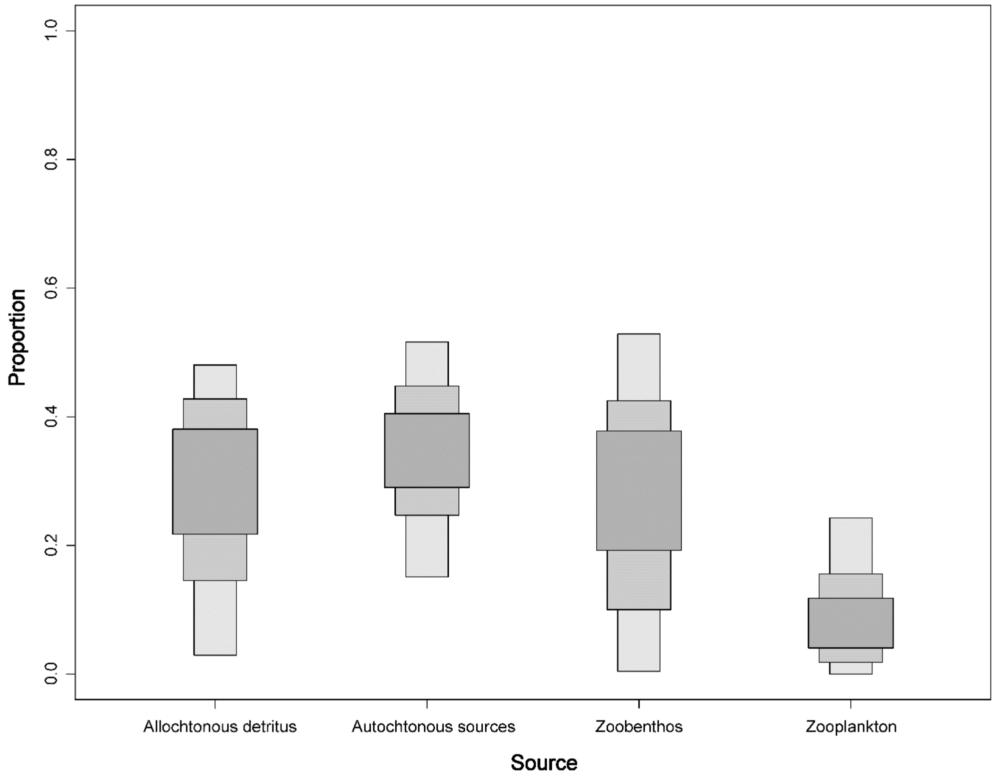


***Figure S2.*** *The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of omnivorous fish in the flooded gravel pit in Leopoldov, Slovak Republic.*

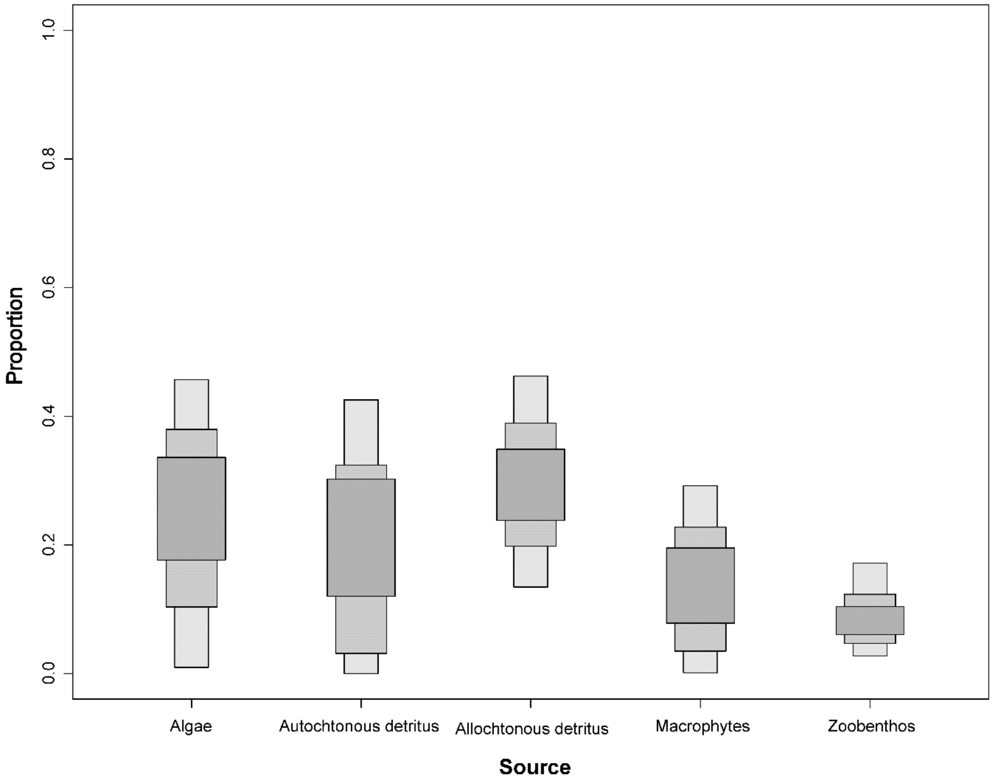


***Figure S3.*** *The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of marbled crayfish in the flooded gravel pit in Leopoldov, Slovak Republic.*

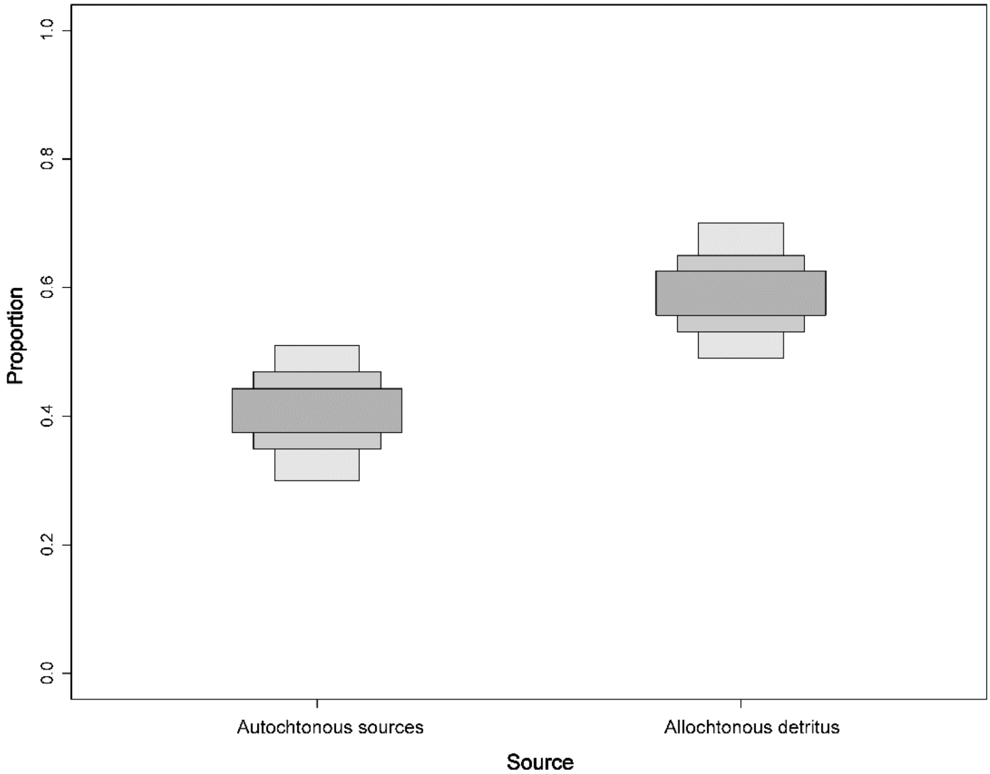


***Figure S4.*** *The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of zoobenthos in the flooded gravel pit in Leopoldov, Slovak Republic.*

# CHAPTER 6

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

# GENERAL DISCUSSION

There is a vast number of non-indigenous (and potentially invasive) species available in the aquarium pet trade in Slovakia (Chapter 2) as well as in other European and Asian countries. Consequently, there is a high risk of deliberate introductions mediated through the owners and breeders and the potential to start a biological invasion. This risk is higher and correlated with the gross domestic product and density of a human population (Perdikaris et al., 2012; Chucholl, 2014). Great European cities are built in close vicinity to the major rivers e.g. Rhine or the Danube River, which exacerbates intorductions due to the easy access to many water bodies of the river basins, while the river itself offers poor management capabilities against an invasion. Special attention should be awarded (as stressed in the Chapter 2) to owners of these non-indigenous species, as they represent a likely introduction pathway and can start a potential biological invasion. Occasional releases of non-indigenous species should not be practiced and encouraged by any means. Maintaining this problem through informative campaigns should be a priority in the prevention of deliberate species introductions. Many aquarists as well as the public are poorly informed regarding biological invasions, specifically how they start and their far reaching implications on native communities and ecosystems. It is evident that this lack of information can have dire consequences, while the root of the problem of anthropogenic species introductions, remains underestimated and poorly studied. Further analysis could solve this problem and contribute as a preventativestrategy to prevent further species introductions. Regulation through the legislation still remains incomplete, although some actions are carried out, namely recently applied Commission Implementing Regulation No. 2016/1141, banning some of the stressed highly-invasive species in the EU. Despite these regulatory mechanisms, there is still insufficient efficacy with this kind of regulation and the problems still persist (Ruokonen et al. 2018, Faulkes 2018, Patoka et al. 2018). Furthermore, most of these kinds of regulatory mechanisms are seriously delayed. Regulation implementation has so far only come to action after the invasion has already started, or the indigenous species has faced serious population declines. The crayfish plague could serve as a good example. Recent considerations of the Ministry of the Environment in Japan to regulate non-indigenous crayfish introduced to the Japanese islands, while there has already been a report of crayfish plague in the country originating from the red swamp crayfish individuals in the wild (Martín-Torrijos et al. 2018) and the signal crayfish (Usio et al., 2007). Due to persisting demand in crayfish in the aquarist trade, the Indonesian crayfish production for example, could seriously endanger the indigenous crayfish species in the region (Putra et al. 2018) and could have negative consequences in regions of their export.

There are already several records of non-indigenous crayfish species release associated with the aquarium trade discovered in Europe, in most of which cases the species were also identified in the Slovak aquarium trade (Chapter 2). Subsequently, only after the marbled crayfish appeared in the German aquarist trade, did the first records of its release in the wild become documented near the Rhine River early in 2003 (Marten et al. 2004). Further records of the species came from Saxony (Germany) at a lowland brook belonging to the Elbe River basin in 2009 (Martin et al. 2010) and the lake Moosweither near Freiburg (Upper Rhine catchment) (Chucholl and Pfeiffer, 2010). The majority of marbled crayfish records are from Germany (Chucholl et al. 2012; Bohman et al. 2013) with the numbers growings not only nationally, but continentally as well, along with increasing research(Kouba et al., 2014). Records of non-indigenous crayfish are now occurring from many other countries, mainly e.g. from the Czech Republic, Hungary, Romania and others (Patoka et al. 2016, Uderbayev et al. 2017, Pârvulescu et al., 2017; Weiperth et al. 2018).

For example, the red swamp crayfish first introduced in Spain in 1973, is already widely distributed in western parts of Europe, (Holdich et al. 2009; Kouba et al., 2014; Weiperth et al. 2018), but also in Africa and various islands (Souty-Grosset et al. 2006; Gherardi, 2006; Putra et al. 2018). The species is currently listed in the top 100 worst invasive species in Europe (Vilà et al., 2009). Despite its commercial utilization, the species soon reached the aquarium trade market and became available to aquarists all over Europe, including Slovakia (Chapter 2). Owing to its interesting colouration, this species is in high demand in the aquarist trade, posing further danger to its possible introductions and subsequent spread. The problem regarding the red swamp crayfish was greatly ignored considering warnings about the species potential negative impact on the freshwater ecosystems from the end of the 90s (Gutiérrez-Yurrita et al., 1998; Nyström et al. 1999). Even though the species is considered restricted to warmer waters, the red swamp crayfish flourishes in the colder climates and at higher altitudes (Chucholl, 2011).

Several additional non-indigenous crayfish species occur in the European wild as a result of human-mediated introductions derived from the the aquarist trade, with lower probability of their establishment in the continental parts of Europe. Nevertheless, they should not be overlooked as they can pose a risk in specific habitats (e.g. natural thermal streams or lakes) and can also have pan-European importance. Similarly to the aquarist trade, fishing can also promote exotic crayfish species introductions and a good example is the *Faxonius immunis* (Hagen, 1870) crayfish now occurring in Germany from Strasbourg to Mannheim in the Rhine River, discovered in the mid-1990s (Schrimpf et al. 2013).

Although non-indigenous freshwater crayfish species successfully established viable populations under European climatic conditions; data on the occurrence of other related crustacean taxa in the wilderness or urbanized areas of Europe remains scarce. So far, no such record is known from Slovakia, although thermally polluted waters are suggested to support their occurrence (Klotz et al. 2013; Weiperth et al. 2019). The red cherry shrimp, however, originates not in the tropics, but its habitats are small to medium-sized streams in East and central China (Klotz et al., 2013). It is hypothesized that the red cherry shrimp could extend into colder waters as documented in other non-indigenous species with high temperature demands (Veselý et al., 2015). This could have major impacts as the shrimp species found in the aquarist trade have been discovered to host the crayfish plague pathogen (Svoboda et al. 2014a). For example, the above mentioned red cherry shrimp was recorded in the cool water at an adjoining brook in Miskolctapolca close to its original introduction into a thermal pond (Weiperth et al. 2019). The red cherry shrimp together with 11 other freshwater shrimp species were also discovered in the Slovak aquarium trade (Chapter 2). Recent research postulated evidence that the freshwater crab (Svoboda et al. 2014b; Putra et al. 2018) species can also become crayfish plague carriers, which suggest a possible new transmission pathway of the zoospore of this disease in Europe and elsewhere. Five non-indigenous freshwater crab species were found in the Slovak aquarium trade, but unlike crayfish or shrimps the crabs were less frequent on the market. Schrimpf et al. (2014) already confirmed that Chinese mitten crab (*Eriocheir sinensis* H. Milne-Edwards, 1853), a highly migratory species penetrating freshwaters up to a distance of several hundred kilometers, serves as a vector of crayfish plague pathogen spread. Thus, non-indigenous freshwater shrimps and crabs in the aquarium trade should also be considered as potential crayfish plague reservoirs (Panteleit et al., 2017) and should not be overlooked in the pet trade issues or in research. However, not all potential vectors of the crayfish plague, or well-known species of the crayfish plague pathogen, are infected with this disease. For example, the absence (or detectable abundance of the crayfish plague pathogen) of the marbled crayfish in the Slovak populations found in the wilderness (Chapter 3 and 4).

Aquarium pet trade species are a high introduction risk and represents a new risk for our nature. This new risk is enhanced by the ever increasing assortment of the non-indigenous species in the aquarist trades and absence of its strict control and regulation in the EU and other countries in the world. The market will respond to new legislation regulations with new species introductions on the market, to replace the old banished species, that have yet to be considered illegal by the legislation. Areas with higher human population density, with higher gross domestic product and growing socio-economic aspects are the introduction risk hotspots (Perdikaris et al. 2012; Chucholl, 2014). On the other hand, rising economics in the eastern European countries or in Asia, open new threads as the majority lack the appropriate legislation for the management of the non-indigenous species in commercial sectors, with the majority currently remaining unknown to the scientific community (e.g. Uderbayev et al., 2017). The species in the aquarium trade are generally selected to fulfill the basic demands of easy breeding, favouring the common conditions of our aquaria and have, as a result of artificial selection, become more tolerant. This selection increases the chances of an establishment of introduced species, but further studies are needed to elucidate this hypothesis as the majority of the ecological research is carried out on wild populations. Autoecological data are thus needed to compare the ecological features of the artificially bred species and those living in the wild, especially for those that are at risk of starting biological invasions. As the species tolerance to general conditions increases, it also increases the possibility of establishing a viable reproducing population (if introduced to favorable conditions) and potentially expansion in its new environment. Introduction of the marbled crayfish in Slovakia is a good example of introductions mediated *via* aquarium enthusiasts (Chapter 3, Chapter 4). Slovakia is believed to be another one of the countries in the center of these potentially troubling events of the ever changing nature and consequences of human impacts.

The marbled crayfish presence in Central Europe is an excellent example of successful introductions of an ornamental non-indigenous species (Chapter 3, Chapter 4). Although, original prognoses questioned its survival in the wild, especially in temperate climate (Martin et al., 2010b), it is now recognized as an established invader both in various parts of Europe (Chucholl et al., 2012; Kouba et al., 2014) and in Madagascar (Jones et al., 2009; Kawai et al., 2009). Due to its parthenogenetic reproduction strategy, theoretically no more than one individual is needed to establish a viable population (Scholtz et al., 2003). Based on our data and on findings from Germany (Chuchol and Pfeiffer, 2010; Chucholl et al., 2012), it is evident that the species survives and successfully reproduces in Central European climatic conditions. Its overwintering ability, with successful survival at ~ 2.5 °C for three months, was also confirmed experimentally (Veselý et al., 2015). As the marbled crayfish is widely available in the aquarium pet trade in Europe and e.g. in Madagascar for multiple purposes (Andriantsoa et al., 2019) this raises concerns of its further introductions (Chucholl, 2013, 2014; Patoka et al., 2014a. b), and spread also under various climates. It is noteworthy to mention that the native range of the closest relative to the marbled crayfish, the slough crayfish *Procambarus fallax* (Hagen, 1870), stretches in the subtropics in the USA (Martin et al., 2010).

However, even if no new marbled crayfish populations become established by means of human aids in near future in Slovakia, the already known populations are an obvious threat, as they may serve as a foothold for the spread of the species in the Danube basin. Several North American non-indigenous invasive crayfish are known for their considerable capacity for active dispersal. For example, the congeneric red swamp crayfish and the signal crayfish can potentially survive desiccation periods of up to several hours (Banha and Anastácio, 2014). This may promote crayfish passive dispersal over long distances, but also allow crossing of terrestrial barriers to new suitable habitats, as documented by Herrmann et al. (2018) for various species, such as the spiny-cheek crayfish (Puky, 2014), and the marbled crayfish

(Chucholl et al., 2012). Moreover, water birds may possibly serve as translocation vectors for juvenile crayfish, but also fisherman equipment could serve to accidentally translocate the living crayfish individuals. Small juveniles of the red swamp crayfish were reported to climb to mallard feet and remain there for up to several minutes and can survive in the open air for up to three hours (Águas et al., 2014).

If the marbled crayfish manages to successfully colonize rivers in the Danube basin (Chapter 3) (which seems likely as the population in the thermal stream in Opatovce is not separated from the Nitra River by any barrier, the other populations are in a close vicinity of the Váh River and its side channel) and the population near the Danube river itself in Bratislava (Chapter 4), the species' relatively fast dispersal can be expected unless restricted by environmental factors. The newest discovered site with the marbled crayfish in Bratislava occurs in the immediate vicinity of the Danube, separated only by a pumping station that periodically releases its waters to one of the river tributaries. The Danube is already colonized by the non-indigenous spiny-cheek crayfish which invaded this river section in the last two decades (Lipták and Vitázková, 2014) and the signal crayfish fostering its colonization in the Danube from the Morava River (Petrusek and Petrusková, 2007; Lipták and Vitázková, 2014). We have not confirmed syntopic occurrence of these species yet, but we consider that confirmation of marbled crayfish in the side arm of the Danube is just a matter of time. Factors that can drive (or have already driven) the species into the Danube include water pumping by the station in case of increased water level or floods, periodical checking of pumps for functionality, intentional translocation of the marbled crayfish individuals by humans, and passive or active migrations of the marbled crayfish individuals(Chucholl et al., 2012; Lipták et al., 2016). The ability of the marbled crayfish to act as an *A. astaci* vector deserves considerable attention as well. The marbled crayfish population near Bratislava discussed in Chapter 4 is in close vicinity to the spiny-cheek crayfish population stretching to the other side of the dam where the marbled crayfish occur. Based on the DNA analysis, one of the spiny-cheek crayfish was infected with the crayfish plague pathogen (Agent level 3). The documented presence of *A. astaci* in that species corresponds to its infection status elsewhere in the Danube (Kozubíková et al. 2010; Pârvulescu et al. 2012). Although no *A. astaci* infection was detected in our studies with marbled crayfish, a complete absence of the pathogen cannot be ascertained. Upon marbled crayfish contactwith infected spiny-cheek crayfish, we expect a horizontal transmission of *A. astaci* between the two host species (James et al. 2017). This means that the marbled crayfish expansion in the Danube catchment will likely be accompanied by the expansion of the crayfish plague pathogen, which causes mass mortalities of susceptible crayfish species and contributes to the decline of indigenous crayfish stocks in the country (Holdich et al. 2009), predominantly the narrow-clawed crayfish confirmed several kilometers downstream (Lipták, 2014). This might have severe consequences for remaining stocks of the indigenous narrow-clawed crayfish (Pârvulescu et al., 2012, 2015). Furthermore, competition with the already present non-indigenous crayfish species is also expected (Hossain et al., 2019). Infected marbled crayfish have already been confirmed in the aquarium pet trade, laboratory cultures, and the wild (Keller et al., 2014; Mrugała et al., 2015; Andriantsoa et al., 2019), and genotyping of the pathogen suggested that the species got infected by horizontal transmission from another species (Mrugała et al., 2015). Due to the potential of the marbled crayfish to rapidly expand its range, it is possible that it might spread the infection into habitats that the other non-indigenous North American species have yet to reach. However, given the characteristics of the marbled crayfish and their role in the ecosystems in general, species spread is inevitable posing significant consequences for a broad range of taxa. This is a serious issue, since the Danube possesses habitats for diverse biota, being a unique ecosystem of European importance.

New reports from Hungary already indicate the presence of the marbled crayfish in the Danube River (Szendőfi et al., 2018), which may be the starting point of the Danube's colonisation. Investigations of the crayfish plague carrier status is ongoing (A. Kouba, pers. comm.). The colonization potential of invasive crayfish can be dramatic, spreading downstream, and even upstream, at an alarming rate (Bubb et al., 2004). A good example is the colonization of the Danube River by the spiny-cheek crayfish in Hungary and adjoining countries (Puky and Shád, 2006; Pârvulescu et al., 2012; Lipták and Vitázková, 2014). The expansion of marbled crayfish may be further enhanced by passive dispersal along the rivers, particularly downstream by currents and floods. Single individuals of sexually reproducing crayfish invaders, even dispersing over long distances, are highly unlikely to establish a population unless a mated mature female or female with a clutch survives the translocation (note that it remains unclear under which conditions facultative asexual reproduction, reported for spiny-cheek crayfish, takes place (Buřič et al., 2013). However, due to the obligate asexual reproduction of the marbled crayfish, this taxon is not limited by the Allee effect at very low population densities, and even dispersal of juvenile individuals may allow their subsequent reproduction in newly colonized sites. Floods (such as those occurring in the Váh basin in 2006 and 2010) may thus not only allow the crayfish to spread from the gravel pits to the river systems, but also facilitate their rapid downstream dispersal.

Thermal streams, both fed by natural warm springs and those thermally polluted by human activities (i.e., cooling water from industry), represent a specific category of habitats that may support introductions of ornamental aquatic species in temperate regions (Chapter 3) (Emde et al., 2016; Weiperth et al., 2017, 2018). Many of such species are elsewhere limited by the low water temperatures and are unable to proliferate outside the thermal streams, however, some of them tolerate a wide range of temperatures and may disperse successfully out of these introduction sites. Numerous cases of establishment of aquarium crustaceans, in particular crayfish, have been documented in such habitats across Europe. The red swamp crayfish in a thermal stream in Austria (Petutschnig et al., 2008) and the non-indigenous redclaw crayfish in an oxbow lake in Slovenia (Jaklič and Vrezec, 2011) so far seem restricted to thermal waters. In Germany, establishment of two aquarium shrimp species, one of which may also tolerate lower temperatures, has been documented in a stream fed by cooling water from a coal power plant (Klotz et al., 2013). Recent finding of the red swamp crayfish in the Hungarian wilderness, confirms the overestimated temperature limitations for the non-indigenous crayfish species (Gál et al. 2018). In the case of the marbled crayfish in a thermal stream in Slovakia, the temperature does not seem to be a limiting factor (Lipták et al., 2016), as apparent from the other established marbled crayfish populations in Central Europe (Chucholl et al., 2012).

Marbled crayfish juveniles observed in autumn, and the presence of medium-sized individuals in our samples, indicate at least two seasonal clutches of the marbled crayfish at the studied sites (Chapter 3, Chapter 4). It is estimated that under the laboratory conditions, the marbled crayfish can complete up to seven reproduction cycles during its 2 to 3 year lifespan, with a generation time of about 6–7 months (Vogt, 2010). The amount of juveniles increases with each cycle in relation to size increase of the maternal individuals (Vogt, 2011), and may reach very high values for large females. Under laboratory conditions, Vogt (2011) reported the maximum number of 427 juveniles in one clutch. Some field-collected individuals were nevertheless even more fecund: one female from Madagascar studied by Jones et al. (2009) carried approximately 530 eggs (Jones et al., 2009), and Chucholl and Pfeiffer (2000) reported as many as 724 eggs in a single marbled crayfish clutch from a German population. Thus, 455 juveniles carried by one marbled crayfish from the Koplotovce site in Slovakia do not seem to be exceptional, even under Central European conditions, and this number confirms a substantial reproduction potential of this invasive species. The marbled crayfish females in the vicinity of Bratislava (Chapter 4) carried in average 420 eggs or juveniles with a positive correlation with a size of the female crayfish. Number of eggs could outreach 700 eggs per individual.

In general, most of the marbled crayfish-inhabited sites with well-established populations are lentic habitats relatively isolated from the main water courses (Chucholl et al. 2012; Patoka et al., 2016). Some of its life history characteristics provide significant advantages even compared to other non-indigenous crayfish species already present in the Danube, usch as the spiny-cheek crayfish and the signal crayfish (Scholtz et al., 2004). The marbled crayfish distribution in Slovakia discussed in Chapters 3 and 4 may not be the last from this region with relatively high density of human population and the fact that the crayfish popularity in the aquarist trade remained unnoticed until recently (Perdikaris et al., 2012; Chucholl, 2014; Gebauer et al., 2018).

Chapter 5 indicates that the marbled crayfish utilizes a wide range of food sources, likely impacting and modifying the food web structure in the ecosystem. In this study, we hypothesized that (i) marbled crayfish utilize sources on multiple trophic levels and macroinvertebrates, macrophytes and detritus are likely the most important food items, (ii) marbled crayfish are an important food source for higher trophic levels, and (iii) may act as key species in energy transfer from detritus to higher trophic levels. This study aimed to provide novel insights into the trophic ecology of the marbled crayfish by investigating its trophic role in a recently colonized lentic ecosystem, through stable isotope analysis of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$). In line with our hypotheses, detritus (both autochthonous and allochthonous) was found to be the most important diet source for marbled crayfish, while other sources such as zoobenthos, algae and macrophytes were utilized to a lesser extent. Moreover, the marbled crayfish was also an important food source for predatory fish, thus playing a multi-trophic functional role in the ecosystem. As a result of its dominance in the benthic community, the marbled crayfish has a high potential to negatively impact local species diversity and ecosystem functioning (Creed et al., 2009; Ruokonen et al., 2014; Lipták et al., 2017), as documented for the red swamp crayfish elsewhere (Gutiérrez-Yurrita et al., 1998).

We revealed that the marbled crayfish serves as an important decomposer, processing allochthonous and autochthonous matter in the ecosystem. This concurs with Usio (2000), stressing the importance of a New Zealand crayfish *Paranephrops zealandicus* (White, 1847) in leaf processing in a temperate stream. Due to its preference for detritus, the marbled crayfish may compete with other organisms (e.g. native crayfish, collectors, shredders) utilizing allochthonous and autochthonous detritus (Dorn and Wojdak, 2004; Ercoli et al., 2015). The marbled crayfish also utilizes algae and macrophytes, which is consistent with the behaviour of other non-indigenous crayfish species introduced to Europe, such as the signal crayfish and the red swamp crayfish (Nyström et al., 1999; Carreira et al., 2014). Although other studies reported especially strong negative effects or a high dependence of non-indigenous crayfish species on the molluscan taxa (e.g. Nyström et al., 1996, 1999; Glon et al., 2017), this was not confirmed in our research (Chapter 5). Zoobenthos contributed little to the diet of the marbled crayfish, possibly due to its generally low population densities at the locality, which is under a severe predatory pressure by benthofagous fish.

The marbled crayfish rapidly became a new element of the European fauna, but this invasive species may have negative effects to native crayfish counterparts as well as entire communities. The utilization of a wide range of food sources by this highly plastic species may contribute to its successful establishment under a wide range of conditions. Moreover, marbled crayfish can form high density populations and become very abundant in a short time

(Janský and Mutkovič, 2010; Lipták et al., 2017). As we hypothesized in Chapter 5, marble crayfish can exploit an ecosystem at various trophic levels potentially impacting on a large range of organisms within the community. As a large-bodied macroinvertebrate, the marbled crayfish acts as a predator as well as prey for organisms at higher trophic levels. Based on our results, the marbled crayfish can be considered as a keystone species, with the ability to transport energy from the bottom of the chain to top predators, potentially driving important changes in the invaded ecosystems, as demonstrated in the red swamp crayfish (Geiger et al., 2005). The impacts of non-indigenous crayfish species associated with their trophic role may vary depending on the plasticity of the invading crayfish (Ruokonen et al., 2014) and their life stages or thermal regimes experienced (Carreira et al., 2017). Thus, the negative effects on the invaded ecosystem can be established in many perspectives and fronts. Therefore, more research is needed on the feeding dynamics and seasonal behaviour of marbled crayfish, specifically field and experimental studies dealing with marbled crayfish under various biotic and abiotic conditions.

**Conclusions**

This thesis includes four publications. The first deals with the occurrence of non-indigenous crustaceans including crayfish in the Slovak aquarist trade and determines the species availability and frequency at the market. Further two publications characterize the marbled crayfish populations discovered in natural ecosystems in Slovakia and gives a basic description of its population ecology and reproduction parameters. Furthermore, the impact of this invader was analyzed based on the carbon $^{13}C$ and nitrogen $^{15}N$ stable isotopes in a lentic ecosystem previously invaded by the marbled crayfish.

The main conclusions from these studies are:

1. Slovak aquarist trade is represented by 26 different crustaceans, including 9 exotic crayfish species. The most common crayfish species occurring in the Slovak aquarist trade includes the marbled crayfish, the red swamp crayfish, Florida crayfish and the orange form of the Mexican dwarf crayfish.
2. One of the most frequently occurring crayfish in the aquarist trade in Slovakia is the marbled crayfish, which now occurs in at least four different localities with established and reproducing populations. Before this study the marbled crayfish were assumed incapable of surviving the temperate winter. Our study was among the first to prove that these species can survive under the climatic conditions of continental Europe, capable of successfully reproducing and expanding their populations.
3. The marbled crayfish can affect an ecosystem to a great extent by utilizing a wide range of energy sources and thus to affect the local cenoses on different trophic levels. As a result, the marbled crayfish can utilize the energy from the lower levels of the trophic chain and shift it to higher trophic levels in the ecosystem. This research presents the first comprehensive study on trophic role of this prominent species.

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## ENGLISH SUMMARY

### Non-indigenous crayfish species in Slovakia

***Boris Lipták***

Human activities have largely impacted the environment and its biota to the extent that biodiversity declines can be seen worldwide. Biological invasions significantly contribute to these processes. Slovakia is a rapidly developing country stretching along the northern parts of the Pannonian basin and western Carpathian Mountains. Geological characteristics predetermine its extraordinarily high species richness thanks to largely preserved regions and habitats of high biological and conservational value. As a result, a strong population of the stone crayfish occurs in the western part of the country, while there is a countrywide distribution of the noble crayfish. On the contrary, the country's narrow-clawed crayfish is on the edge of extinction. The main reason behind the decline of this species is the expansion of the non-indigenous crayfish species transmitting the crayfish plague pathogen. Although sites of the stone and the noble crayfish occur in the upper parts of the river basins, there is a high risk of crayfish plague outbreaks, since the established spiny-cheek crayfish populations are confirmed chronic carriers of the causative agent.

Aquarist trade is increasingly recognized as an important pathway for the non-indigenous species introductions, out of which some may establish and become invasive. Freshwater crayfish, shrimp and crab species were recorded in the aquarist trade in Slovakia, counting altogether 26 different species. The marbled crayfish was one of the most frequently traded species. We identified several new sites of the marbled crayfish occurrence with established reproducing populations in very close vicinity of major rivers in the country. One of the newly identified populations is located in the Chorvátske rameno canal, which is separated from a sidearm of the Danube River only by a bank with an installed pumping station. This sidearm harbors a flourishing spiny-cheek crayfish stock infected with the crayfish plague. Marbled crayfish can become a crayfish plague carrier acquiring the pathogen from the infected spiny-cheek population in the Danube and rapidly spreading the disease along the river, thereby endangering the remaining populations of the narrow-clawed crayfish. More sites with the marbled crayfish are expected to occur in the country. Given their parthenogenetic reproduction, theoretically, a single individual is sufficient to establish a new sustaining population.

Considering the high availability of the marbled crayfish in the pet trade industry and the rising numbers of established populations in the wild, research clarifying its potential impacts on the invaded ecosystems was warranted. We provide the first study investigating the trophic position and food preferences of the marbled crayfish in its well-established populations. Based on carbon $^{13}C$ and nitrogen $^{15}N$ stable isotopes analysis marbled crayfish were identified in the middle of the trophic chain with polyphagic diets. Marbled crayfish were found to utilize algae, allochthonous and autochthonous detritus, zoobenthos and macrophytes, thus being a strong competitor to a wide scale of organisms depended on the same food sources. The marbled crayfish transmit the energy from the bottom of the trophic pyramid to higher trophic levels as it was found to be a prey for top fish predators. This species can form dense populations and become a dominant component of the benthic fauna, thus affecting the entire invaded ecosystem. Its trophic niche width confirms high plasticity of the species, sustaining its populations in a wide range of different habitats. The marbled crayfish is thus a highly adaptable invader that can threaten not only the indigenous crayfish species by means of competition and the spread of crayfish plague, but also entire freshwater ecosystems and their biota.

## CZECH SUMMARY

### Nepůvodní druhy raků na Slovensku

***Boris Lipták***

Lidská činnost významnou měrou změnila životní prostředí a jeho biotu do té míry, že můžeme celosvětově pozorovat pokles biodiverzity. Biologické invaze k těmto procesům významně přispívají. Slovensko je rychle se rozvíjející zemí nacházející se na severu Panonské pánve a západní části Karpat. Geologické charakteristiky regionu předurčují jeho mimořádnou druhovou bohatost díky dosud zachovaných regionů s biotopy o vysoké biologické a ochranářské hodnotě. Díky tomu se na západě země stále vyskytují prosperující populace raka kamenáče a napříč zemí i populace raka říčního. Naopak rak bahenní je na Slovensku na pokraji vyhynutí. Hlavní příčinou poklesu jeho početnosti je šíření nepůvodních druhů raků přenášejících patogen račího moru. Přestože se populace raků kamenáčů a raků říčních nacházejí v horních částech povodí, riziko propuknutí račího moru je vysoké, jelikož etablované populace raka pruhovaného jsou potvrzenými chronickými přenašeči původce tohoto onemocnění.

Akvaristický obchod je stále více shledáván jako významná cesta introdukce nepůvodních druhů, z nichž některé se mohou etablovat a invazivně šířit. Ve slovenském akvaristickém obchodě bylo zaznamenáno 26 druhů sladkovodní druhy raků, krevet a krabů. Mramorovaný rak byl jedním z nejčastěji obchodovaných druhů. Identifikovali jsme několik nových lokalit výskytu mramorových raků s etablovanými rozmnožujícími se populacemi v těsné blízkosti významných slovenských řek. Jedna z nově nalezených populací se nachází v Chorvátském ramenu, které je od postranního ramene Dunaje odděleno pouze hrází s instalovanou čerpací stanicí. Toto postranní rameno je osídleno prosperující populací raka pruhovaného infikovanou račím morem. Díky kontaktu s infikovaným rakem pruhovaným se rak mramorový může snadno stát nositelem tohoto onemocnění a začít jej šířit, což by přispělo k ohrožení zbytkových populací raka bahenního v Dunaji. Lze očekávat, že se na Slovensku vyskytuje více doposud neobjevených lokalit s rakem mramorovaným. Vezmeme-li v úvahu jeho partenogenetickou reprodukci, je teoreticky i jediný jedinec dostačující pro založení nové populace.

Vzhledem k vysoké dostupnosti raka mramorovaného v akvaristickém obchodě a rostoucímu počtu volně žijících populací bylo důležité realizovat výzkum hodnotící potenciální dopady raka mramorovaného na invadované ekosystémy. Poskytujeme první studii zkoumající trofickou pozici a potravní preferenci raka mramorovaného v etablované populaci. Pomocí stabilních izotopů ($^{13}C$ a $^{15}N$) bylo zjištěno, že se rak mramorovaný vyskytuje ve středu potravního řetězce a je všežravý. Rak mramorovaný konzumoval řasy, allochthonní a autochtonní detrit, zoobentos a makrofyta, čímž je silným konkurentem pro širokou škálu organismů závislých na stejných potravních zdrojích. Rak mramorovaný přenáší energii ze základny potravinové pyramidy do vyšších trofických úrovní, jelikož je kořistí rybích predátorů. Tento druh může vytvářet početné populace a tvořit tak dominantní složkou bentické fauny, a tím ovlivňovat celý invadovaný ekosystém. Jeho široká trofická nika potvrzuje vysokou plasticitu druhu a podporuje výskyt jeho populací v širokém spektru habitatů. Rak mramorovaný je tedy vysoce adaptabilní invazivní druh, který může ohrožovat nejen původní druhy raků prostřednictvím kompetice a šíření račího moru, ale i celé sladkovodní ekosystémy a jejich biotu.

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## TRAINING AND SUPERVISION PLAN DURING THE STUDY

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| **Period** | 2nd October 2015 until 19th September 2019 |

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| Crayfish aquaculture | 2017 |
| Applied Hydrobiology | 2018 |
| Pond Aquaculture | 2018 |
| English Language | 2018 |

| **Scientific seminars** | **Year** |
|---|---|
| Seminar days of FFPW | 2015<br>2016<br>2017<br>2018 |

| **International conferences** | **Year** |
|---|---|
| Lipták, B., Janský, V., Kouba, A., 2015. Update of the non-indigenous crayfish species in Slovakia. In: European Crayfish Conference: Research and Management, 9–12 April 2015, Landau, Germany, p. 52. | 2015 |

## CURRICULUM VITAE

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