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Crayfish in changing biotic and abiotic conditions

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Crayfish

Crayfish are well known freshwater animals having a global native distribution with the exception of Antarctica and continental Africa. Crayfish belong to the subphylum Crustacea, order Decapoda, being divided into two superfamilies – Astacoidea and Parastacoidea. The superfamily Astacoidea, occupying the northern hemisphere, consists of two families – Astacidae and Cambaridae. Family Astacidae is composed of two subfamilies – Astacinae distributed in Europe and Pacifastacinae with a distribution on the west coast of North America. Cambaridae, with three subfamilies, are mainly distributed along the east coast of North America from far north to Central America (Cambarellinae and Cambarinae), while Cambaroidinae lives in East Asia. Generally, the diversity of the superfamily Astacoidea is concentrated in North and Central Americas, while it is particularly low in Europe. The superfamily Parastacoidea, having almost 200 described representatives, occupies the southern hemisphere with Australia and Papua New Guinea being biodiversity hotspots (Holdich and Crandall, 2002; Kawai et al., 2016; Kozák et al., 2015). However, this traditionally accepted taxonomic division remains in a state of flux. Among others, a disjunction distribution of families is particularly apparent in the case of northern hemisphere crayfish. Development of new molecular methods is offering avenues to solve this issue (Grandjean et al., 2017). Nowadays, there are well above 600 crayfish species described with several new species being discovered yearly. As expected, new species descriptions are usually related to the most speciose families – Cambaridae and Parastacidae. From the beginning of this year alone at least 4 new species have been described (Loughman et al., 2017; Lukhaup et al., 2017; Mccormack and Ahyong, 2017; Schuster and Kendrick, 2017).

In general, crayfish are large and among the longest lived invertebrate animals, capable of occupying most lotic and lentic freshwater ecosystems (Holdich and Crandall, 2002; Kawai et al., 2016; Kozák et al., 2015). In addition, some species became part of the stygo- or triglofauna, being able to occupy subterranean habitats (Hobbs et al., 1977). Crayfish can excavate substrate to create a suitable-sized refuge or even create burrows for protection against unfavourable environmental conditions, protection against predators or as a suitable habitat for reproduction or moulting, likely as a result of their marine ancestry (Berrill and Chenoweth, 1982; Dalosto et al., 2013; DiStefano et al., 2009). Additionally, if conditions in the aquatic environment are not suitable (e.g. oxygen deficiency, toxic wave), crayfish are able to escape to the terrestrial ecosystem surrounding a given water ecosystem for a period of time and return when conditions become suitable (Gherardi et al., 2000; Smart et al., 2002), thus imparting sufficient ecological plasticity enabling them to successfully occupy a variety of habitats (Alcorlo et al., 2004, 2008; Correia, 2002; Holdich and Crandall, 2002).

Trophic webs in almost every ecosystem contain many units which link together (Vander Zanden and Rasmussen, 2001), and in aquatic systems crayfish are one of the most important (Momot, 1995). Crayfish are often considered as keystone species as well as ecosystem engineers and geomorphic agents in freshwaters due to their ability to change inhabited ecosystems to suit their requirements. They are omnivorous animals capable of influencing, both directly and indirectly, other trophic levels through their ability to feed on species belonging to various trophic levels, while crayfish themselves are a food item for a wide range of mammals, birds, fish, turtles, snakes and others (Dorn and Wojdak, 2004; Lodge et al., 2012; Pringle and Hamazaki, 1998; Twardochleb et al., 2013). Given this, their role within the ecosystem is of much importance, especially considering that they may represent up to 85% of benthos biomass (Nyström et al., 2001). Compared to specialist feeders (predators, herbivores etc.), the omnivorous diet of crayfish makes it difficult to evaluate their exact trophic position in any given food web. Furthermore, omnivory has been recognized as an important aspect of both aquatic and terrestrial food webs (Polis and Strong, 1996). Generally, crayfish food resources are varied and include, for example, detritus, algae, macrophytes, zoobethos and dead fish (Twardochleb et al., 2013). In some cases, mostly when food resources are extremely limited, crayfish are even able to feed on terrestrial sources. Grey and Jaskson (2012) reported that red swamp crayfish *Procambarus clarkii* have the ability to feed up to 40 m from the shoreline on terrestrial vegetation surrounding Lake Naivasha, Kenya. In addition, feeding preferences can change during ontogenetic development. Momot (1995) found that juvenile crayfish are primarily carnivorous compared to older specimens, which is in line with our findings (a manuscript in the state of compilation). On the other hand, some recent studies suggest that there is no significant shift between crayfish size categories (Stenroth et al., 2006). Moreover, crayfish foraging behaviour and movements increase habitat connectivity (Ruokonen et al., 2012; Statzner et al., 2000). Taking this information as a whole, crayfish occupy a central position in aquatic ecosystems (Twardochleb et al., 2013).

Many crayfish fulfil the criteria for surrogate species with respect to environmental control and conservation biology (Caro and O'doherty, 1999). Indeed, crayfish have been considered as health indicators, umbrella species, or flagship species (Füreder et al., 2003, Füreder and Reynolds, 2003). Recently, there has been an influx of novel non-indigenous crayfish species to European systems (Holdich et al., 2009, Kouba et al., 2014). It may be rather appropriate to call these new species "pirate flagships", due to their ability to out-compete indigenous counterparts and negatively impair entire ecosystems. Biological invasions are a common occurrence, impacting an ecosystems development (McKinney and Lockwood, 1999, Strayer et al., 2006), with this process determined by the rules of invasion which separate the winners from the losers (Moyle and Light, 1996). On the other hand, introduced species could devastate links in ecosystems, leading to their collapse (McKinney and Lockwood, 1999). Contemporary biological invasions have become one of the worst threats to global biodiversity, bringing a wide range of far-reaching ecological, economic and sociological consequences (Clavero and García-Berthou, 2005; Dukes and Mooney, 1999; Lee, 2002; McKinney and Lockwood, 1999). Moreover, freshwater ecosystems are experiencing a loss of biodiversity at a significantly higher rate than those in the most affected terrestrial ecosystems (Grosholz, 2002; Ricciardi and Rasmussen, 1999). Some crayfish species represent prominent invaders in freshwater ecosystems, particularly in Europe and various parts of North America (Holdich et al., 2009). The pathway of non-indigenous crayfish dispersal is mediated by humans and can be attributed mostly to aquaculture, utilization of crayfish as a fishery bait (Lodge et al., 2000) and the pet trade which includes, among others, keeping animals both in home aquaria and garden ponds (Chucholl, 2013, Patoka et al., 2016a, Patoka et al., 2014). Non-indigenous crayfish colonize or use water courses as bio corridors to reach new locations (Karatayev et al., 2008, Leuven et al., 2009), causing irreversible changes in indigenous freshwater crayfish communities (García-Berthou et al., 2005; Holdich et al., 2009). Dispersion of North American crayfish species is particularly problematic due to the spread of crayfish plague, a disease caused by an oomycete *Aphanomyces astaci*, which is usually lethal to all crayfish not originating from North America (Kozubíková et al., 2011; Svoboda et al., 2017; Unestam and Weiss, 1970). Generally, non-indigenous species live in higher densities, they are able to survive less favourable conditions and are more aggressive than indigenous species (Bondar et al., 2005; Pintor et al., 2008; Vorburger and Ribi, 1999). Thus, native biota such as zoobenthos, macrophytes, molluscs, amphibians and fish are under elevated direct (e.g. mainly eggs and early stages) and indirect (e.g. competition for food and shelter) pressures (Angeler et al., 2001; Ilhéu et al., 2007; Nyström et al., 2001). A combination of burrowing and consumption of aquatic plants leads to an undesirable increase in water turbidity (Matsuzaki et al., 2009;

Rodríguez et al., 2005). This may alter the entire ecosystem and its food webs. To sum up, further spread of non-indigenous species will lead to the loss of biodiversity and ecological services in affected ecosystems. Since there is no effective method enabling the eradication of crayfish once established in an environment (except for e.g. total destruction of habitat, or utilization of biocides in closed localities where the active compounds are not exclusively crayfish specific (Bernd et al., 2011, Peay et al., 2006)), elimination of crayfish dispersion and measures avoiding further human-mediated introductions are of key importance.

Overall, crayfish are charismatic creatures of high interest to humans (Gherardi, 2011; Patoka et al., 2016b). Due to the abovementioned reasons, crayfish deserve our closer attention since they are threatened by multiple drivers (Bland, 2017; Richman et al., 2015). Therefore, we decided to test an array of selected abiotic and biotic factors that directly or indirectly influence particular native and/or non-native crayfish, in addition to assessing crayfish as a model group for freshwater animals.

Abiotic and biotic factors

Human activities induce environmental changes through pollution, habitat alteration, nutrient enrichment, and global warming. These changes are often rapid and pose major threats to global biodiversity, functioning of ecosystems, and ultimately to the fate of mankind (Pereira et al., 2010). It is therefore crucial to understand how these drivers impact organisms and their interactions to better predict the consequences of climate change on the global biota (Harley et al., 2006; Kennish, 1997; Neves and Angermeier, 1990; Poff et al., 2002; Porter et al., 2013). While organisms are often exposed to multiple stressors that affect them both directly and indirectly through biotic interactions, most studies deal only with only one factor. This leaves a gap in our understanding of how and when multiple drivers impact species and their interactions (Sentis et al., 2017, Sentis et al., 2014). On the other hand, testing multiple stressors make sense only if the effect of any single environmental driver is principally known, thus allowing the effect of multiple drivers to be distinguished from one other. Therefore, we argue that investigating the effect of both single and multiple drivers on biota should be of paramount importance to ecologists, enabling a better understanding, and thus improved predictions, regarding the consequences of rapid environmental changes on communities.

Tolerance to low winter temperatures

Temperature strongly influences reproduction of crayfish such as mating, egg development, and the number of reproductive cycles per year. Also, foraging activity together with ingestion and moulting rate is influenced during the season by temperature (Brown et al., 2004; Holdich and Crandall, 2002; Kozák et al., 2015). Moreover, survival and spreading of ectotherm animals is temperature dependent (e.g. overwintering ability of warm water crayfish in new cold areas). Capinha and Anastácio (2011) suggest the mean annual temperature and the mean annual temperature of the coldest quarter of the year are good predictors of future distribution of crayfish. Similar approaches are, for example, included in risk assessments of aquatic animals in the pet trade (Kalous et al., 2015; Kopecký et al., 2016; Patoka et al. 2016b). Central Europe and other similarly cold regions were considered relatively immune to the establishment of invasive warm water non-indigenous crayfish species, owing to the temperature barrier presented by continental winter. We consider the lack of a comparative study addressing this issue as a serious gap in our knowledge, and one which limits our ability to predict the possibilities of non-native crayfish establishment.

Drought

Climate change is expected to have significant impacts on hydrologic regimes and freshwater ecosystems (Carpenter et al., 1992; Erwin, 2009). Drought, a parameter mostly connected with temperature and precipitation in a given area (Mishra and Singh, 2010), is an important driver determining the success of organisms in a particular ecosystem (Moyle and Light, 1996). Freshwater animals evolved several strategies to withstand droughts (Brock et al., 2003; Moyle and Light, 1996). Among others, organisms modified their life strategies: producing eggs or spores resistant to desiccation before the parental generation die (Brock et al., 2003; Williams, 1998), organisms migrate to suitable habitat before the drought period begins (Baptista et al., 2010), and animals evolve physiological adaptations to withstand drought period, or find or make a refuge (Haag and Warren Jr, 2008; Magoulick and Kobza, 2003; Verberk et al., 2008). As mentioned, crayfish species are able to borrow tunnels, although the length, shape or direction differs across species (Crandal et al., 2000; Martin et al., 2008) The direction in which burrows are constructed could be an important characteristic to distinguish successful crayfish survivors in terms of unexpected drought period. Crayfish confronted with drought mostly burrow vertical tunnels, while crayfish living in permanent aquatic environments burrow almost horizontal or do not tunnel at all (Tarr, 1884). Therefore, we can expect that crayfish inhabiting hydrologically dynamic aquatic systems have a higher chance of surviving unpredictable periods of severe droughts compared to crayfish occupying aquatic ecosystems where drought periods are rare. Studies addressing interspecific comparison in terms of burrowing abilities in general and burrowing under drought conditions in particular are scarce (Dorn and Trexler, 2007; Dorn and Volin, 2009). This was quite apparent in assessments of both native and non-native crayfish species present in the European wild, hence attracting our attention.

Salinity

Salinity is an important abiotic factor responsible for crucial processes in animals. Feeding rate, growth, reproduction, survival or spreading into new localities strongly depends on water salinity (Ball, 1998; Costa-Dias et al., 2010; Snell, 1986). In general, species can be divided into euryhaline and stenohaline organisms, which specify their ability to adapt to a wide or narrow range of salinities, respectively (Croghan, 1976). A saline environment is generally toxic for freshwater organisms and the magnitude of toxicity depends on the ionic composition, i.e., the ratio of cations to anions together with the pH (Bailey and James, 2000, Frey, 1993). On the other hand, freshwater organism with marine ancestry may tolerate a wide range of salinities, while this pattern strongly varies across and within taxa (Hart et al., 1991). Freshwater organisms, despite possible detrimental effects, can use saline environments as a bio corridor or as a feeding niche (Jaszczołt and Szaniawska, 2011; Kornis et al., 2012; van Ginneken and Maes, 2005). However, time spent in a saline environment is limited and often related to a specific part of their life cycle (juvenile stages and reproduction are usually vulnerable to higher salinity) due to physiological constraints (Anger, 2003; Holdich et al., 1997). Salinity tolerance of crayfish strongly varies across the species (Holdich and Crandall, 2002). Species living in semi-arid or arid areas usually tolerate higher salinities than species distributed in humid areas. Similarly, species occupying marsh areas should be able to withstand a wider range of salinities (Alcorlo et al., 2008; Jones, 1989). However, exceptions are to be expected, and the mere presence in a given area does not guarantee high salinity tolerance (Holdich et al., 1997). Therefore, we can conclude that salinity is an important barrier to the spreading of freshwater organisms into new areas (Croghan, 1976;

Leppäkoski and Olenin, 2000). However, when evaluating whether a given organism is able to cope with a saline environment, we should consider its life history features together with general ecological information (e.g. distribution, abundance and population structure). This background should provide an accurate picture of the salinity tolerance of a species.

Marbled crayfish *Procambarus fallax* f. *virginalis* is an emerging invader not only in Europe (Faulkes, 2015a,b; Patoka et al., 2016a). Nevertheless, its establishment in Ukraine (Novitsky and Son, 2016), and broad availability in the local (Kotovska et al., 2016) as well as Russian pet trade (Vodovsky et al., 2017), raised an array of significant questions. Eastern Europe comprises a large part of the range of indigenous crayfish species belonging to the genus *Astacus*, forming freshwater populations but also inhabiting the Azov, Black and Caspian Seas, as is the case of the narrow-clawed crayfish *A. leptodactylus* and the thick-clawed crayfish *A. pachypus* (Holdich et al., 2009; Kouba et al., 2014). Due to the lack of information, we considered an evaluation of marbled crayfish salinity tolerance as a much needed contribution to the species biology.

Crayfish plague

Non-indigenous species may alter native communities via predation, competition, hybridization, or habitat modification, however they may also threaten native biota through the transmission of pathogens, parasites and parasitoids (Daszak et al., 2000; Peeler et al., 2011). The crayfish plague agent, an oomycete *Aphanomyces astaci*, is an example of such introduced exotic pathogens. Nowadays, four different *A. astaci* genotype groups (A, B, D and E) exist, each differing in virulence, are known to currently occur in Europe (Svoboda et al., 2017). Crayfish plague is undoubtedly one of the most devastating emerging diseases in European freshwaters, also listed among the worst invasive species in Europe as well as globally (Hulme et al., 2010; Lowe et al., 2000). Due to a long co-evolutionary history with *A. astaci*, North American crayfish species have evolved defence mechanisms against the growth of *A. astaci* mycelium in their cuticles (Cerenius et al., 2008). The opposite situation is found in Asian cambarids, European astacids and all parastacids. Thousands of populations of native European astacid crayfish have been lost in recent decades, and many more have been substantially reduced, largely due to direct or indirect effects of the so called “old non-indigenous species” presence (spiny-cheek crayfish *Orconectes limosus*, signal crayfish *Pacifastacus leniusculus*, and red swamp crayfish; *sensu* Holdich et al., (2009)). These not only influence their European counterparts by competition, but also through the spreading of the crayfish plague pathogen, a disease causing mass mortalities in all crayfish species not originating from North America (Edgerton et al., 2002, Lodge et al., 2000).

After confirming the ability of Australian yabby *Cherax destructor* to withstand winter temperature conditions of Central Europe (Veselý et al., 2015), we questioned its resistance towards three strains of *A. astaci* that are present in the region, using another susceptible native crayfish species, the noble crayfish *Astacus astacus*, for comparative purposes.

Interactions under global changes

In general, temperature is one of the most important global change drivers influencing and altering ecological communities worldwide (Corvalan et al., 2005). Warming can have both positive and negative effects on targeting ecosystems/communities. Its effects depend on the amount of heat delivered relative to the shape and optimum of an organisms thermal performance curves (Schulte et al., 2011). Also temperature affects the speed of chemical reactions and thus warming directly influences biological rates (metabolism, growth, feeding) of ectotherm species with important consequences for their survival and spatial distribution (Dukes and Mooney, 1999; Moyle and Light, 1996). According to Binzer et al. (2012) warming increases risk of starvation and biomass loss of top predators in oligotrophic ecosystems, since metabolism increases faster than ingestion, leading to energetic losses and thus starvation. Interestingly, this detrimental effect of warming may be dampened by an increase in resource availability (e.g. increasing prey density) at the lower trophic levels. These predictions and empirical studies suggest that warming could lead to the disproportional loss of species at higher trophic levels with weaker effects at lower trophic levels (i.e. producers and bacterivores) (Petchey et al., 1999). This is concerning since the insurance hypothesis proposes that biodiversity should dampen the effects of environmental variation, due to species substitution (MacArthur, 1955; Yachi and Loreau, 1999). Still, warming could strongly influence higher trophic levels in terms of intraguild homogenization or a decrease in species richness (Griffin et al., 2013). This effect could be critical for an ecosystem because predators ensure ecosystem services and temperature fluctuations strongly influence the strength of interactions in the ecosystem (Duffy et al., 2007; Schmitz, 2007). Moreover ecosystems usually consist of multiple predators which share the same prey and their interaction determine links in ecosystems, however the warming effect could strongly unbalance this steady state (Barrios-O’Neill et al., 2015; Sih et al., 1998).

The increase in resources at lower trophic levels (i.e. nutrient enrichment) presents another anthropogenically driven challenge to communities and ecosystems (Boukal et al., 2007; Rosenzweig, 1971). We can imagine nutrient enrichment in two ways. First, nutrient enrichment in terms of inorganic particles such as phosphorus or nitrogen, which are major drivers of aquatic ecosystem eutrophication (Kalff, 2002). Second, nutrient enrichment can be presented as an increase of prey density (Sentis et al., 2017; Sentis et al., 2014). In both cases, nutrient enrichment leads to changes to the ecosystem (Boukal et al., 2007; Kalff, 2002; Rosenzweig, 1971). In terms of eutrophication, we could expect oxygen deficiency leading to decaying processes and also loss of diversity including crayfish (Kalff, 2002).

Generally, nutrient enrichment can modify the population growth rate and densities of interacting species, both of which have important consequences for their survival. For example, theoretical studies of Rosenzweig (1971) and Boukal et al. (2007) showed that consumer-resource population densities move from stable equilibrium to oscillations with enrichment which, in turn, increase species extinction risk. This phenomenon is known as “paradox of enrichment” (Rosenzweig, 1971). This phenomenon strongly depends on temperature: “paradox of enrichment” is typical for lower temperatures, while enrichment at higher temperatures tends to resurrect consumers at higher trophic levels from starvation (Binzer et al., 2012). The relative impact of temperature and enrichment on species survival and population stability is further modified by body sizes of the constituent species (Binzer et al., 2016). Interestingly, the shape of the functional response (i.e. the relationship between prey density and predator feeding rate), shows system stability at lower trophic levels, due to effects of changing population densities. According to Rall (2008) and Brose (2006), predator-prey systems with predators displaying a type II functional response (Holling, 1959) are more sensitive to the destabilizing effect of enrichment than systems with type III functional response. Additionally, complex food web systems are more stable compared to a system with low connectance, but still systems with type II functional response remain highly unstable (Rall et al., 2008).

In general, ecosystems usually consist of multiple predators that share the same prey. Moreover, interactions between predators and their prey determine links in the ecosystem (Barrios-O’Neill et al., 2015; Sih et al., 1998). Crayfish as a key stone species link most trophic levels in aquatic ecosystems (Twardochleb et al., 2013). The strength of interactions with other organisms depend on their role as predator or prey (Holdich and Crandall, 2002; Kawai et al., 2016). As mentioned above, crayfish could drastically change links in ecosystems and also change the composition of biota due to intensive pressure on prey and the environment (Dorn and Wojdak, 2004; Pringle and Hamazaki, 1998; Twardochleb et al., 2013). This issue in not only associated with non-indigenous crayfish species invading ecosystems throughout Europe, but also native species spreading into new ecosystems, causing changes in composition of present biota.

Consumers from different functional groups such as predators and omnivorous scavengers which are ubiquitously distributed in ecosystems usually shape prey density and behaviour in different ways (Wasserman et al., 2016). However, the pressure on prey could vary if more than one predator is present. Thus, the strengths of species interactions in ecological communities can rarely be predicted based on a single-predator effect (Sentis et al., 2017; Wasserman et al., 2016). According to Griffin and Silliman (2011) predator diversity could enhance top down control and stabilized food webs in the face of global change. However Finke and Snyder (2010) suggest that changing predator composition could affect the whole food web. In addition, Finke and Snyder (2010) suggest that communities consisting of more generalist predators would be able to exploit resources at higher rate than communities consisting of specialists. Thus, crayfish acting as omnivorous species, and also their ability to scavenge, could decrease energy loss in an ecosystem caused by interactions among predators (a manuscript in the state of compilation). Moreover global drivers such as temperature or nutrient enrichment could strongly affect single as well as multi trophic systems (Corvalan et al., 2005; Rosenzweig, 1971). Therefore, predicting the future of ecosystems strongly depends on our ability to predict the effect of global drivers on biotic interactions, such as those we explored in the last study.

THE AIMS OF THE THESIS

The overall aim of this thesis was to investigate an array of emerging abiotic and biotic factors influencing freshwater crayfish

The specific objectives were to:

1) Determine foraging activity and survival success of four pet traded warm-water crayfish under simulated conditions reflecting temperature during temperate winters.
2) Assess the ability of three indigenous and five non-indigenous crayfish species to survive and construct burrows for protecting themselves under conditions of simulated severe drought.
3) Assess salinity tolerance of marbled crayfish.
4) Compare ability of yabby and noble crayfish to withstand different inoculum doses and strains of crayfish plague *A. astaci* pathogen.
5) Investigate the effects of temperature and nutrient enrichment on a multiple predator community involving crayfish as a model organism.

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CHAPTER 2

HARDY EXOTICS SPECIES IN TEMPERATE ZONE: CAN "WARM WATER" CRAYFISH INVADERS ESTABLISH REGARDLESS OF LOW TEMPERATURES?

Veselý, L., Buřič, M., Kouba, A., 2015. Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures? Sci. Rep. 5, 16340.

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Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures?

Lukáš Veselý, Miloš Buřič & Antonín Kouba

The spreading of new crayfish species poses a serious risk for freshwater ecosystems; because they are omnivores they influence more than one level in the trophic chain and they represent a significant part of the benthic biomass. Both the environmental change through global warming and the expansion of the pet trade increase the possibilities of their spreading. We investigated the potential of four "warm water" highly invasive crayfish species to overwinter in the temperate zone, so as to predict whether these species pose a risk for European freshwaters. We used 15 specimens of each of the following species: the red swamp crayfish (*Procambarus clarkii*), the marbled crayfish (*Procambarus fallax* f. *virginalis*), the yabby (*Cherax destructor*), and the redclaw (*Cherax quadricarinatus*). Specimens were acclimatized and kept for 6.5 months at temperatures simulating the winter temperature regime of European temperate zone lentic ecosystems. We conclude that the red swamp crayfish, marbled crayfish and yabby have the ability to withstand low winter temperatures relevant for lentic habitats in the European temperate zone, making them a serious invasive threat to freshwater ecosystems.

Invasive species are one of the most serious threats to global biodiversity[1,2]. The introduction of exotic species outside of their native range may have far-reaching ecological consequences on local communities as well as causing unpredictable impacts, even for humans[3–5]. Most non-indigenous species have had minimal negative effects and they usually fail to become established, or if they become naturalized, they constitute only a small portion of the community with no significant effects[6,7]. However, some of them are able to adapt to new conditions[6] and strongly affect the ecosystems they invade[8].

Crayfish may represent up to 85% of the local zoobenthic biomass[9] and they are considered as keystone species[3,10]; they are strong ecosystem engineers, modifying the environment to suit themselves[11,12]. Based on previous studies, the introduction of non-indigenous crayfish species have caused major and almost irreversible changes in native freshwater crayfish stocks[13–15]. In addition to imposing strong competitive pressures on the native crayfish populations, they also may alter entire ecosystems and their food webs[13,14]. Red swamp crayfish, *Procambarus clarkii,* has been well studied and their negative impact is often cited as an example[16,17]. Although not as well studied, other alien crayfish species , e.g., the marbled crayfish *Procambarus fallax* f. *virginalis,* yabby *Cherax destructor* and the redclaw *Cherax quadricarinatus* etc. are considered to cause detrimental impacts once established in the natural environment[8], these new invaders warrant more attention relative to potential regulation or management.

The aquarium trade is considered to be one of the main pathways for introduction[18]. As all four species are regarded as "warm water", global warming may increase their potential to become established in previously unavailable or unsuitable habitats[19,20]. The ability of non-indigenous species to withstand

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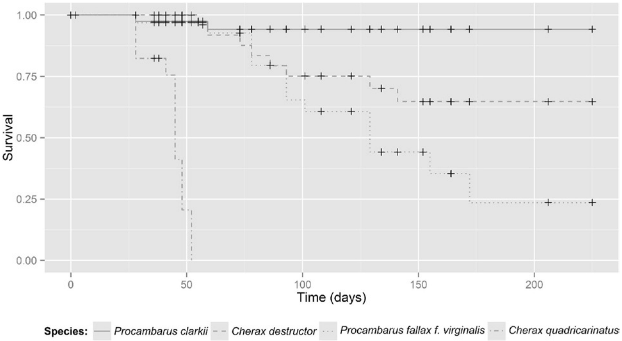

Figure 1. Survival analysis plot of chosen non-indigenous crayfish.

temperatures outside their natural optima (e.g., low winter temperature in the temperate zone) is a necessary prerequisite for survival and spread. Several studies have been carried out in Europe on the spread and reproduction of invasive poikilothermic organisms[21,22], but only a few concerning crayfish[19,20,23]. However, while the distribution models based on laboratory studies are not fully predictive, they provide evidence that European conditions might already be suitable for warm water crayfish with further increasing suitability in the future.

We focused on four non-European crayfish species that can be considered as "warm water" from the Central European perspective; their recent geographic distribution supports this view[24]. The red swamp crayfish is native to the southeastern United States and Mexico[25,26]. Its European occurrence is concentrated in to the southwestern part of the continent, having invaded at least 15 territories ranging from Spain to Germany[24]. The native range of the marbled crayfish is unknown; however, peninsular Florida and southern Georgia have been suggested, due to its close affinity to *Procambarus fallax* originating from that region[27]. Records in Europe are rapidly increasing, with reports of populations having been established in Germany and Slovakia[24,28]; thus, indicating the potential to spread to other European countries. The yabby, *Cherax destructor,* is the most widespread crayfish species in Australia; it is native to semiarid and arid parts of the continent[29]. Its original distribution was southern and eastern Australia (the states of New South Wales, Victoria[30], but it may also be found in Queensland and a part of the Northern Territory[31]). The redclaw, *Cherax quadricarinatus,* is native to the northern part of Australia and to southeastern New Guinea[32]. In Europe, these Australian crayfishes so far have a limited distribution (Spain, Italy, and Slovenia) where the redclaw inhabits an oxbow lake with an elevated temperature due to thermal springs[24,33]. All these species are widely kept as pets, both in Europe and North America[34–36] and all but one (marbled crayfish) are used in aquaculture. Animal escapes or releases have been already proved to be the most likely sources of introductions in several crayfish species[24]. In this study, we tested the overwintering capability of the four species so as to predict the potential risk of their establishment in the freshwater ecosystems of temperate Europe.

Results

Marked differences in survival were observed among the tested species ($\chi^2 = 136$, df = 3, $p = <0.001$; Fig. 1; see Table S1). We found that the red swamp crayfish was the most capable of withstanding the experimental conditions; with 13 out of 15 surviving specimens. Moderate resistance was found in yabby, where 7 out of 15 animals survived. All the mortality occurred during the exposure to the hardest winter conditions (2–3 °C; days 55–141). Similarly, ten marbled crayfish died between 78 to 129 days when the temperature was the lowest. Only one marbled crayfish survived the whole experimental trial. The lowest survival was recorded in redclaw, demonstrating they were the least tolerant of the low temperature conditions; all specimens died between days 41 to 52 when the temperature declined to 2-3 °C. Survival was not related to size; no significant correlation was found between crayfish winter survival and their weight, carapace or postorbital lengths (One-way ANOVA $p = 0.11–0.81$).

Differences in survival were found in males and females of yabby ($\chi^2 = 2.9$, df = 1, $p = 0.048$; Fig. 2; see Table S2). Individuals of both sexes withstood the experimental conditions; however, males survived at a higher rate. No such differences were observed for redclaw ($\chi^2 = 0.3$, df = 1, $p = 0.61$) and red swamp crayfish ($\chi^2 = 0.1$, df = 1, $p = 0.80$).

Foraging activity of crayfish was also recorded during the experiment (Fig. 3). We observed that at least two red swamp crayfish were feeding even during the coldest period, but generally around four

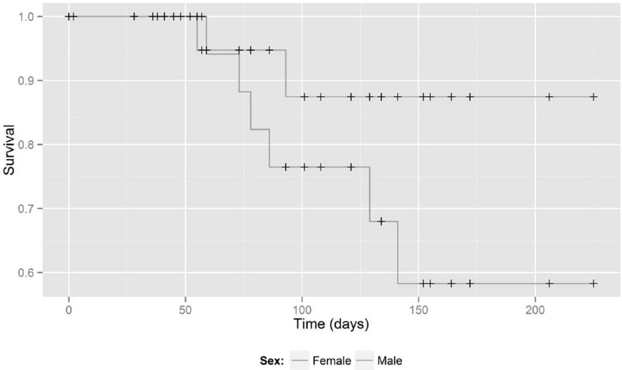

Figure 2. Survival analysis plot of *Cherax destructor*.

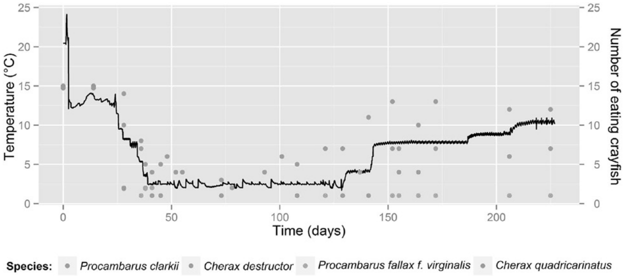

Figure 3. Temperature and foraging activity of chosen non-indigenous species during the experiment.

specimens fed at this time. Numbers of feeding crayfish rose substantially with increasing temperature. In case of the yabby, food intake decreased during acclimatization. Finally, only one specimen irregularly ate during the coldest period. Marbled crayfish almost did not eat during coldest period. In fact, only the one surviving specimen occasionally ate. Feeding activity was low in the redclaw and completely stopped after 28 days i.e. during acclimatization.

Discussion

Catford *et al.*[37] suggests that, after propagule pressure the first set of factors that must be met for species establishment are abiotic conditions, importantly including temperature and other environmental conditions. In this study we examined one of most important factors that influences or determines the freshwater biota composition. We found that three out of four species survived simulated winter conditions (Fig. 1; see Table S1). Red swamp crayfish had the highest survival rate and foraging activity, followed by yabby and marbled crayfish. Redclaw suffered high mortalities and did not survive the lowest temperatures.

Both a distribution model[19] and use of "avatar" species[38] suggested Europe could be a suitable place for red swamp crayfish establishment. Similarly, our results revealed winter mortality is exceptionally low compared to the other tested species. Red swamp crayfish were able to withstand the coldest period with a temperature of around 2.5 °C, similar to the one occurring in waterbodies that freeze during the winter, where its survival has already been documented[30]. In the central part of Europe, populations are established in Austria and Germany. However, the Austrian occurrence is represented by a single population settled in a thermal stream[39]. Chucholl[40] describes the life history of this species in new cold habitats situated in southern Germany and highlights its high plasticity. Unfortunately, no exact data about the course of winter temperatures are mentioned. Thus, our study provides interesting insight into red swamp crayfish temperature resistance in this respect. The red swamp crayfish could be a real problem for European temperate zone ecosystems as exemplified by substantial damage caused to local freshwater communities in southwestern Europe[41]. This non-indigenous species exhibits extended environmental resistance, substantial plasticity, extreme aggressiveness and high population densities that favor the displacement of native European crayfish[42–44]. In addition, its ability to resort to riparian and terrestrial feeding when the freshwater sources are depleted has been documented[17]. Moreover, it is also capable of transmitting the crayfish plague pathogen to native European crayfish species[45].

With respect to winter survival it is also necessary to mention that there is more than one factor determining population success. For example, the temperature during the whole season is also important. If the temperature during the season is not warm enough for egg development (degree days), the species also would fail to become established[46]. According to Suko[47] red swamp crayfish demonstrate a link between warmer water temperatures and increased reproductive success in terms of multiple reproduction. It is partly caused by faster egg development in warmer temperature[48]. However, not only this species could represent this trends. All species involved in the trial are considered as warm water species and this trend could be highly expected among them.

Observed foraging activity in the coldest period is also surprising. Croll & Watts[49] studying the effect of temperature on feed consumption and nutrient absorption reported that the red swamp crayfish were generally lethargic at 8 °C. Food consumption was minimal and remained low even at 14 °C compared to southern white river crayfish (*Procambarus zonangulus*). Populations of the latter species and some closely related taxa are also present in European open waters[24,50]. Winter food intake, likely occurring in both aforementioned species, might have negative impacts on local communities although it could be much reduced by the low temperature.

We also found that one red swamp crayfish male, at the end of acclimatization period when the temperature reach 4 °C, molted. It did not eat its exuvium and its carapace remained soft but the individual crayfish survived until the end of the experiment. Although its survival in natural conditions may be difficult as an uncalcified crayfish will be an easy prey for potential predators, it still illustrates the substantial physiological abilities of the species.

Considering the native range and recent distribution of yabby in Europe, covering Spain and Italy[24], the discovery its substantial resistance to experimental conditions is surprising. Although winter mortality was higher compared to red swamp crayfish, the yabby still exhibited a good ability to survive. According to Capinha *et al.*[51] to date, yabby could occupy nine times higher suitability areas outside the species current ranges. On the other hand it is predicted to have lower climatic suitability in Italy[52] and with a view to future Spain seems to be less suitable for yabby in terms of climatic change[19]. Moreover according to model prediction the mean annual temperature and mean temperature of the coldest quarter seems to be the best predictor of future distribution[53].

Considering our results, we expect that the establishment of new populations of yabby in the temperate zone of Europe is highly possible. The female's mortality was higher compared to the male. On the other hand, winter conditions pose a new challenge for a warm water species and the survivors' sex ratio could be changed due to a different life strategy in term of multiple reproductions in one season and the related higher energy and nutrient demands in females. Still, the number of female survivors could be sufficient to establish a new population.

According to Withnall[31] "*Cherax destructor* is adapted to a wide range of water temperatures, between 1 °C and 35 °C. It does not grow at water temperatures below 15 °C and falls into a state of partial hibernation (i.e. metabolism and feeding cease) when water temperature drops below 16 °C". These results are in contrast to ours, where foraging activities were observed even in the coldest period, although a certain degree of variability among the studies is expected. Finding the substantial resistance of yabby to low water temperatures opens an array of topics, including assessment of its life history at both recently known and possibly new localities, and presumed impacts on European freshwater communities.

Marbled crayfish suffered massive mortality and only a single specimen survived in our experiment. However, due to its parthenogenetic reproduction strategy, theoretically no more than one individual is needed to establish a viable population and potential invasiveness is therefore extremely high[54,55]. The parthenogenetic reproduction strategy combined with fast growth, early maturation, high fecundity, and a capacity for competing with other crayfish species[56,57] provides this crayfish species an enhanced chance of success. Marbled crayfish are already present and flourish in Germany[58], Slovakia[59] and likely also in other European states e.g., Italy[60] and Croatia[61] and future spread throughout the Europe is predicted[28].

Redclaw will clearly not be a threat to European temperate zone ecosystems in the near future which is in accordance with the distribution model of Larson and Olden[38]. The likelihood of its establishment in the southwestern part of Europe may increase along with the temperature rise caused by a global warming, unless the region is fully occupied by species carrying the infectious mold *Aphanomyces astaci*. In this context, also the presence and competition with freshwater crab species might act as a limiting factor[62,63]. According to previous study from Israel where survival of redclaw was tested in earthen ponds during the winter, 60% of specimens survived[64]. Such climatic conditions are generally comparable to Mediterranean European winters, suggesting the ability of redclaw to survive in this region and consequently spread as far as the resistance to the low temperatures is considered.

Conclusion

To sum up, all the species mentioned are widely traded as pets in Europe and North America[34–36], which might facilitate their further spread throughout Europe[24]. Only red swamp crayfish demonstrated high survival in experimental conditions and exhibited feeding activity even during the coldest period. Thus, this species could be regarded as a major threat to lentic freshwater habitats in Central Europe. Considering the native range and recent distribution of the yabby in Europe, its ability to withstand low temperatures indicates a potential to spread and should highlight the need for consideration of this species' environmental plasticity. Finally, our results clearly indicate that the redclaw will not pose a risk for European temperate zone.

Methods

Given current condition the study was conducted at the Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic, from the second half of December 2013 to the first half of July 2014. No specific permissions were required for the locations and activities involved in this study. The study did not involve endangered or protected species. All experimental manipulations (rearing, capture and measurements) were conducted according to the principles of the Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, based on the EU harmonized animal welfare act of Czech Republic. The above mentioned Ethical Committee (IACUC) specifically approved this study. The principles of laboratory animal care and the national laws 246/1992 and regulations on animal welfare were followed (Ref. number 22761/2009-17210).

Crayfish were placed individually into one-liter plastic boxes which was placed into the temperature regulated incubator. Half of the box, as well as lid, were covered with black tape to provide shelter. The lids were perforated (10 holes per lid with size 0.5 cm^2 per hole) to ensure air exchange; winter temperature and light regimen were set according to lentic conditions in temperate continental Europe. The temperature was recorded at one hour intervals by a datalogger MINIKIN (Environmental measuring systems, Brno, Czech Republic). Crayfish were fed during each control (see below) with a slice of carrot and also, once a month, with worms (*Tubifex tubifex*); unconsumed food was removed. Crayfish feeding, surviving and molting were recorded. Absolute food consumption was not recorded, only its occurrence.

Experimental design.

Fifteen adult specimens of each of the four non-indigenous crayfish species were used (Table 1). The sexes were generally equally selected with exception that the red swamp crayfish had more males (12 males vs. 3 females) and only females were used for the marbled crayfish. Each specimen was measured and weighed to the nearest 0.1 mm and 0.1 g, respectively.

The 226-day experiment was divided into three parts. During the initial 40-d acclimatization period, the temperature was decreased from 25 to 3 °C. In the second part, winter lentic conditions were simulated by maintaining temperature at 2–3 °C for 90 days. During the final two months spring conditions were simulated by gradually increasing temperature from 2–3 °C to 10 °C. In the first three months crayfish were checked once per week because of the predicted high mortality. After this period, the animals were checked each 14 days till the end of the experiment. In order to maintain water quality, water was changed by tempered tap water during the controls. Water quality parameters of water before exchange ($n = 2$) were as follows: ANC$_{4.5}$ (acid neutralization capacity) $= 1.0 \pm 0.1$ mmol·l^{-1}; ammonia nitrogen 0.2 ± 0.0 mg·l^{-1}; nitrites < 5.00 mg·l^{-1}; nitrates 0.03 ± 0.0 mg·l^{-1}; calcium 24.4 ± 1.9 mg·l^{-1}; phosphorus 0.1 ± 0.0 mg·l^{-1}; magnesium 2.4 ± 0.2 mg·l^{-1}.

Statistical analysis.

For statistical evaluation of the dataset we used the R-statistics software with packages: survival[65], Kmsurv[66], GGally[67] and ggplot2[68]. Non-parametric survival analysis (Kaplan-Meier method) was performed for all species as well as for both sexes in each species (if applicable). One-way ANOVA was performed to test whether length of carapace or weight of specimen influenced survival during the winter.

	CL (mm)	W (g)
Procambarus clarkii	32.3 ± 3.1	9.1 ± 2.0
Procambarus fallax f. virginalis	24.0 ± 2.7	4.4 ± 1.4
Cherax destructor	28.7 ± 2.7	8.5 ± 3.2
Cherax quadricarinatus	34.8 ± 5.6	9.5 ± 4.4

Table 1. Mean size expressed as carapace length (CL) and weight (W) of chosen species.

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Author Contributions

A.K., design experiment, M.B., contributed on manuscript writing, L.V., wrote main part of manuscript and analyzed data.

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CHAPTER 3

THE SIGNIFICANCE OF DROUGHTS FOR HYPORHEIC DWELLERS: EVIDENCE FROM FRESHWATER CRAYFISH

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The significance of droughts for hyporheic dwellers: evidence from freshwater crayfish

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Freshwater biodiversity is globally threatened by various factors while severe weather events like long-term droughts may be substantially devastating. In order to remain in contact with the water or stay in a sufficiently humid environment at drying localities, the ability to withstand desiccation by dwelling in the hyporheic zone, particularly through vertical burrowing is crucial. We assessed the ability of three European native and five non-native crayfish as models to survive and construct vertical burrows in a humid sandy-clayey substrate under a simulated one-week drought. Three native species (*Astacus astacus*, *A. leptodactylus*, and *Austropotamobius torrentium*) suffered extensive mortalities. Survival of non-native species was substantially higher while all specimens of *Cherax destructor* and *Procambarus clarkii* survived. The native species and *Pacifastacus leniusculus* exhibited no ability to construct vertical burrows. *Procambarus fallax* f. *virginalis* and *P. clarkii* constructed bigger and deeper burrows than *C. destructor* and *Orconectes limosus*. In the context of predicted weather fluctuations, the ability to withstand desiccation through constructing vertical burrows into the hyporheic zone under drought conditions might play a significant role in the success of particular crayfish species, as well as a wide range of further hyporheic-dwelling aquatic organisms in general.

Freshwater ecosystems occupy less than 1% of the Earth's surface, but support approximately one tenth of the world's species and one third of all vertebrates[1]. These systems provide a wide range of valuable services also to human populations. The increased demand on freshwater resources has led to a freshwater crisis in both human and biodiversity perspectives[2]. While the conservation status and distribution of freshwater taxa is less well-known than that of terrestrial species[3], there is growing evidence that freshwater taxa are at greater risk of extinction than those in terrestrial or marine ecosystems[4–6], making freshwater conservation a priority[7].

Freshwater crayfish (Crustacea, Decapoda, Astacida) are considered not only keystone species in freshwaters but also strong ecosystem engineers modifying the environment to suit themselves. Indigenous crayfish species (ICS) have been often designated as e.g., bioindicator, umbrella, or even flagship species in aquatic conservation[7,8]. Three prominent crayfish invaders of North American origin (spiny-cheek crayfish *Orconectes limosus*, signal crayfish *Pacifastacus leniusculus*, and red swamp crayfish *Procambarus clarkii*) have been introduced to Europe between 1890 and the mid-1970s and became particularly widespread across the continent[9]. Thousands of populations of native European astacids have been lost, and many more have been substantially reduced, largely due to direct or indirect effects of the presence of non-indigenous crayfish species (NICS). These not only influence their European counterparts by competition but especially spread the causative pathogen of crayfish plague (an oomycete *Aphanomyces astaci*), causing mass mortalities to crayfish not originating from North America. Apart from imposing strong competitive pressures on native crayfish populations, these invaders possess the ability to alter food webs and entire ecosystems[10]. The main reason for NICS introductions in Europe was initially their expected commercial use (fisheries and aquaculture)[11]. In recent years, however, introductions of further NICS have usually involved escapes or intentional releases of aquarium-bred specimens[12,13], making the situation more inauspicious.

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Besides the expectation of high extinction rates in crayfish in general[7,14], interactions with non-native crayfish are the leading cause of decline of the native counterparts[15,16]. Explanation of these displacements have been generally based on the evaluation of one, or a combination, of four biotic mechanisms: competition, predation, reproductive interference and disease transmission[15], as well as lower environmental requirements in non-native crayfish[11]. However, an array of factors threatening biodiversity and aquatic ecosystems in general is much wider and more complex, including climate change and severe weather events like long-term droughts, unexpected floods, fires, heavy storms etc.[7,17] having far reaching consequences[18,19]. Indeed, the role of abiotic disturbances such as long-term droughts on aquatic biota has remained overlooked and poorly understood for a long time[19–21], and such knowledge is still scarce and fragmentary[22–24]. In the context of ongoing and predicted weather fluctuations[25,26], the ability to withstand desiccation and particularly to be involved in vertical burrowing under severe drought conditions might play a significant role in the success of various crayfish. Similar kinds of adaptations have been documented for unionid mussels, gill-breathing snails[27,28] and a wide range of aquatic insects[19,21].

In this study, we assessed the ability of three European ICS and five NICS as models to survive simulated drought conditions and to construct vertical burrows in a humid sandy-clayey substrate as a protection against drought conditions.

Results

Substantial differences were detected by means of survival analysis among studied species ($\chi^2 = 44.3$, df = 7, $p \leq 10^{-6}$). All specimens of red swamp crayfish, yabby and marbled crayfish survived the simulated one-week drought but certain post-treatment mortality was modeled for marbled crayfish during a one-week observation in aquaria with a final survival of 85.7 ± 13.2% (mean ± SD; Fig. 1). Relatively high survival rates of spiny-cheek crayfish and signal crayfish at the end of the simulated one-week drought (84.6 ± 10.0 and 88.9 ± 10.5%, respectively) were followed by post-treatment mortality resulting in final values of 42.3 ± 21.7 and 59.3 ± 18.5%, respectively. Narrow-clawed crayfish (*Astacus leptodactylus*) and stone crayfish (*Austropotamobius torrentium*) suffered substantial losses during simulated drought (25.0 ± 21.7 and 30.0 ± 23.9% survival, respectively) but the values remained stable thereafter. All noble crayfish (*Astacus astacus*) died within 5 days of simulated drought.

No attempts at vertical burrowing were observed in ICS (noble, stone and narrow-clawed crayfish) and signal crayfish. The remaining species exhibited different degrees of burrowing activity (Fig. 2). Crayfish usually constructed a single burrow in the suggested position ("initial burrow"). One red swamp crayfish and one yabby created two burrows and two other red swamp crayfish, both males, even dug three burrows in different places. The most prominent burrow was always located in the originally suggested depression. Only red swamp crayfish exhibited the ability to close the burrow entrance by means of a mud plug in our experiment. One out of twelve males created the plug but females were more active (n = 7) in doing this ($Z = -2.050$, $p = 0.040$).

Differences in burrowing between the sexes of particular species were detected only in red swamp crayfish (Fig. 2). Although the relative volume was comparable ($t_{24,\,22} = -0.670$, $p = 0.491$), females constructed deeper burrows than males ($t_{24,\,22} = -2.989$, $p = 0.007$). Marbled and red swamp crayfish constructed bigger ($p < 0.020$

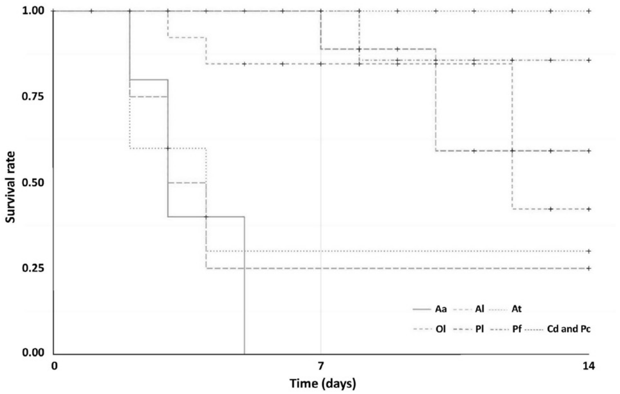

Figure 1. Kaplan-Meier survival analyses of crayfish species involved in the experiment. Legend refers to the particular species as follows: Aa–noble crayfish *Astacus astacus*, Al–narrow-clawed crayfih *Astacus leptodactylus*, At–stone crayfish *Austropotamobius torrentium*, Ol–spiny-cheek crayfish *Orconectes limosus*, Pl–signal crayfish *Pacifastacus leniusculus*, Pf–marbled crayfish *Procambarus fallax* f. *virginalis*, Cd–yabby *Cherax destructor*, and Pc–red swamp crayfish *Procambarus clarkii*.

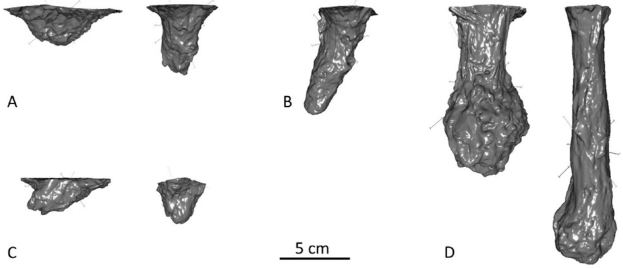

Figure 2. Examples of constructed burrows in yabby (**A**), marbled crayfish (**B**), spiny-cheek crayfish (**C**), and red swamp crayfish (**D**). 3D models of burrows of males (if present) are located on the left side of respective species. Further examples of burrows are available in supplementary materials (Fig. S1).

and $p < 10^{-4}$, respectively) and deeper burrows ($p < 0.002$ and $p < 0.004$, respectively) than did yabby and spiny-cheek crayfish (Fig. 3).

Discussion

We conducted the first comparative study evaluating burrowing activity under drought conditions in both selected native and non-native crayfish species currently present in Europe. Taking the results as a whole, it should be understood that the outlined crayfish desiccation capacities and burrowing abilities possess a degree of simplification and are related to the experimental set up, thus crayfish responses might vary under specific conditions. For instance, crayfish facing desiccation at localities might be exposed to even worse conditions, e.g. in terms of higher temperature and lower air humidity[29,30], the substrate might not be plastic enough for stability of burrows or the presence of coarse particles might prevent burrowing as such, i.e. substrate composition matters[24,31]. Also desiccation capacities and burrowing abilities of small-bodied juvenile animals are expectedly lower compared to sub-adults and young adults. Smaller animals have less water reserves compared to their relatively big body surface, allowing their desiccation; their physical ability to manipulate relatively big substrate particles is lower[29,30]. On the contrary, burrowing capacities of large-bodied specimens are likely also low due to difficulties with movement out of water (among others, the presence of big claws). Nevertheless, we believe it is unexpected that the patterns of desiccation capacities and burrowing abilities presented would change substantially among species under specific conditions. Also, the terminal stages of drought events when free water becomes unavailable at the localities are similar at both lotic and lentic sites, thus some degree of generalization is warranted.

Elevated desiccation capacities under different conditions have been documented among a wide range of NICS[29,32,33]. Our results clearly document substantially reduced survival of European ICS compared to NICS under simulated drought conditions, with absolute resistance in red swamp crayfish and yabby (Fig. 1), both considered to be warm-water species well adapted to conditions even in semiarid and arid regions[34,35]. Marbled crayfish suffered only post-treatment mortality with a final modeled survival of above 80%. The closely related slough crayfish *P. fallax* was found to cope less successfully with drought conditions than the Everglades crayfish *P. alleni*[24,36]; the latter has also been found accidentally released into European waters, although its establishment is considered unlikely[37]. High survival was also achieved by signal crayfish and spiny-cheek crayfish after the simulated drought followed by some post-treatment mortality (Fig. 1).

Resistance to desiccation is a necessary prerequisite for burrowing that mediates successful survival during severe droughts. A certain degree of burrowing is a habit present among crayfish. Less burrowing species just create short, unbranched burrows (or depressions) in the substratum, under stones, logs etc. They may also excavate burrows in the sides of clay banks[38–40]. Nevertheless, such burrowing activity might not be as adequate for survival as vertical burrowing under severe long-term droughts. European ICS species and signal crayfish exhibited no ability to construct vertical burrows. On the other hand, red swamp crayfish and marbled crayfish constructed bigger and deeper burrows than yabby and spiny-cheek crayfish (Fig. 3).

Considering their desiccation resistance and burrowing abilities, the red swamp crayfish is the most tolerant species we compared. It is worth mentioning that red swamp crayfish is also the only species in our experiment exhibiting the closing of the burrow entrance with a mud plug[31,41], particularly in females, which also created deeper burrows than males (Fig. 2). Females frequently use burrows for egg incubation. Females with eggs are not usually submerged in the ground water due to low available levels of dissolved oxygen, and oxygen diffuses directly from the burrow atmosphere while egg are fanned by swimmeret movements[42]. However, deeper burrows constructed by females in our experiment suggest the possible importance of having better access to the water. Even signal crayfish and white-clawed crayfish *Austropotamobius pallipes* eggs artificially stored in a humid environment require incubation in aquatic conditions at least during final stages of embryonic development, likely due to increased metabolic waste excretion[43], thus, at least periodical egg submergence can be expected in crayfish.

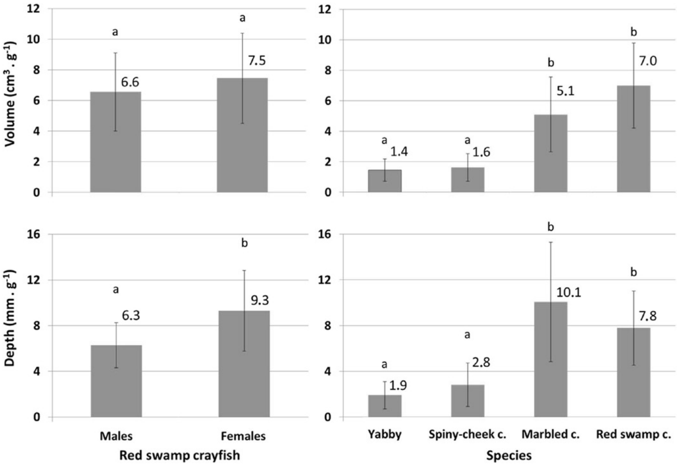

Figure 3. Relative volume and depth of burrows in burrowing crayfish species involved in the experiment. Significant differences between the sexes were detected only in case of red swamp crayfish–left column. Interspecific values are shown in the right column. Data are presented as mean ± SD. Values with differing letters within each graph are significantly different ($P < 0.05$).

Besides red swamp crayfish, further NICS involved in the study exhibit combined strategies focusing on increased desiccation capacity (yabby, marbled crayfish and signal crayfish) and burrowing (marbled crayfish and to a lesser extent also yabby and spiny-cheek crayfish). The lowest success belongs to ICS in particular and signal crayfish in terms of burrowing. We consider desiccation capacity and burrowing as further, still largely overlooked factors[7,11,17], whose importance will rise with ongoing and predicted weather fluctuations in the future[25,26]. Descriptions of current status and projections of droughts in European freshwater habitats together with current and future distributions of crayfish are beyond the scope of this article, however, the first suggestion might be that such events will be particularly pronounced among ICS in the warmer (Mediterranean) regions of the continent. Nevertheless, local extremes cannot be neglected and their importance will likely rise in the future too. For instance, a long-lasting drought hit Europe in 2015. It particularly affected Central and Eastern Europe while in some regions it was the driest (North Slovakia) and in others (Czech Republic and Poland) it was the second driest summer of the last 50 years–following 2003[44]. Expanding from its importance during droughts, burrowing also plays a role in overwintering which might increase the probability of establishment of non-indigenous species[45,46]. Following crayfish as a model group of freshwater organisms, a similar mode of action can be expected in further hyporheic-dwelling aquatic biota e.g. unionid mussels and clams, and a wide range of aquatic insects, as well as crabs and fish.

Methods

Container preparation. To create a suitable test substrate, sixteen kilograms of sand (České štěrkopísky Inc., Čavyně, Czech Republic) with a humidity of 5.2% and 24 kg of WBT clay (Keraclay, Plc., Brník, Czech Republic) with a humidity of 7.1% were thoroughly mixed by hand (=60% clay proportion expressed on a wet weight basis). For size distribution of sand and clay particles see Table S1. Aged tap water was added to get a final humidity of 16.5%. Our preliminary experiment revealed that the clay itself and a mixture with 80% clay proportion are too plastic to facilitate manipulation by crayfish. On the other hand, a substrate with 40% clay proportion was not stable enough for burrowing, which confirms the importance of substrate composition for successful burrowing[24,31]. The resultant humid mixture was used to fill a series of plastic containers (inner diameter = 34.0 cm, height = 44.5 cm) to a depth of ca. 34 cm. To better simulate natural conditions when certain areas with residual water persist at the drying-up localities[47], a shallow "initial burrow" (diameter 2.6 cm, depth 1.3 cm; volume 6.9 cm^3) was created in the margin of the container and 5 mL of water was added to stimulate burrowing in the suggested position. A single crayfish individual (see respective species and numbers below) was placed in the container. Each container was covered by a 0.5 cm thick polystyrene lid in order to prevent acute desiccation of the experimental animal. The air relative humidity (RH) reached at least 99% within an hour after coverage. The coverage was implemented in order to enhance survival of susceptible indigenous crayfish species allowing supposed burrowing. Natural conditions necessarily possess lower air humidity but there is a certain time period before free water becomes unavailable. It opens a space for animals to prepare a burrow, find water pools etc. For comparison, highly tolerant red swamp crayfish exposed to room air of approximately 50% RH showed mortality after 3–7 days' exposure[30] and all animals died at 30% RH and 24 °C within about a day[29]. The experimental temperatures (mean ± SD) of air and the sandy-clayey mixture were 20.2 ± 0.3 and 20.3 ± 0.1 °C, respectively. Temperature was registered hourly using Minikin loggers (Environmental Measuring Systems, Brno, Czech Republic). Fairly similar temperatures are often used in laboratory experiments and are relatively high—representing warm periods of the year when the most pronounced droughts usually occur. Although the temperatures might be even higher during such events[29,30], we considered values close to 20 °C the best compromise, taking the requirements of the most sensitive species involved (the stone crayfish) into account[48,49].

Experimental animals. We selected intact (with all walking legs including well developed chelae) intermoult specimens of three European ICS and five NICS. These were usually adults based on biometry and secondary sexual characters, but a few subadults might also have been involved. The sex ratio was balanced except for marbled crayfish *Procambarus fallax* f. *virginalis* where only females occur. For reasons of conservation and following a lack of vertical burrowing activities (see results above), only limited numbers of ICS (n = 4 for each species) were used. Noble crayfish *Astacus astacus* were caught from the pond U Sudu (Těšínov u Protivína, Czech Republic; 49° 20′ N, 14° 28′ E) under permit no. KUJCK 4820/2011 OZZL/4/Ou, Regional Office of South Bohemian Region. Narrow-clawed crayfish *A. leptodactylus* were obtained from the limestone quarry Kosov (Jarov u Berouna, Czech Republic, 49° 56′ N, 14° 3′ E) under permit no. 123564/2012/KUSK, Regional Office of Central Bohemia Region, and stone crayfish *Austropotamobius torrentium* came from Zubřina brook (Havlovice, Czech Republic; 49° 12′ N, 14° 17′ E) based on permit no. ŽP/2450/2011, Regional Office of Plzeň Region. Both signal crayfish *Pacifastacus leniusculus* and spiny-cheek crayfish *Orconectes limosus* (n = 10 for both species) were caught from the wild populations in the Vysočina Region and from the Lipno Reservoir, South Bohemian Region, respectively. Marbled crayfish (n = 12), yabby *Cherax destructor* (n = 14), and red swamp crayfish *P. clarkii* (n = 24) were obtained from laboratory cultures. Considering the categorization by Hobbs[50], all crayfish species involved in our experiment belong to the tertiary burrower category. Some members of this group are often incorrectly referred to as non-burrowers[39] but they may respond to habitat drying by excavating shallow simple burrows into the hyporheic zone, although experiencing population declines and local extinctions during severe droughts[22,51–54].

Crayfish were individually acclimatized for three days in a bucket with 8 L of aerated tap water, without feeding. Animal wet weights (to the nearest 0.1 g) and carapace lengths (to the nearest 0.1 mm) were determined and crayfish were placed in the experimental container for a one week period simulating drought conditions (for crayfish biometry see Table S2). Crayfish survival was evaluated daily. After one week's exposure, surviving animals were collected and transferred to aquaria with water for one week to evaluate post-treatment mortality.

All experimental manipulations were conducted according to the principles of the Institutional Animal Care and Use Committee (IACUC) of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, based on the EU harmonized animal welfare act of Czech Republic. Nevertheless, no specific permissions were required for the locations and activities considering taxa involved in this study.

Creation and measurement of casts. Gypsum casts of any burrows excavated were created after removal of animals. If direct collection of crayfish from burrows was impossible, a small amount of carbonated water was added to the burrow in order to evict animals, which led to success in most cases. If collection of animals was prevented (as occurred only with several specimens of red swamp crayfish), a new independent replication was conducted. Any excess water was removed from the burrow bottom by blotting with absorbent paper. Depth of casts was measured by a digital caliper to the nearest mm. Casts were further scanned by an Artec Spider™ hand-held 3D laser scanner (Artec Group, Luxenbourg) located at the Department of Cybernetics, Faculty of Applied Sciences, University of West Bohemia in Pilsen with a stated resolution of 0.1 mm and accuracy up to 0.03 mm. The scanner is based on the structured light principle and provides a 3D mesh of the object as an output, generated in real world coordinates (mm). The resulting STL (STereoLithography) mesh was imported to the Artec Studio, version 10 (Artec Group, Luxembourg) where the volume of the 3D mesh was calculated. As size naturally varies within and among crayfish species, relative data reflecting weight of respective animals were used in presentation of burrowing activity (volume and depth).

Statistical analysis. Non-parametric Kaplan-Meier survival analyses were performed in the R-statistics software (version 3.2.4, R Development Core Team 2015), with the packages: "KMsurv" and "survival". In addition, for graphical visualisation the packages "GGally" and "ggplot2" were employed. In assessing sex differences, the ability to close the burrow entrance by means of a mud plug was assessed as 1 or 0. Due to lack of normality and homoscedasticity of this dataset (evaluated with Kolmogorov–Smirnov and Levene's tests, respectively; these tests uniformly used further if appropriate for testing assumptions of parametric tests), a non-parametric Mann-Whitney U was applied. Intersex differences in term of relative burrow depth and volume were compared with Student's t-test. Because of heteroscedasticity in data, the non-parametric Kruskal-Wallis test followed by multiple comparisons of mean ranks for all groups was applied for interspecific comparisons (values of both sexes were pooled among species for this purpose). These data were analyzed using Statistica 12.0 (StatSoft, Inc.). The null hypothesis was rejected at $\alpha = 0.05$ in all tests of this study.

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Author Contributions

A.K. conceived the experiment and wrote the core part of the manuscript. J.T., L.V., M.F. and J.Př. conducted the experiment. P.C. analyzed the burrows. J.Pa. and M.B. improved earlier versions of the manuscript.

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CHAPTER 4

SALINITY TOLERANCE OF MARBLED CRAYFISH *PROCAMBARUS FALLAX* F. *VIRGINALIS*

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RESEARCH PAPER **OPEN ACCESS**

Salinity tolerance of marbled crayfish *Procambarus fallax* f. *virginalis*

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Abstract – Eastern Europe comprises a significant part of the native ranges for indigenous crayfish species (ICS) belonging to the genus *Astacus*. This region has been largely overlooked by astacologists and considered relatively immune to the impacts of non-indigenous crayfish species (NICS). The recent discovery of two marbled crayfish *Procambarus fallax* f. *virginalis* populations in Ukraine has changed this view. Increased propagule pressure (mainly due to pet trade) has raised concerns of NICS which may negatively impair the ecosystems of Azov, Black and Caspian Seas and their tributaries inhabited by ICS. In this study, we provide the first insight into salinity tolerance of marbled crayfish. We performed a 155-day experiment using 5 different salinities (6, 9, 12, 15, and 18 ppt) and a freshwater control. Evaluation of survival, growth and reproduction suggests that marbled crayfish have a lower salinity tolerance than other crayfish species, which may limit their invasive potential in brackish environments. However, its ability to survive for more than 80 days at 18 ppt opens up the possibility of gradual dispersion and adaptation to brackish conditions. Our study highlights the need for further studies elucidating the potential for marbled crayfish to negatively impair these ecosystems.

Keywords: brackish environment / growth / reproduction / moult / survival

Résumé – Tolérance à la salinité de l'écrevisse marbrée *Procambarus fallax* f. *virginalis*. L'Europe de l'Est comprend une partie importante des régions abritant des espèces indigènes d'écrevisses (ICS) appartenant au genre *Astacus*. Cette région a été largement ignorée par les astacologues et considérée comme relativement protégée des impacts des espèces d'écrevisses non indigènes (NICS). La découverte récente de deux populations d'écrevisses marbrées *Procambarus fallax* f. *virginalis* en Ukraine ont changé cette vision. Le potentiel d'augmentation de la pression des propagules (principalement en raison du commerce des animaux de compagnie) a soulevé des préoccupations concernant les NICS qui pourraient nuire aux écosystèmes des mers d'Azov, Noire et Caspienne et à leurs affluents habités par des ICS. Dans cette étude, nous fournissons le premier aperçu de la tolérance à la salinité des écrevisses marbrées. Nous avons effectué une expérience de 155 jours en utilisant 5 salinités différentes (6, 9, 12, 15, 18 ppt) et un contrôle d'eau douce. L'évaluation de la survie, de la croissance et de la reproduction suggère que les écrevisses marbrées ont une tolérance à la salinité plus faible que les autres espèces d'écrevisses, ce qui peut limiter leur potentiel invasif dans les milieux saumâtres. Cependant, sa capacité à survivre pendant plus de 80 jours à 18 ppt ouvre la possibilité d'une dispersion progressive et d'une adaptation aux conditions saumâtres. Notre étude souligne la nécessité de poursuivre les études permettant d'éclaircir le potentiel de l'écrevisse marbrée à nuire négativement à ces écosystèmes.

Mots-clés : environnement saumâtre / croissance / reproduction / mue / survie

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1 Introduction

Salinity is an important abiotic factor influencing crucial processes of animals such as feeding, growth, and reproduction, which determines their long-term survival, distribution and success in ecosystems (Snell, 1986; Ball, 1998; Sousa *et al.*, 2006, 2007, 2008; Costa-Dias *et al.*, 2010). Species are generally divided into euryhaline and stenohaline organisms, which specify their ability to adapt to a wide or narrow range of salinities, respectively (Croghan, 1976). Basically, freshwater species do not invade marine environments. However, species with marine ancestry may tolerate a wide range of salinities, while this pattern strongly varies across and within taxa (Hart *et al.*, 1991). Despite physiological changes and potential detrimental effects of elevated salinities to freshwater organisms (Heugens *et al.*, 2001; Nielsen *et al.*, 2003), they can migrate through saline ecosystems (van Ginneken and Maes, 2005; Jaszczołt and Szaniawska, 2011; Kornis *et al.*, 2012). However, this window of opportunity is often limited, and related to a specific part of their life cycle (juvenile stages and reproduction are usually vulnerable to higher salinity) due to physiological constrains (Holdich *et al.*, 1997; Anger, 2003). Thus salinity is of paramount importance for the spread of freshwater animals into new areas (Croghan, 1976; Leppäkoski and Olenin, 2000) due to, for example, possible dispersion to new watersheds through different estuaries. Also, the ionic composition of water, *i.e.*, the ratio of cations to anions together with the pH, strongly affects the magnitude of saline toxicity to freshwater organisms (Frey, 1993; Bailey and James, 2000). Therefore, life history features together with general ecological information (*e.g.*, distribution, abundance and population structure) should be taken into account when evaluating the effects of salinity.

Biological invasions are a significant threat to native biodiversity worldwide, with important ecological and economic impacts (Simberloff *et al.*, 2013; Seebens *et al.*, 2017). These impacts are particularly important in freshwater ecosystems (Strayer, 2010; Sousa *et al.*, 2014; Moorhouse and Macdonald, 2015). Conversely, estuaries and coastal areas have been overlooked in this regard within the last decades (Cohen and Carlton, 1998; Grosholz, 2002). Nowadays, these heavily invaded ecosystems are used as biological corridors for species that are able to withstand saline conditions (Grosholz, 2002). To our knowledge there are only a few studies dealing with invasion of freshwater species into saline environments (Leppäkoski and Olenin, 2000; Gonçalves *et al.*, 2007). Interestingly, some of these few studies assess the colonization of brackish waters by red swamp crayfish *Procambarus clarkii* in Europe, *e.g.*, Sousa *et al.* (2013) and Meineri *et al.* (2014). Considering biological invasions, the EU parliament and Council have listed some non-indigenous invasive species considered to be of high concern to European biodiversity (EU Regulation No. 1143/2014; Commission Implementing Regulation No. 2016/1141). It lists 23 animals of which freshwater crayfish form a remarkable group of five representatives. This clearly highlights the invasive potential, ecological and economic importance of at least some members in this animal group, as documented mainly in Europe and various parts of North America (Lodge *et al.*, 2000; Holdich *et al.*, 2009). Marbled crayfish *Procambarus fallax* f. *virginalis* is one of these listed animals. Its all-female stocks exclusively reproduce via apomictic parthenogenesis, thus producing clones that exhibit fast growth, early maturation, and high fecundity (Seitz *et al.*, 2005; Martin *et al.*, 2010). As presumed from its North American origin, it has also been proven to be a chronic carrier of crayfish plague *Aphanomyces astaci* pathogen (Keller *et al.*, 2014; Mrugała *et al.*, 2014). Marbled crayfish is a capable burrower (Kouba *et al.*, 2016) with the ability to overwinter in the temperate zone (Veselý *et al.*, 2015; Lipták *et al.*, 2016). Marbled crayfish were first discovered in the German aquarist trade in the mid-1990s, from where it dispersed (Scholtz *et al.*, 2003). The pet trade is an important pathway for the spread of non-indigenous taxa, and marbled crayfish are one of the most frequent and environmentally risky crayfish traded (Patoka *et al.*, 2014). Due to irresponsible or uninformed hobbyists, it may intentionally or accidentally be introduced into the wild (Chucholl, 2013; Patoka *et al.*, 2014). Indeed, reports on the presence of single specimens in the wild occurred at the beginning of the new millennium, followed by confirmed established populations in Germany and Slovakia in 2010. Since then, the number of invaded European countries has substantially increased (Patoka *et al.*, 2016, and references cited therein).

Eastern Europe possesses the entire native ranges, or at least their significant parts, for indigenous crayfish species (ICS) belonging to the genus *Astacus*, especially thick-clawed crayfish *Astacus pachypus* (Kouba *et al.*, 2014). This region has been largely overlooked by astacologists and considered relatively safe from the adverse impacts of gradually expanding non-indigenous crayfish species (NICS) (Perdikaris *et al.*, 2012). Discovery of two distant marbled crayfish *Procambarus fallax* f. *virginalis* populations in Dnipropetrovsk and Odessa, Ukraine in 2015 drastically changed this view (Novitsky and Son, 2016). Pet trade surveys provide extended lists of NICS, often of North American origin, both in the Ukraine (Kotovska *et al.*, 2016) and Lower Volga region of the Russian Federation (Vodovsky *et al.*, 2017). This has raised concerns of NICS potential to negatively impact the unique ecosystems of Azov, Black and Caspian Seas as well as their tributaries that are inhabited by ICS. Therefore, our goal was to investigate survival, growth, and reproduction of marbled crayfish in a range of salinities. This information will be important for implementation of possible management measures regarding the spread of this species in Eastern Europe, but also in estuaries elsewhere.

2 Material and methods

2.1 Experimental design and data acquisition

We conducted a 155-day experiment, lasting from the second half of May to the second half of October 2016, on salinity tolerance in marbled crayfish *Procambarus fallax* f. *virginalis*. This time of year normally includes a seasonal peak in reproduction (Vogt, 2015). The experiment was conducted at the Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic and we used animals from our own laboratory culture. Ten specimens (five in two replicates) for each of 6 experimental treatments, 60 animals in total, were used. The experiment was divided into two parts. Animals were first acclimated in a step-wise manner for 5 days to the target levels of salinity (final salinities of 6, 9, 12, 15, and 18 ppt, respectively). On the first day of acclimation crayfish were moved from fresh water to a saline environment of 6 ppt.

Subsequently, salinities were gradually elevated by 3 ppt per day until the target levels were reached. During the acclimation period all crayfish were divided into 12 static aquaria (described below) with 5 specimens per aquarium (10 aquaria with saline conditions and two aquaria with fresh water serving as a control). The aquaria (36 × 29.5 × 54 cm) were always filled with 16 L of aged tap water with or without salt added depending on the treatment. For ion composition of source water, see Table 1. Aquaria were covered by a plastic lid to limit water evaporation and aerated. To minimize aggression, the shelters were provided. For this, two blocks of joined polypropylene tubes, each containing five tubes (length 10 cm, inner diameter 35 mm), were added to each aquarium. The base of each block was represented by three longitudinally joined tubes with a further two tubes positioned pyramidal in the second layer.

The second part of the experiment started immediately after acclimation. Crayfish were held in the same aquaria as in the acclimation period and individually marked with nail polish on specific places on the carapace (Buřič *et al.*, 2008). Every day observations noting the number of individuals alive in each lot, the number of moulting and the presence of eggs were performed. After visual checking, impurities (*e.g.*, faeces and unconsumed food) were gently siphoned. Offspring was counted at the second developmental stage. When crayfish moulted, the marking was renewed when the animal re-calcified their exoskeleton. To maintain water quality, all baths were changed twice per week (Tue and Fri). The light regime was 12L:12D. Water temperature was recorded hourly by means of a temperature sensor MINIKIN (Environmental measuring systems, Brno, Czech Republic) and kept at 20.6 ± 0.7 °C. Crayfish were fed daily in excess with commercial dry feed for aquarium fish enriched with algae (Sera Granugreen, Sera GmbH, Germany) and frozen chironomid *Chironomus* sp. larvae.

We decided to generalize our experimental design by adding common salt (NaCl p.a., Penta s.r.o., Czech Republic), considering that marine water has specific profiles of salts, and these ions represent the bulk of the compositions. The range of tested salinities was chosen based on specific conditions in the target region and by the continental context. Average salinities of 10–12, 12–13 and 17–18 ppt correspond to the Azov, Caspian and Black Seas, respectively (Berdnikov *et al.*, 1999; Jazdzewski and Konopacka, 2002; Pourkazemi, 2006). Thus, salinity values tested in this experiment are relevant both for the mentioned Seas and estuaries elsewhere.

Prior to the experiment, crayfish were measured (digital calliper; Proma CZ Ltd., Mělčany, Czech Republic) and weighed (analytical balance; Kern & Sohn GmbH, Balingen, Germany) to the nearest 0.1 mm and 0.1 g, respectively. Mean ± SD carapace length (29.9 ± 2.3 mm) and weight (5.3 ± 1.5 g) of marbled crayfish did not differ among all experimental groups, *i.e.*, saline and control groups ($F_{5,54} = 0.39$, $p = 0.95$ and $F_{5,54} = 0.53$, $p = 0.87$, respectively). Following re-calcification (usually 2–3 days after moulting), crayfish biometry was re-measured. For assessment of growth, the following indices were calculated:

$$\mathrm{SGR} = \frac{(\ln(W_t) - \ln(W_i) \times 100)}{T}\ (\%\,\mathrm{day}^{-1}). \qquad (1)$$

Firstly, we counted specific growth rate (SGR; Eq. (1)), where W_t is weight at time t, W_i is initial weight and T is time in days.

Table 1. Ion composition of aged tap water used in experiment. Water analyses conducted in the accredited laboratory of the AGRO-LA, spol. s r.o., Jindřichuv Hradec, Czech Republic.

Ion	Concentration (mg L^{-1})
Bicarbonates	67.1
Sulphates	45.5
Calcium	31.6
Chlorides	9.44
Nitrates	8.65
Sodium	8.6
Magnesium	2.92
Potassium	2.42
Iron	0.113
Manganese	0.05
Nitrites	0.01
Total	**176.4**

$$L_m = \frac{(L_a - L_b) \times 100}{L_b}\ (\mathrm{mm}). \qquad (2)$$

Secondly, we counted absolute carapace length increment at moult (L_m; Eq. (2)), specifically for each moult separately. L_a is carapace length after moult and L_b is length before moult.

2.2 Statistical analysis

Non-parametric survival analysis (Kaplan–Meier method) was performed for all groups, using survival package (Therneau and Grambsch, 2000). To confirm normality in data (Kolmogorov–Smirnov test) one-way ANOVA followed by Tukey's HSD test was performed to compare initial biometry, and absolute length increments and SGR values among groups if applicable. Relationships between initiated and successful moult as well as ovulation were evaluated by means of a Spearman-rank correlation. All analyses were conducted in R version 3.2.5 (R Core Team, 2016). The null hypothesis was rejected at $\alpha < 0.05$ in all tests in this study.

3 Results

Marbled crayfish survival rate differed among tested conditions ($\chi^2 = 31.3$, df = 5, $p \leq 0.001$; Fig. 1). At the end of the experiment, survivors were found only in three experimental groups – control ($n = 8$), 9 and 12 ppt, each having a single specimen after 155 days. In other salinity conditions (6, 15, and 18 ppt) no crayfish survived but the last specimens died at different times (146, 87, and 91 days, respectively). When control was removed from survival analysis, we found no differences among salinity conditions ($\chi^2 = 7$, df = 4, $p = 0.13$). Additionally, neither carapace length ($F_{5,54} = 1.03$, $p = 0.31$) nor weight ($F_{5,54} = 0.66$, $p = 0.41$) significantly influenced marbled crayfish survival among salinity conditions.

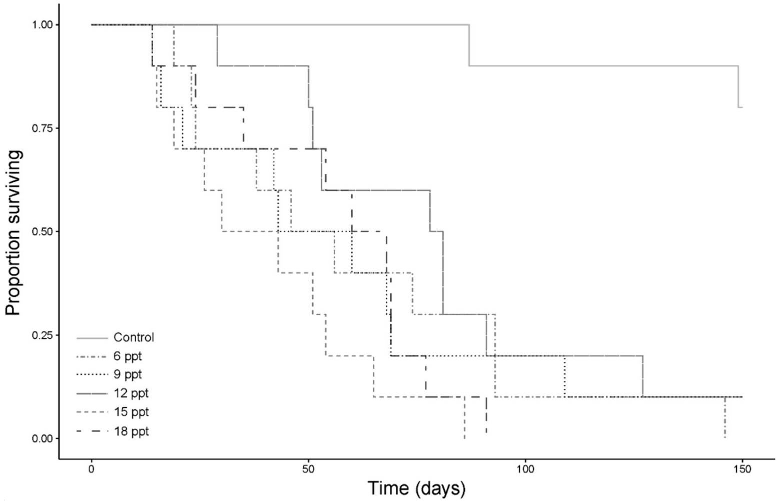

Fig. 1. Survival analysis plot of marbled crayfish *Procambarus fallax* f. *virginalis* kept under different salinities.

Table 2. Growth and reproduction indices of marbled crayfish *Procambarus fallax* f. *virginalis* kept under different salinities. Growth indices presented as mean ± SD. Reproduction indices refer to number of specimens. Values with differing superscripts in given column are significantly different (one-way ANOVA, Tukey's HSD test, $p < 0.05$).

Group	SGR (% day^{-1})	Absolute length increment at moult (mm)		Moult		Ovulation		Juveniles
		1st	2nd	1st initiated/ success	2nd initiated/ success	1st initiated/ success	2nd initiated/ success	
Control	0.22 ± 0.12^b	7.28 ± 3.97^b	7.33 ± 4.43	9/8	5/5	9/8	1/1	44–141
6 ppt	0.04 ± 0.09^a	2.17 ± 3.23^a	0	10/3	0/0	3/0	0	0
9 ppt	0	0	0	2/0	0/0	3/0	0	0
12 ppt	0.02 ± 0.08^a	0.85 ± 2.56^a	0	2/1	0/0	4/0	0	0
15 ppt	0	0	0	2/0	0/0	1/0	0	0
18 ppt	0	0	0	1/0	0/0	1/0	0	0

Salinity negatively influenced physiological processes such as growth, moulting and reproduction of marbled crayfish (Tab. 2). Salinity significantly decreased all measures of moulting (number of initiated, number of successful; Spearman-rank correlation, $p < 0.05$), SGR ($F_{1,30} = 10.74$, $p < 0.001$), and L_m ($F_{1,30} = 9.37$, $p < 0.001$). Only in the control five specimens successfully moulted twice. Salinity negatively influenced ovulation rate and reproduction success of marbled crayfish (Spearman-rank correlation, $p < 0.05$). Females ovulated in all experimental groups, while successful reproduction (reaching 2nd developmental stage) was confirmed only in the control. One female reproduced twice. No apparent cannibalism was observed during the experiment.

4 Discussion

Growth and reproduction are the most important processes expressing fitness and adaptation of species (Guan and Wiles, 1999). Most crayfish are able to survive in saline environments from a few days to a few months, while the effects of salinity on physiological processes differ among crayfish species and families (Jones, 1989; Holdich *et al.*, 1997; Alcorlo *et al.*, 2008). According to Jaszczołt and Szaniawska (2011) spiny-cheek crayfish *Orconectes limosus* are able to successfully reproduce and grow at salinity up to 7 ppt, but growth could be limited in more saline conditions. These results are in line with Holdich *et al.* (1997) who assessed growth in signal crayfish

Pacifastacus leniusculus and narrow-clawed crayfish *Astacus leptodactylus* sensu lato at salinity of 7 ppt. Nevertheless, salinity levels higher than 14 ppt were lethal for eggs in both species. According to Casellato and Masiero (2011), red swamp crayfish reproduce at salinities up to 25 ppt, but there is a negative correlation between salinity and the number of eggs (Alcorlo *et al.*, 2008). Newsom and Davis (1994) suggest elevated salinity as a factor causing higher growth in red swamp crayfish, due to lower energy spent on osmoregulation, which concurs with Sharfstein and Chafin (1979) suggesting salinity of 3–9 ppt as possible for culture of this species. Similarly, Australian species belonging to Parastacidae show analogous patterns where they are generally capable of growth and reproduction under saline conditions (Jones, 1989). For example, Anson and Rouse (1994) found the hatching ability of redclaw *Cherax quadricarinatus* to be from 1 to 20 ppt but hatching rate reduces as salinity increases. Additionally, high salinity (up to 18 ppt) reduced growth and caused lethargy of tested redclaw specimens (Jones, 1989). In comparison to the above-mentioned studies, marbled crayfish exhibited lower survival, growth and no reproduction even in the lowest salinity (6 ppt). Furthermore, increasing salinity contributed to high direct mortality during moulting. It is likely that osmotic stress negatively influenced moulting for which Na^+ and Cl^- are particularly important (Wheatly and Gannon, 1995; Bissattini *et al.*, 2015). The imbalance in ions composition, in particular, might have altered osmoregulation, resulting in a high mortality rate during moulting in our experiment. Nevertheless, it should be taken into account that all mentioned studies had different acclimation periods at each salinity level or used different methods of salt addition (gradual application *vs.* salt shock) and maintenance making possible comparisons among studies very difficult and highly context dependent.

Currently, marbled crayfish is considered a possible result of either hybridization between slough crayfish *Procambarus fallax* and other species of the genus *Procambarus*, or rather of autopolyploid origin (Martin *et al.*, 2016). It is usually regarded as *P. fallax* (Hagen, 1870) f. *virginalis* (Martin *et al.*, 2010). However, Vogt *et al.* (2015) suggest elevation of marbled crayfish to the species level. Slough crayfish and Everglades crayfish *Procambarus alleni* are North American species with a distribution centre in Florida, USA (Taylor *et al.*, 2007). They are probably the closest relatives to marbled crayfish (Martin *et al.*, 2016). Sometimes they live in sympatry in fresh waters (Hendrix and Loftus, 2000; Martin *et al.*, 2010), but it seems that salinity is an important factor in the separation of these two species in brackish conditions (Hendrix and Loftus, 2000). Everglades crayfish can inhabit saline environments in a range of 0–18 ppt (Hendrix and Loftus, 2000), but we are not aware of any study evaluating salinity tolerance in slough crayfish. Considering the relationship of marbled crayfish with the later may partly explains its low salinity tolerance. We found no differences in survival among salinities. It seems that long-term establishment in saline environments such as estuaries or coastal areas are not possible for marbled crayfish. Nevertheless, the ability to withstand saline environments at least for more than 80 days suggests that the species might inhabit watersheds in the vicinity and gradually adapt to more saline conditions using brackish waters as a biological corridor. This might promote its spread to coastal areas and estuaries and then colonisation of different river basins. Also, different water compositions could either reduce or enhance physiological or survival conditions of marbled crayfish, depending on pH, and cations and anions composition (Frey, 1993; Bailey and James, 2000). Furthermore, the short generation time of marbled crayfish might promote its quick adaptation to local conditions. Nevertheless, taking our experimental design as a whole (duration of adaptation and own experiment, numbers and size class of crayfish and salinity values used, etc.), further research is needed since salinity stress and salinity fluctuations may be amplified by other environmental conditions such as temperature, oxygen, and pH (Gilles and Pequeux, 1983).

5 Conclusion

Marbled crayfish is a successful invader with high ecological plasticity, capable of colonizing new habitats (Martin, 2015). We provide first insights into salinity tolerance of marbled crayfish. To sum up, marbled crayfish are probably unable to invade saline ecosystems due to their low survival, reduced growth and prevented reproduction. However, acclimation to natural conditions might lead to higher salinity tolerance due to the broader range of ions which are regulated by different pathways. Even so, its long-term survival in saline conditions has been proved. This might enable its spread in saline ecosystems, which in addition to their short generation time, could potentially lead to its local adaptation in the future.

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CHAPTER 5

MAY *CHERAX DESTRUCTOR* CONTRIBUTE TO *APHANOMYCES ASTACI* SPREAD IN CENTRAL EUROPE?

Mrugała, A., Veselý, L., Petrusek, A., Viljamaa-Dirks, S., Kouba, A., 2016. May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe? Aquat. Invasions 11, 459–468.

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Research Article

May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe?

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Abstract

Transmission of the crayfish plague pathogen *Aphanomyces astaci* endangers native European crayfish. This pathogen, spread mainly by its natural hosts, North American crayfish, has also been detected in the aquarium trade in Europe. As the trade in ornamental crayfish is nowadays considered a key introduction pathway of non-European crayfish, it may contribute to crayfish plague spread. Non-American crayfish have been assessed as highly susceptible to the pathogen, and thus unlikely to participate in *A. astaci* spread from aquarium facilities. However, moderate resistance to this disease has been suggested for the Australian yabby *Cherax destructor*. This widely traded crayfish species exhibits high potential to establish in Central Europe, and has been assessed as a high-risk species with regards to its invasiveness. We investigated resistance of juvenile *C. destructor* towards three *A. astaci* strains differing in virulence (representing genotype groups A, B and E), present in Central European waters. *Cherax destructor* was exposed to two doses of *A. astaci* zoospores (10 and 100 spores ml^{-1}) and its mortality was further compared with that of the juvenile European noble crayfish *Astacus astacus*. While some survival among *C. destructor* individuals was observed after exposure to the least virulent *A. astaci* strain (genotype group A), total mortality of Australian crayfish was noted after infection with the two more virulent strains. However, in contrast to *A. astacus*, the mortality of *C. destructor* was significantly delayed. These results suggest that under favourable conditions *C. destructor* may contribute to crayfish plague spread in Central Europe.

Key words: *Astacus astacus*, temperate zone, crayfish plague, aquarium trade, survival test, Australian crayfish, non-indigenous species

Introduction

Increasing numbers of commodities traded all over the world result in deliberate or unintentional introductions of non-native species outside of their natural ranges (Hulme 2009). Besides such impacts as predation, competition, hybridization, and habitat modification, these non-native species may threaten native competitors through transmission of pathogens, parasites and parasitoids (Daszak et al. 2000; Peeler et al. 2011). The disease emergence driven by non-native species introductions may happen in a twofold manner, either by expanding the geographic range of pathogenic agents or by facilitating host-switching (Peeler et al. 2011). In other words, non-native organisms may bring new diseases to their novel ranges or may act as reservoirs of existing parasites (Strauss et al. 2012).

Freshwater ecosystems are particularly vulnerable to biological invasions (Ricciardi and Rasmussen 1999; Shea and Chesson 2002), with the key drivers of non-native species introductions being aquaculture and the associated trade of live organisms for direct consumption, ornamental purposes, or even research (Copp et al. 2007; Gozlan 2008; Peeler et al. 2011). Consequently, all these pathways also contribute to the introduction of exotic pathogens (Peeler et al. 2011; Rodgers et al. 2011). The crayfish plague agent, an oomycete *Aphanomyces astaci* Schikora, is an example of such introduced exotic pathogens. It is undoubtedly one of the most devastating emerging diseases in European freshwaters, also listed among worst invasive species in Europe as well as globally

(Lowe et al. 2004; DAISIE 2009). Its unintentional introduction from North America to Europe resulted in substantial declines and local extinctions of native crayfish populations (Holdich et al. 2009). Although the origin of *A. astaci* involved in the first mass mortalities of European crayfish populations remains unknown, further spread of this pathogen has been, to a large extent, facilitated by stocking and subsequent expansion of three North American crayfish species: the spiny-cheek crayfish *Orconectes limosus* (Rafinesque, 1817), the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), and the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Holdich et al. 2009). Natural dispersal and human-aided translocations of these crayfish have resulted in a wide spread of the crayfish plague infection in Europe. Even though import and stocking of North American crayfish are nowadays illegal in many European countries, additional non-indigenous crayfish species have been introduced through illegal introductions, garden pond escapes, and releases of aquarium or bait specimens (Chucholl 2013 and references therein).

Import, trade and transport of ornamental non-indigenous crayfish species are forbidden or restricted in many European regions (Svobodová et al. 2010). Nevertheless, the market for ornamental crayfish has grown rapidly in some Central European countries in the recent decade, and keeping crayfish as pet species became a popular hobby (Chucholl 2013; Patoka et al. 2014). Consequently, the trade in ornamental crayfish has recently gained in importance as a key introduction pathway of non-European species (Peay 2009; Chucholl 2013). In particular, populations of the marbled crayfish *Procambarus fallax* f. *virginalis* Martin, 2010, introduced through this pathway, have already established across Central Europe and the number of invaded countries is gradually increasing (Kouba et al. 2014; Samardžić et al. 2014; Lipták et al. 2016; Lőkkös et al. 2016; Novitsky and Son 2016). In addition, specimens of other popular ornamental crayfish species including the yabby *Cherax destructor* Clark, 1936, the redclaw *Cherax quadricarinatus* (von Martens, 1868), and the Florida crayfish *Procambarus alleni* (Faxon, 1884) have been discovered in the wild in Europe (Souty-Grosset et al. 2006; Holdich et al. 2009; Jaklič and Vrezec 2011; Gross 2013). Moreover, the recent report of *A. astaci*-infected crayfish in the German aquarium trade (Mrugała et al. 2015) highlighted that the ornamental trade may not only act as an introduction pathway for non-indigenous crayfish species, but also as a reservoir of the crayfish plague agent. The pathogen may be introduced from household aquaria, aquarium facilities, and garden ponds either with discarded water, or with infected crayfish. Although most of the *A. astaci* infections were detected in North American crayfish, other crayfish species such as Australian *C. quadricarinatus*, with infection acquired through horizontal transmission from other species, may also be purchased (Mrugała et al. 2015). This finding clearly demonstrates that releases of any non-European crayfish species, even those considered vulnerable to crayfish plague, may potentially contribute to the spread of *A. astaci*.

Thanks to a long co-evolutionary history with *A. astaci*, North American crayfish species have evolved defence mechanisms against growth of *A. astaci* mycelium in their cuticles (Cerenius et al. 2003). In contrast, crayfish of European, Asian and Australian origin that lack efficient immune responses are considered highly susceptible (Unestam 1969, 1972, 1975; reviewed in Svoboda et al. 2016). However, a differential susceptibility towards *A. astaci* has been also observed in populations of the European noble crayfish, *Astacus astacus* (Linnaeus, 1758), and has been linked to differences in *A. astaci* virulence (Makkonen et al. 2012, 2014; Becking et al. 2015). Four different *A. astaci* genotype groups (A, B, D and E), at least some of them differing in virulence, are known at present in Europe (Huang et al. 1994; Diéguez-Uribeondo et al. 1995; Kozubíková et al. 2011) but the actual variation of this pathogen is probably higher (see Grandjean et al. 2014). A lowered virulence towards European crayfish species was observed in some strains from genotype group A isolated from infected European crayfish and implicated in latent *A. astaci* infections carried by *A. astacus* (Viljamaa-Dirks et al. 2011, 2013). The other three groups apparently exhibit substantially higher virulence and have been involved in numerous crayfish plague outbreaks in European crayfish populations (Filipová et al. 2013; Kozubíková-Balcarová et al. 2014; Rezinciuc et al. 2014).

In addition to variation in the pathogen's virulence, a variation in susceptibility towards *A. astaci* may be apparently present in non-American crayfish host species. Early studies by Unestam (1969, 1975) indicated that two crayfish species, the narrow clawed crayfish *Astacus leptodactylus* Eschscholtz, 1823 and *C. destructor,* seem less susceptible to *A. astaci* than the noble crayfish. Chronic *A. astaci* infections were indeed observed in various populations of the former species (Kokko et al. 2012; Pârvulescu et al. 2012; Schrimpf et al. 2012; Svoboda et al. 2012), and even the pathogen strain from genotype group B has been reported from infected specimens in Turkey (Svoboda et al. 2014a). The strain used by Unestam (1975) in the experimental exposure of *C. destructor* to *A. astaci* also belonged to genotype group B (see Huang et al. 1994). However, a strain from genotype group D was used in a successful eradication of established populations of *C. destructor* in Spain (Souty-Grosset et al. 2006), suggesting that a substantial variation may exist in susceptibility of this crayfish to various *A. astaci* genotypes.

Cherax destructor, endemic to south-eastern Australia, has successfully spread outside of its native range throughout the whole continent (Coughran and Daly 2012), and its presence in Western Australia poses a threat to the endemic crayfish species (Beatty et al. 2005). It seems likely that it may also spread rapidly and impose a wide range of negative impacts on native species and freshwater ecosystems in other continents. In Europe, established populations of this Australian crayfish are already known from Spain and Italy, where this species is farmed (Holdich et al. 2009; Scalici et al. 2009; Kouba et al. 2014). Its survival in European temperate climate was believed to be constrained by low winter temperatures. However, a recent study revealed that it is capable of surviving Central European winters (Veselý et al. 2015). *Cherax destructor* is a common ornamental crayfish in these regions and some specimens probably originating from aquarium releases have already been reported from the wild (Hefti and Stucki 2006; Souty-Grosset et al. 2006). Its wide availability in the pet trade coupled with biological characteristics of a successful invader have resulted in its assessment as a high-risk species (Chucholl 2013; Papavlasopoulou et al. 2014; Patoka et al. 2014). In this context, the trade in ornamental crayfish should be considered a potential entry pathway of *C. destructor* to Central European open waters.

Cherax destructor released from household aquaria and/or aquarium facilities may not only threaten the native fauna as a prominent predator and competitor, but may also contribute to *A. astaci* spread in a twofold manner 1) through an introduction of already infected *C. destructor* individuals into the natural environment, and 2) through an increase in *A. astaci* prevalence if crayfish populations come into contact with pathogen zoospores. For these reasons, we tested whether this Australian crayfish species indeed shows a decreased susceptibility towards *A. astaci* infection. Juvenile *C. destructor* were exposed to *A. astaci* strains representing three genotype groups involved most often in crayfish plague outbreaks in Central Europe (Kozubíková-Balcarová et al. 2014), including two highly virulent strains and one of lower virulence (Becking et al. 2015), and patterns of its mortality were compared with similarly-aged *A. astacus* highly susceptible to crayfish plague.

Methods

Studied crayfish and Aphanomyces astaci *strains*

The yabby, *Cherax destructor*, originated from an experimental culture and were kept at the Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic. The noble crayfish, *Astacus astacus*, were caught with permission of the nature conservancy authorities from the pond Pařez (Kaliště, Czech Republic; 49°36′N, 15°19′E). Before the experiment, *A. astacus* were adapted to the communal rearing conditions in the laboratory for 3 weeks. All crayfish were approximately 4 months old at the beginning of the experiment; their total length ranged from 20 to 40 mm.

The crayfish were exposed to three *A. astaci* strains (Al7, Pec14 and Evira4805a/10; as in Becking et al. 2015), representative of genotype groups A, B and E present in Central European freshwaters (for discussion on nomenclature of *A. astaci* genotype groups, see Svoboda et al. 2016). These strains are kept in Petri dish cultures with RGY agar (Alderman 1982) at the Department of Ecology, Charles University in Prague, Czech Republic.

Experimental design

The infection trial was conducted in an experimental facility of the Research Institute of Fish Culture and Hydrobiology in Vodňany between November 2014 and February 2015. The crayfish were kept separately in glass dishes with 400 ml of aged tap water, which was changed every week. Water temperature (mean ± SD: 15.6 ± 0.4°C) was registered hourly using a data logger (Minikin, Environmental Measuring Systems, Brno, Czech Republic). No aeration was provided to prevent airborne pathogen cross-contamination among vessels; to check for possible oxygen depletion, oxygen content (8.0 ± 0.7 mg l^{-1}) was measured in two additional dishes with crayfish that were managed in an identical manner. Each glass dish was further covered with an aluminium foil. Feeding with two pellets (Biomar Inicio plus 1.5) took place three times per week. The crayfish were monitored daily; dead crayfish and exuviae were removed immediately and stored in 96% ethanol. The experiment was terminated after 100 days. All crayfish that survived the trial were euthanized and also stored in 96% ethanol.

In total, 60 individuals of *C. destructor* and 30 of *A. astacus* were exposed to three *A. astaci* strains with two different zoospore concentrations of 10 and 100 spores ml^{-1} in six different treatments (i.e. spore concentration/*A. astaci* strain combinations). *Astacus astacus*, due to their confirmed high susceptibility to crayfish plague pathogen (Unestam 1969; Holdich et al. 2009), were used as a positive control to evaluate *A. astaci* virulence and infectiveness. Production of *A. astaci* zoospores was induced according to Cerenius et al. (1988). The motility of spores was checked, and spores were counted using the Bürker counting chamber. Appropriate volumes of the zoospore suspension were then added to the glass dishes with crayfish. For two *A. astacus* individuals (from treatments with Al7 and Evira4805a/10 strains and a dose of 10 spores ml^{-1}) the spore addition was accidentally omitted. Consequently, in each experimental trial 10 *C. destructor* and 4–5 *A. astacus* were used. In addition, 10 *C. destructor* and 6 *A. astacus* were treated as a pathogen-free control group.

DNA extraction and A. astaci *detection*

All crayfish used in the experiment were analysed for the presence of *A. astaci* infection. Additionally, 20 specimens of *C. destructor* from the same source as the experimental animals were tested before the experiment to rule out a chronic presence of the crayfish plague pathogen in this stock. Prior to dissection, total length (from the tip of the rostrum to the end of the telson) of each specimen was noted. Furthermore, the crayfish specimens were carefully examined for any presence of melanized spots as melanization is a common immune defence mechanism in crustaceans (Cerenius et al. 2008) and may indicate crayfish immune reaction to *A. astaci*. From each crayfish, the DNA was extracted using the DNeasy tissue kit (Qiagen) from up to 50 mg subsamples of mixed tissues (containing the soft abdominal cuticle, legs with basal joints, whole tail fan and any melanized tissues) ground in liquid nitrogen (as in Mrugała et al. 2015). The same procedure was also used for DNA isolation from the whole crayfish exuviae.

The detection of *A. astaci* infection was performed with TaqMan MGB quantitative PCR (qPCR) on the iQ5 BioRad thermal cycler as described in Vrålstad et al. (2009); with minor modifications of the original protocol to reduce likelihood of false positive results (as in Svoboda et al. 2014a).

Statistical analyses

The statistical analyses were performed in R version 3.2.2 (R Development Core Team 2015), with the package “survival” (Therneau and Grambsch 2000). To evaluate the differences in mortality rate between both crayfish species as well as two zoospore doses after exposure to each *A. astaci* strain the “survdiff” function was used. The significance level was set at 0.05. Non-parametric Kaplan-Meier survival analyses were performed using the “survfit” function. In addition, for graphical visualisation the packages “GGally” (Schloerke et al. 2014) and “ggplot2” (Wickham 2009) were employed.

Results

Aphanomyces astaci DNA was not detected in any crayfish used as a negative control, and in *C. destructor* individuals tested prior to the beginning of the experiment.

In comparison to *A. astacus*, the experimental exposure to all three *A. astaci* strains indicated higher resistance of *C. destructor* to the crayfish plague pathogen (Figure 1). Considerable differences in mortality rates were observed between the two tested species after infections with each *A. astaci* strain (Al7, group A: χ^2=22.1, df=3, p≤0.001; Pec14, group B: χ^2=43.3, df=3, p≤0.001; Evira4805a/10, group E: χ^2=90.2, df=3, p≤0.001). The detailed information about mortality of both crayfish species is provided in Table 1.

Infection with the least virulent *A. astaci* strain (Al7, genotype group A) resulted in deaths of two and six *C. destructor* individuals challenged with 10 and 100 spores ml^{-1}, respectively (Figure 1A). No statistical difference in mortality rate was found between the groups (χ^2=2.4, df=1, p=0.122). The first dead crayfish were found on the 34th day post-inoculation in both treatments. In the treatment with the lower spore concentration, crayfish died either during moulting or a few days afterwards. Similarly, two crayfish individuals died several days after moulting in the other treatment. Moderate to exceptionally high agent levels (A4–A7) were detected in the dead crayfish. In some crayfish individuals that survived the experimental infection, a higher pathogen load was detected in exuviae in comparison to crayfish bodies (Table 2).

The infection with the two more virulent *A. astaci* strains (Pec14 and Evira4805a/10) resulted in a total mortality of *C. destructor* individuals; without a statistical difference in mortality rate between the two spore concentrations (χ^2=0, df=1, p>0.8 for both strains). In each treatment, *A. astaci* infections reached very high to exceptionally high agent levels (A6 and A7) except for two individuals in which the pathogen loads were moderate and high (A4 and A5). In the treatment with the Pec14 (group B) strain, the first dead crayfish were recorded four to six days post-inoculation, but *C. destructor* died on average 46.4 ± 17.1 days (mean ± SD) after exposure to 10 spores ml^{-1} and 15.4 ± 12.5 days when challenged with 100 spores ml^{-1} (Figure 1B). While no

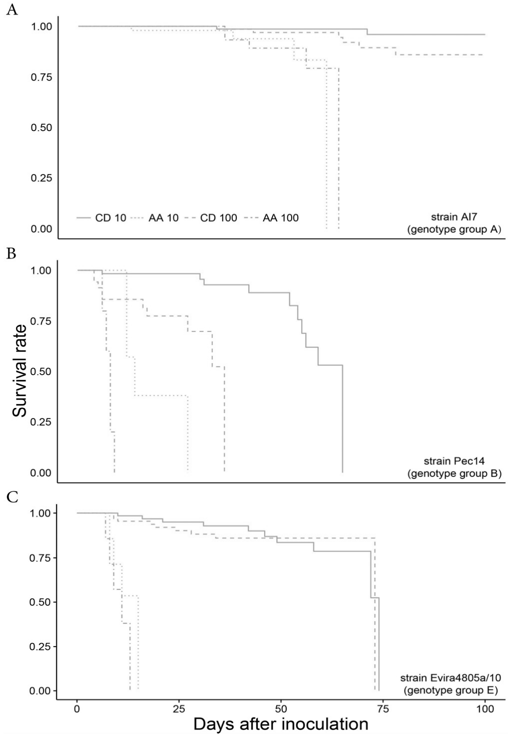

Figure 1. Kaplan-Meier survival analyses for both crayfish species: *Astacus astacus* (AA) and *Cherax destructor* (CD) after infection with three *A. astaci* strains (Al7, Pec14 and Evira4805a/10 representing genotype groups A, B and E, respectively) in two zoospore doses of 10 and 100 spores ml^{-1}. Curves are marked accordingly (e.g., AA10 standing for the crayfish species *A. astacus* and 10 spores ml^{-1}).

crayfish moulted after exposure to 10 spores ml^{-1}, one individual had moulted in the treatment with the higher spore concentration; this most likely contributed to its death two days later.

In the treatment with *C. destructor* individuals infected with the Evira4805a/10 strain (group E), mortality occurred on average 41.9 ± 22.4 and 24.0 ± 19.1 days post-inoculation with spore doses 10 and 100 spores ml^{-1}, respectively (Figure 1C). Whereas no moulting was observed in crayfish exposed to 100 spores ml^{-1}, five *C. destructor* moulted and died shortly afterwards in the treatment with 10 spores ml^{-1}. External body examination indicated that the remaining crayfish exposed to 10 spores ml^{-1} might have died prior to moulting.

All *A. astacus* individuals infected with *A. astaci,* died. After exposure to the zoospores of the Al7 strain, mortality occurred on average 41.0 ± 20.9 and 46.8 ± 12.6 days post-inoculation in the treatment with 10 and 100 spores ml^{-1}, respectively (Figure 1A). No statistical difference in mortality rate was observed between the treatments (χ^2=0.2, df=1, p=0.648). The molecular detection of crayfish plague pathogen indicated high and very high infection levels (A5–A6). In the treatment with 10 spores ml^{-1}, all crayfish died either on the same day or a few days after they moulted, which most likely contributed to their overall faster mortality. In the treatment with 100 spores ml^{-1}, no exuviae were collected but two crayfish individuals died during moulting.

Similarly, a total mortality was observed after exposure to the two more virulent *A. astaci* strains. Very high and exceptionally high agent levels (A6–A7) were detected. No crayfish had moulted during the experiment. The first dead *A. astacus* were recorded on the 12th and 6th day post-inoculation with the zoospores of the Pec14 strain, and 100% mortality was reached on the 27th and 9th day, in concentrations of 10 and 100 spores ml^{-1}, respectively (Figure 1B). Furthermore, exposure to the Evira4805a/10 strain resulted in the first crayfish deaths on 8th and 7th day; no *A. astacus* survived longer than 15th or 13th day of the experimental trial (Figure 1C). Whereas no difference in mortality rate

Table 1. Results of experimental infection with three *A. astaci* strains. N: number of crayfish individuals exposed to zoospores. Semi-quantitative agent levels based on the estimated amount of PCR-forming units (PFU) in the reaction (according to Vrålstad et al. 2009) are provided: A0 no *A. astaci* DNA, A1 ($PFU < 5$), A2 ($5 \leq PFU < 50$), A3 ($50 \leq PFU < 10^3$), A4 ($10^3 \leq PFU < 10^4$), A5 ($10^4 \leq PFU < 10^5$), A6 ($10^5 \leq PFU < 10^6$, A7 ($PFU \geq 10^6$).

Species	Treatment (spore ml^{-1})	N	Agent level in dead/surviving crayfish	Days to death	
				Average (days)	Mortality rate
Astacus astacus	Al7 (10)	4	A5-A6	41.0 ± 20.9	100%
	Al7 (100)	5	A5-A6	46.8 ± 12.6	100%
	Pec14 (10)	5	A6	15.4 ± 6.5	100%
	Pec14 (100)	5	A6-A7	7.6 ± 1.1	100%
	Evira4805a/10 (10)	4	A6-A7	10.8 ± 3.1	100%
	Evira4805a/10 (100)	5	A6-A7	10.0 ± 3.2	100%
Cherax destructor	Al7 (10)	10	A4-A6/A0-A2	52.5 ± 18.5	20%
	Al7 (100)	10	A4-A7/A0-A3	58.8 ± 16.8	60%
	Pec14 (10)	10	A6-A7	46.4 ± 17.1	100%
	Pec14 (100)	10	A6-A7	15.4 ± 12.5	100%
	Evira4805a/10 (10)	10	A5-A7	41.9 ± 22.4	100%
	Evira4805a/10 (100)	10	A4-A7	24.0 ± 19.1	100%

Table 2. Results of the qPCR analysis after an experimental infection with the least virulent Al7 strain. The *A. astaci* infection levels detected in *C. destructor* individuals that survived the 100-day long exposure and their exuviae sampled during the experiment are presented.

Concentration (spore ml^{-1})	Crayfish	Agent level in crayfish body	Moulting 1		Moulting 2	
			Day of moulting	Agent level in exuviae	Day of moulting	Agent level in exuviae
10	1	A0	24	A3	98	A0
	2	A0	23	A1	80	A0
	3	A0	25	A0	79	A0
	4	A0	8	A0		
	5	A0	3	A4	57	A0
	6	A0	2	A3	80	A0
	7	A2	68	A4		
	8	A0	33	A0	98	A0
100	1	A0	39	A0		
	2	A1				
	3	A3	4	A6		
	4	A0	99	A4		

was observed between the treatments with the two spore concentrations after infection with the Evira4805a/10 (χ^2=1.3, df=1, p=0.258), the difference in mortality rate after exposure to the Pec14 strain was highly significant (χ^2=20, df=1, p≤0.001).

Prior to death, individuals of both crayfish species tended to lose their limbs (claws and legs) after the challenge with the two more virulent *A. astaci* strains. Infection with the Al7 strain was followed by limb loss only in challenged *A. astacus* (regardless of the spore dose) and one *C. destructor* from the treatment with 100 spores ml^{-1}. Molecular analyses revealed exceptionally high pathogen load (more than 10^6 PFU) in that crayfish individual.

The external examination of crayfish bodies revealed the presence of macroscopic melanized spots in 73% of challenged *A. astacus*. These spots were mainly present on the soft abdominal cuticle, basal joints, legs, and on the tail fan. In contrast, only seven out of 60 *C. destructor* individuals were found with melanized spots on their body, associated with broken limbs and injured uropods. No visible melanization was present in the control animals.

Discussion

The potential interactions of *Cherax destructor* with three *Aphanomyces astaci* genotype groups occurring in Central European freshwaters were assessed for the first time. As suggested by Unestam (1975), we confirmed an elevated resistance of *C. destructor* to the crayfish plague pathogen in comparison to European *Astacus astacus*. Depending on the pathogen virulence, this may lead to chronic infections or delayed mortalities in *C. destructor* populations. Therefore, it seems possible that under certain conditions this Australian crayfish species may contribute to *A. astaci* spread in Central Europe.

Long co-evolutionary history between pathogens and their hosts often results in lowered virulence of pathogens and higher resistance of hosts (May and Anderson 1990), a mechanism that explains balanced host-pathogen relationship between North American crayfish species and the crayfish plague pathogen (Cerenius et al. 2003). Recent field observations, however, provided evidence of latent *A. astaci* infections in most European native crayfish species, including *A. astacus* in Finland (Viljamaa-Dirks et al. 2011), *A. leptodactylus* in Turkey and Romania (Svoboda et al. 2012; Pârvulescu et al. 2012), the stone crayfish *Austropotamobius torrentium* in Slovenia (Schrank, 1803) (Kušar et al. 2013), the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858) in Italy (Manfrin and Pretto 2014) as well as several crayfish species in Croatia (Maguire et al. 2016). This confirms that even crayfish species generally considered highly susceptible may carry this pathogen without quickly progressing to acute infection. This phenomenon has been linked to a decreased virulence of some *A. astaci* strains belonging to genotype group A (Makkonen et al. 2012, 2014; Viljamaa-Dirks et al. 2013, 2016) but apparently other genotype groups may also be involved (see Svoboda et al. 2014a).

The variation in host resistance may contribute to chronic infections as well, as highlighted by considerably different survival rates of *C. destructor* and *A. astacus* after infection with the Al7 strain in our study. Only some *C. destructor* individuals died during the experimental trial, in contrast to a total mortality observed in infected *A. astacus*. In most *C. destructor* and *A. astacus* individuals, mortality occurred either during or shortly after moulting, with the possible reasons being 1) high physiological demands of this process and likely associated moulting-dependent variation in immune responses (Cheng et al. 2003; Liu et al. 2004), 2) an increased availability of a suitable substrate for colonization by zoospores (carapace with lower Ca^{2+} content of premoult or freshly moulted crayfish; Aydin et al. 2014), or 3) an intensive spore release during moulting of infected animals (Strand et al. 2012; Svoboda et al. 2013). Interestingly, however, most *C. destructor* individuals were able to substantially reduce *A. astaci* infection level through moulting. Makkonen et al. (2012) speculated that inefficient attachment and germination of *A. astaci* spores and/or an effective crayfish immune response after infection by less virulent crayfish plague strains may limit pathogen growth. Both mechanisms also likely contributed to *C. destructor* ability to withstand and limit infection of the *A. astaci* strain of genotype group A, as observed in our experimental trial.

In comparison to adult crayfish, juvenile individuals moult at a considerably higher rate (Reynolds 2002). In freshwater shrimps, frequent moulting was considered an important factor in their apparent resistance to *A. astaci* infection (Svoboda et al. 2014b). Similarly, it was suggested that frequent moulting of juvenile crayfish is a reason for decreased pathogen prevalence within this age class (Vrålstad et al. 2011), although selective mortality of infected individuals could result in the same prevalence patterns. Our results suggest that moulting may influence the progress of infection differently in hosts with varying levels of susceptibility. In *A. astaci* hosts exhibiting increased resistance (as North American crayfish species, freshwater shrimps or the *C. destructor* tested here) it seems that moulting may lead to reduction of infection levels, while in noble crayfish (and possibly other highly susceptible hosts), it contributes to extensive mortality.

The effect of differently virulent *A. astaci* strains on *A. astacus* resistance has been assessed in several laboratory experiments (Makkonen et al. 2012, 2014; Becking et al. 2015). Although differences were apparent between some of the Finnish strains used by Makkonen et al. (2012), on the whole their results confirm the generally lowered virulence of *A. astaci* strains from genotype group A. Moreover, the use of geographically distant *A. astaci* strains in different experimental studies, originating either from Fennoscandian *A. astacus* (Makkonen et al. 2012, 2014; Viljamaa-Dirks et al. 2016) or from *A. leptodactylus* of Armenian origin (Becking et al. 2015), provides a further evidence that the long-term interactions between *A. astaci* and European crayfish may have resulted in the pathogen's decreased virulence (Jussila et al. 2014). Interestingly, although we have used the same *A. astaci* strain (Al7) as in the study by Becking et al. (2015), in contrast to results of that study, all *A. astacus* individuals died in the present one. This highlights that caution is needed when comparing results from different experiments, as many factors apart from the overall strain virulence may influence mortality of the same host species. These include, among others, design and length of experimental trials, spore concentrations of an infective agent, age and physiological state of tested crayfish, or their population of origin. Use of juvenile individuals, longer infection trials, and higher spore dosages could have contributed to the higher *A. astacus* mortality rate seen in the present experiment.

Although all *C. destructor* individuals exposed to *A. astaci* strains from the two more virulent genotype groups (B and E) died, the delayed mortality may be an indicator of its ability to slow down the progress of *A. astaci* infection. Unestam (1975) hypothesised that melanin deposition may be correlated with some degree of resistance to *A. astaci* infection in Australian yabby. In our study, the melanization on *C. destructor* individuals was sporadically observed and was mainly associated with broken limbs or injured uropods. This was most probably not directly associated with *A. astaci* infection, as melanization is a common invertebrate immune response towards any damage (Cerenius et al. 2008). Three non-exclusive reasons may explain the lack of observable

A. astaci-associated cuticle melanization: 1) intensive moulting of juvenile crayfish, 2) micromelanization of areas of hyphal penetration (Aquiloni et al. 2011) that could be missed by the naked eye, and 3) less expressed and thus less competent immune systems of the young crayfish used in our experiment in comparison to adults. We presume that the immune response towards penetrating hyphae, i.e., encapsulation of hyphae by haemocytes and subsequent inhibition of its growth by capsule melanization (Unestam and Weiss 1970; Unestam and Nylund 1972), may be less effective in juvenile than in adult crayfish (as already observed in other groups of invertebrates; e.g., Dikkeboom et al. 1985; Dyrynda et al. 1995). If that is true, it may be expected that adult *C. destructor* may even more efficiently inhibit growth of *A. astaci* mycelium in their cuticles. Research focusing on differences in immunological responses between juvenile and adult crayfish is, however, crucial to test this hypothesis. In any case, our results clearly demonstrate that a difference in resistance towards *A. astaci* exists between European *A. astacus* and Australian *C. destructor*, with the latter being able to slow down the infection progress even of the two more virulent *A. astaci* strains.

Cherax destructor that might successfully establish in Central European waters (Veselý et al. 2015) may become infected with crayfish plague via zoospores present in the ambient water. Crayfish survival will then depend not only on the virulence of the transmitted *A. astaci* strain, but also on the amount of zoospores an individual will be exposed to, as shown by the faster mortality rate of crayfish exposed to higher zoospore concentrations (observed in our study as well as in Alderman et al. 1987; Makkonen et al. 2014; Becking et al. 2015). *Aphanomyces astaci* monitoring in open waters revealed relatively small concentrations (usually not more than 1–50 spore l^{-1}) in lakes inhabited by North American crayfish species (Strand et al. 2014). Substantial increases in spore release were reported during episodes of moulting and crayfish death (Strand et al. 2012; Makkonen et al. 2013; Svoboda et al. 2013), or acute disease outbreaks in European crayfish species (up to 500 spore l^{-1}; Strand et al. 2014). Fluctuations in ambient spore concentration may be decisive for potential survival of *C. destructor* in the presence of *A. astaci*. However, we hypothesise that yabby may survive coexistence with American crayfish species, as our specific experimental conditions imposed much higher pathogen pressure on the tested *C. destructor* individuals than generally encountered in the wild.

Introduction of this popular ornamental crayfish into Central European freshwaters may pose a substantial risk to native European crayfish species. *Cherax destructor* potential to survive Central European winters (Veselý et al. 2015; Kouba et al. 2016), together with its environmental plasticity known from Australia (Beatty et al. 2005), indicate a high potential for crayfish to establish populations in temperate Europe. Bearing this in mind, the prevention of *C. destructor* establishment in Central Europe should be given priority, as this prominent invader from Australia may cause a wide range of negative impacts on whole ecosystems (Coughran and Daly 2012), and also likely contribute to the spread of *A. astaci* in Europe.

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CHAPTER 6

TEMPERATURE AND RESOURCE DENSITY JOINTLY INFLUENCE TROPHIC AND NON-TROPHIC INTERACTIONS IN MULTIPLE PREDATOR-PREY SYSTEM

Veselý, L., Boukal, D., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., Prchal, M., Kozák, P., Kouba, A., Sentis, A., Temperature and resource density jointly influence trophic and non-trophic interactions in multiple predator-prey system. Manuscript

My share on this work was about 40%.

TEMPERATURE AND RESOURCE DENSITY JOINTLY INFLUENCE TROPHIC AND NON-TROPHIC INTERACTIONS IN MULTIPLE PREDATOR-PREY SYSTEM

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SUMMARY

1. Rapid environmental changes such as global warming and nutrient enrichment can affect ecological communities by altering individual life histories and species interactions. Recent studies focusing on the consequences of environmental change on species interactions highlighted the need for a wider, multi-species context that includes both trophic and non-trophic interactions (e.g., predator interference). However, the effects of biotic and abiotic factors on the strengths of trophic and non-trophic interactions remain largely unexplored.
2. To fill this gap, we combined laboratory experiments and functional response modelling to investigate how temperature and resource availability influence the strengths of trophic and non-trophic interactions in multiple predator communities.
3. The biological system under study consisted of dragonfly larvae (*Aeshna cyanea*) and juvenile marbled crayfish (*Procambarus fallax* f. *virginalis*) preying on the common carp fry (*Cyprinus carpio*). We first estimated the functional response of each predator in single-predator experiments and used this information to parse species interaction strength in multiple-predator trials into the trophic and non-trophic component and measure their environmental dependencies.
4. Overall, we found that predator identity, prey density, and temperature all affect trophic and non-trophic interaction strengths. non-trophic interactions among predators were mostly negative, suggesting that predator interference is widespread in aquatic communities. The magnitude of responses varied strongly across predator pairs and temperature independently of prey density.
5. Our results thus highlight the need for further studies required to elucidate the mechanisms linking individual properties and environmental drivers to interaction strengths in food webs involving multiple predators.

Key words: *Interaction strengths, predator-prey, non-trophic interactions, functional response, global warming, enrichment*

Introduction

Human-induced rapid environmental changes such as habitat alteration, eutrophication and global warming pose major threats to global biodiversity, functioning of ecosystems, and ultimately to the fate of mankind (Pereira et al., 2010). It is therefore crucial to understand how these drivers impact organisms and their interactions to better predict the consequences of climate change on Earth biota (Harley et al., 2006; Neves and Angermeier, 1990; Porter et al., 2013). While organisms are often exposed to multiple stressors that affect them both directly and indirectly through biotic interactions, previous studies focused mainly on the effects of a single driver on the biology and ecology of a single species. This leaves a gap in our understanding on how and when multiple drivers impact species and their interactions (Sentis et al., 2016; Sentis et al., 2014).

Temperature and nutrient enrichment are two of the most important global change drivers influencing and altering ecological communities worldwide (Corvalan et al., 2005). Temperature directly influences the biological rates (metabolism, growth and feeding) of ectotherms with important consequences for their fitness and ability to spread to new areas (Brown et al., 2004; Dukes and Mooney, 1999; Irlich et al., 2009; Veselý et al., 2015). Global warming can have both positive and negative effects on species and communities depending on its amplitude relative to the current temperature and the thermal optima of the organisms (Schulte et al., 2011). Moreover, warming can indirectly affect individuals by influencing species interactions. These indirect effects remain poorly understood, especially in the context of multiple environmental drivers (Sentis et al., 2016).

Higher trophic levels are particularly sensitive to temperature changes and warming tend to decreased species richness at these levels (Griffin et al., 2013; Hiddink and Ter Hofstede, 2008). Theoretical and empirical evidence suggests that warming may increase the risk of predator starvation and extinction in relatively nutrient-poor ecosystems as metabolic rate typically increases faster than ingestion rate with warming (Binzer et al., 2012; Fussmann et al., 2014). As a consequence, this can lead to communities with a higher proportion of species at low trophic levels, i.e., producers and bacterivores (Petchey et al., 1999). This negative effect of warming is important for ecosystem functioning and services because predators regulate prey and pest populations (Duffy et al., 2007; Schmitz, 2007). Altogether, these theoretical and empirical studies suggest that investigating the effects of temperature on predators is particularly important to understand and predict how global warming may affect food-web dynamics and persistence.

Increase in resources at lower trophic levels (i.e., nutrient enrichment), another common human-driven phenomenon, modifies population growth rates and can have important consequences for species survival. In particular, enrichment can lead to the ‘paradox of enrichment’ under which consumer-resource dynamics change from stable equilibrium to oscillations and therefore increase species extinction risk (Boukal et al., 2007; Rosenzweig, 1971). This phenomenon strongly depends on temperature: ‘paradox of enrichment’ is typical of lower temperatures, while enrichment at higher temperatures tends to resurrect consumers at higher trophic levels from starvation (Binzer et al., 2012). The relative impact of temperature and enrichment on species survival and population stability is further modified by body sizes of the constituent species (Binzer et al., 2016). We thus argue that investigating the joint effects of global change drivers is of paramount importance to better understand and predict consequences of rapid environmental changes on ecological communities.

Moreover, ecosystems typically consist of multiple predators sharing the same prey. Their interactions determine links in ecosystems (Barrios-O’Neill et al., 2015; Sih et al., 1998) and the consequences of their alterations caused by warming and other environmental change are insufficiently understood. Predator diversity can enhance top-down control and stabilize food webs facing global change (Griffin and Silliman, 2011), but changing predator composition can negatively affect whole food webs (Finke and Snyder, 2010). Considering species diversity and interactions is therefore important to better understand the effects of global warming on species-rich ecological communities (Gilman et al., 2010; Petchey et al., 1999; Sentis et al., 2016; Worm et al., 2005). In particular, the effects of resource availability and temperature on species interactions in communities including multiple predators remain largely unexplored.

In this study, our goal was to investigate the joint effects of temperature and resource availability (i.e. prey density), on trophic and non-trophic interaction strengths in communities involving multiple predators. Sentis et al. (2017) developed a theoretical approach based on predator functional response to disentangle trophic and non-trophic interactions and investigate their environmental dependencies. Following their approach, we first estimated the functional response of each predator in single–predator experiments and used this information to disentangle trophic and non-trophic interaction strengths in multiple predator trials. We next investigated the effects of temperature and prey density on trophic and non-trophic interactions. These three factors are of paramount importance for individual fitness and population dynamics of most species and can thus influence entire ecosystems by altering species biological rates and interactions (Winder and Schindler, 2004).

Material and methods

We conducted experiments using two predator species, the marbled crayfish *Procambarus fallax* f. *virginalis* (Decapoda; Cambaridae) and larvae of the dragonfly *Aeshna cyanea* (Odonata; Aeshnidae). Marbled crayfish is an invasive benthic actively searching omnivorous species spreading across most freshwater ecosystems in Europe (Patoka et al., 2016). *Aesha cyanea* larvae are widespread predators that can alternate between a 'sit-and-wait' and active foraging strategy and are often top predators in small fishless waters (Corbet, 1999). Both species can feed on invertebrates as well as vertebrates. We used common carp *Cyprinus carpio* (Cypriniformes; Cyprinidae) fry as prey in our experiments.

Experiments were conducted at the Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic in May and June 2015. No specific permissions were required for the locations and activities involved in this study. Dragonfly larvae were collected in a small sandpit pool near Vodňany. Crayfish and fish fry were obtained from our own experimental culture. We standardized prey size (total length: mean ± SD = 6.42 ± 0.20 mm) and dragonfly larvae (hereafter abbreviated as D) size and developmental stage (F-1 instar, total length: 30.1 ± 2.3 mm, wet weight: 0.53 ± 0.12 g). Also we used two sizes of crayfish: small crayfish (abbreviated as SC; mean carapace length: 11.3 ± 0.9 mm, measured from the tip of the rostrum to the posterior edge of cephalothorax; wet weight: 0.45 ± 0.13 g) and large crayfish (abbreviated as LC; mean carapace length: 15.5 ± 1.0 mm; wet weight: 1.12 ± 0.18 g).

Before the experiment, predators and prey were maintained at 16 °C and fed in excess with sludge worms (*Tubifex tubifex*) and brine shrimps (*Artemia salina*), respectively. Dragonfly larvae were housed individually in 0.5-L plastic boxes (85 x 45 x 80 mm) containing a wooden stick as a perching site. Crayfish were kept at low densities (0.8 ind.L^{-1}) sizes separately in 50-L aquaria with access to shelters (> 1 per animal) to avoid excessive competition and cannibalism. Prior to the experiment, predators were kept individually in 0.5-L boxes and starved for 24 hours. Four hours before the experiment, predators were acclimated to the experimental temperature (16 or 20 °C). Prey were hold in trays (256 x 48 x 18 cm, filled by 147.5 L aged tap water) at experimental temperature and entire batches of individuals were exchanged weekly in order to provide similarly sized prey throughout the experiment. Experimental arenas (plastic boxes, 163 x 118 x 62 mm in size) were filled with 1 L of aged tap water and lined with a 1 cm layer of fine sand. All experiments were conducted under 12L: 12D photoperiod.

Experiment 1: Single-predator functional responses

We first quantified functional responses of all predators by measuring their feeding rate at 8 prey densities (3, 12, 25, 40, 70, 110, 160, 220 ind.L^{-1}) and two experimental temperatures (16 and 20 °C). The temperature of 16 °C corresponds to the temperature at which the experimental animals were collected in their natural habitat and 20 °C matches the increase of 4 °C predicted by the IPCC for 2100 (IPCC, 2013). Prey were introduced in the experimental arenas one hour before the experiment to minimize stress effects caused by manipulation. After this acclimation period, predators were released gently into the arenas and the number of remaining prey in each arena was recorded after 24 hours.

We distinguished eaten (N_e), surviving, dead prey (without visible marks of an attack), and attacked prey (killed but not eaten prey). Seven replicates per combination of prey density and temperature were conducted. Moreover, five replicates without predators were conducted for each prey density-temperature combination to control for potential prey "natural" mortality. In this paper we distinguish only eaten and all other prey (i.e., combine the surviving, dead and attacked prey), which reflects the energy flow through the food web. The effect of temperature, prey density and predator identity on the number of killed but not eaten prey is analysed elsewhere (Veselý et al., subm.).

For each temperature regime, a logistic regression between initial prey density (N_0) and predation mortality (N_e/N_0) was performed to identify the shape of the functional response:

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_2 + P_3N^3_0)}{1+\exp(P_0 + P_1N_0 + P_2N^2_0 + P_3N^3_0)} \tag{1}$$

where P_0, P_1, P_2, and P_3 are the intercept, linear, quadratic, and cubic coefficients, respectively, estimated by the maximum likelihood (Juliano, 2001). If $P_1 < 0$, the proportion of prey killed declines monotonically with the initial density of prey, matching a type II functional response. If $P_1 > 0$ and $P_2 < 0$, the proportion of prey killed is a unimodal function of prey density, corresponding to a type III functional response (Juliano, 2001). Our results indicate type II functional response for each predator in both temperatures (Table S1). We thus used Rogers random predator equation (Rogers, 1972), which accounts for prey depletion during the experiment:

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

where Ne is number of prey eaten, N0 is initial prey density per litre, is a predator search rate, h is a predator handling time and t is the duration of experiment in days. To fit the model, we used Lambert W function to solve Eqn. 2 for Ne (for further details see Bolker, 2008):

$$N_e = N_0\frac{w(ahN_0e^{-a(t-hN_0)})}{ah} \tag{3}$$

Background prey mortality in controls was low (proportion dead: mean±SD= 0.69 ± 1.23%), thus the data had not to be corrected.

We then tested whether the search rate (a) and handling time (h) of each predator are influenced by temperature. We evaluated four different functional response models covering all possible combinations of temperature dependence in both parameters (Sentis et al., 2015) using the maximum likelihood method implemented in the 'bbmle' package (Bolker, 2008) and selected the most parsimonious model based on the lowest AICc value (Burnham and Anderson, 2002).

Experiment 2: Multiple predators

We next quantified non-trophic interaction strengths using a multiple predator experiment in which two predators were released simultaneously and allowed to forage and interact freely. We chose three initial prey densities (70, 110, 220 ind.L^{-1}) representing low, medium and high prey densities. All other experimental conditions and methods were the same as in Experiment 1. We tested all three intraspecific scenarios with identical predators (abbreviated as D_D, SC_SC and LC_LC), the intraspecific scenario involving differently sized crayfish (SC_LC) and both interspecific scenarios involving differently and similarly sized predators (D_SC, D_LC). Experiment 2 was conducted alongside Experiment 1 and all replicates within each experiment were randomized to avoid potentially confounding effects of any temporal changes in physiological status, behaviour and developmental stage of the predators and prey.

Interaction strengths

We calculated species interaction strength for all predator scenarios in Experiment 2 as the per-capita mortality of prey R exposed to predator assemblage Z (Berlow et al., 1999):

$$IS(R,Z) = \frac{N_R - N_{R,Z}}{N_R} \tag{3}$$

where N_R and $N_{R,Z}$ are the respective prey densities in the absence and presence of predators.

To test whether interaction strength was influenced by temperature, prey density, and predator identity, we used GLM with quasibinomial distribution that accounts for data overdispersion (Zuur et al., 2009). The most parsimonious model was determined by sequential deletion of the least significant explanatory parameters (or interaction terms) from the full model. Parameter significance was evaluated using F-tests from analysis of deviance with $p = 0.05$ as the threshold value. The final model included only parameters with significant p-values. Finally, we performed post-hoc Tukey test to find differences between all treatments. Moreover, we also tested how temperature, prey density and interaction type (i.e., intra- or inter-specific interactions), within each interaction type influenced interaction strength using GLM with quasibinomial distribution as described above.

Non-trophic interactions

To estimate the strength of non-trophic interactions for a given predator assemblage, we parsed the empirically observed interaction strength IS into a trophic (IS_T) and non-trophic (IS_{NT}) component, $IS = IS_T + IS_{NT}$, where IS_T is the strength of trophic interaction in the absence of non-trophic interactions (Sentis et al., 2016). Positive and negative values of IS_{NT} correspond to predator facilitation and interference, respectively.

To obtain the value of IS_T for each experimental treatment, we used our estimates of the predator functional response parameters from Experiment 1 to parameterise a population dynamics model of exponential decay (Sentis et al., 2016):

$$\frac{dN_C}{dt} = \frac{-a_D(T)N_C N_D}{1 + a_D(T)h_D(T)N_C} - \frac{-a_{LC}(T)N_C N_{LC}}{1 + a_{LC}(T)h_{LC}(T)N_C} - \frac{-a_{SC}(T)N_C N_{SC}}{1 + a_{SC}(T)h_{SC}(T)N_C} \qquad (4)$$

where N is the population density (ind.L^{-1}), h(T) is the prey handling time (day.ind^{-1}) at temperature *T*, *a*(*T*) is the predator search rate (L.day^{-1}) at temperature *T*, and the subscripts *C, D, LC*, and *SC* refer respectively to the densities of the carp fry, dragonfly larvae, large crayfish and small crayfish. We used the package "deSolve" (Soetaert et al., 2016) to integrate equation 4 over time and obtain the numbers of surviving prey and calculated the expected value of IST for each experimental treatment in Experiment 2. Initial prey densities were used as starting values. We then estimated the strength of non-trophic interactions ISNT by subtracting IS_T from *IS*.

We used analysis of covariance (ANCOVA) with predator assemblage as categorical variable and temperature and prey density as continuous variables to assess whether predator assemblage, prey density, temperature, and their interactions affect trophic and non-trophic interaction strengths. The model selection approach was the same as described above. We next divided and analysed the results by interaction type (i.e., intra- or inter-specific interactions) to investigate how interaction type, temperature, prey density, and their interactions influence non-trophic interaction strengths. All analyses were implemented in R version 3.2.5 (R Core Team, 2016) . Box limits in figures of interaction strengths correspond to upper and lower quartiles, horizontal bar to the median, and points show outliers outside the 1.5 times IQR interval.

Results

Experiment 1: Single-predator functional responses

For each predator at each temperature, the relationship between prey density and the number of prey eaten was best described by a Holling type II functional response (Table S1). Moreover, model selection indicated that, for each predator, functional response parameters depended on temperature (Fig. 1, Table S2): search rate increased significantly with temperature whereas handling time decreased with warming, although the latter effect was only significant for the dragonfly larvae (Fig. S3, Table S2). Dragonfly predators also had higher search rates and lower handling times compared to small and large crayfishes.

Experiment 2: Multiple predators

Species interaction strengths

Species interaction strengths were affected by predator assemblage, temperature, and prey density and their interactions (Table 1). The most parsimonious model indicated that temperature influenced interaction strengths independently of prey density but differently among assemblages (Fig. 2). In two intraspecific assemblages (LC_LC and D_D), temperature did not significantly affect interaction strength, whereas warming increased interaction strength in one intraspecific assemblage (SC_SC) and all interspecific assemblages (LC_D, SC_D, and LC_SC). In addition, interaction strengths decreased significantly with prey density independently of predator assemblages or temperature (Fig. S4).

When we grouped predator assemblages by interaction type (intra- or interspecific), we found that both inter- and intraspecific interaction strengths increased significantly with warming, but this increase was steeper for interspecific than for intraspecific interactions (Table 1; Fig. S5).

Non-trophic interaction strengths

Non-trophic interaction strengths (IS_{NT}) were mostly negative, although we also found positive values of IS_{NT} in 6% of the replicates. Positive values of IS_{NT} (range: 0.001–0.30) occurred in some replicates for every predator assemblage except D_D, in which the dragonflies always interfered. The strength of non-trophic interactions varied among predator assemblages, temperature, and prey density treatments (Table 2). The effect of temperature on ISNT depended on predator assemblage: ISNT did not change with temperature in four predator assemblages (D_D, LC_LC, D_LC, and SC_LC), whereas it respectively increased and decreased with temperature in the SC_SC and D_SC assemblages (Fig. 3). The effect of temperature on IS_{NT} also differed between predator assemblage types (Fig. S6): warming substantially decreased IS_{NT} in interspecific pairs, whereas it had the opposite effect in intraspecific pairs. Moreover, IS_{NT} varied with prey density and was lowest at intermediate prey density (Fig. S7).

Discussion

Global change is affecting ecosystem stability and functioning in multiple ways that can be difficult to intuit (Hooper et al., 2005; Tilman et al., 1997). However, we still have very limited understanding on how multiple environmental drivers may influence organisms and their interactions in complex systems involving multiple predator species (Sentis et al., 2016). In this study, we combined experiments and modelling to investigate the influence of temperature and resource availability on the strength of trophic and non-trophic interactions in multiple predator communities.

Temperature-dependent functional responses

Temperature influences the speed of biochemical reactions and thus drives biological rates and interactions (Brown et al., 2004). In this study, we found that warming increased the search rate of all three predators, while the handling time decreased only in the dragonfly larvae. Our results are consistent with a previous meta-analysis of Rall et al. (2012) showing that the search rate is more sensitive to warming than handling time. The latter involves a suite of processes such as handling, ingesting and digesting the prey (Jeschke et al., 2002; Sentis et al., 2013). Although we cannot tease apart the underlying relationships, our results indicate that both species have different thermal response curves for one or more of these processes.

Interaction strengths in multiple-predator assemblages

Interaction strengths are not randomly distributed: food webs are often characterized by many weak and a few strong consumer-resource interactions (Berlow et al., 2004). This pattern is thought to increase food-web stability and prevent species invasions, population oscillations and other disturbances affecting ecosystem stability and functioning (Berlow et al., 2004; Kokkoris et al., 1999). Interaction strengths can be quantified in many different ways but they typically focus on one predator species, while the relationships between interaction strengths involving multiple predator types and community-level properties still need to be explored. We found large variations in interaction strength across the assemblages as did other recent studies (Sentis et al., 2016; Wasserman et al., 2016), but the limited number of predators tested so far is not sufficient to make any conclusions about the underlying distributions of interaction strengths in multiple-predator communities.

Consequences of altered interaction strengths depend on the timescales. In the short term, stronger interactions translate into higher predation rates that could be detrimental to prey populations (de Ruiter et al., 1995). On longer timescales, stronger interactions can lead to amplified population oscillations and population extinctions (Binzer et al., 2012). Predator-prey interaction strengths in our experiment increased with warming and decreased with prey availability in both single- and multiple-predator treatments. This is in agreement with previous studies and implies that enrichment can destabilize communities even when multiple predator species forage together. Moreover, we found that the effect of temperature on species interaction strengths was predator-assemblage specific. Our results thus highlight the need to consider predator diversity and other traits together in order to fully understand the effects of global change on species interaction strengths.

Non-trophic interaction strengths in multiple predator assemblages

Almost all ecological communities consist of multiple predator species that consume different or shared prey (Begon et al., 2006). Different predators may either interfere or facilitate each other, with potentially important implications for population dynamics and community structuring (Arditi et al., 2004; Kéfi et al., 2012). Interference among predators should dampen population oscillations and stabilize food webs (Brose et al., 2006; Rall et al., 2008) due to weaker trophic interactions (Sentis et al., 2016). Our results strengthen the notion that consumer diversity and consumer-consumer interactions crucially affect consumer-prey interactions (Crowley and Martin, 1989; Crumrine and Crowley, 2003; Wasserman et al., 2016). We found predominately negative interactions (i.e., interference) between the predators with only a few individual cases of positive non-trophic interactions (i.e., facilitation) in our experiment. The magnitude of non-trophic interactions varied widely among predator pairs. We attribute these patterns to interactions between predators, because we did not observe any obvious changes in prey behaviour during the experiments (Veselý et al., personal observation).

Our results provide novel insights into the dependence of non-trophic interaction strengths on predator identity and, prey density, and temperature. Unlike Wasserman et al. (2016), we did not always observe stronger interference among conspecifics in comparison to heterospecific predators. Although interference was highest among conspecific dragonfly larvae, the differences between conspecific and heterospecific pairs involving the omnivorous crayfish were much smaller. The effect of varying temperature on the sign and strength of non-trophic interactions is virtually unknown (Sentis et al., 2014). We found that it varies qualitatively with predator identity: temperature had no effect on four out of the six tested predator pairs. In the remaining two predator pairs (small crayfish paired together or with a dragonfly larva), predator interference respectively increased and decreased with warming. Translated into interaction types (i.e., intra- and interspecific assemblages), we found a significant effect of temperature on interference only for interspecific predator pairs. This contrasts with the results of Sentis et al. (2017), who used the same experimental temperatures but found a systemic decline in non-trophic interaction strengths with temperature for all tested predator assemblages.

Similarly to trophic interaction strengths, differences in the magnitude of non-trophic interaction strengths can be driven by predator functional traits and habitat domains (Sentis et al., 2016). In particular, feeding behaviour of top predators and omnivores can differ (Charnov, 1976; Pyke, 1984). We found more intensive interference at 16 °C in predator pairs including dragonfly larvae than in those including only crayfish. However, these differences diminished or entirely disappeared at 20 °C, possibly due to higher metabolic demands of the predators (Lang et al., 2012; Sentis et al., 2016). We conclude that correctly predicting the effects of global warming on communities involving multiple predators is challenging: the effect of temperature on interaction strengths is context-dependent, and more studies are needed to better understand the patterns and mechanisms that underlie the differences.

Last but not least, prey density can also influence interactions among predators. Sentis et al. (2017) found a concave-down relationship between prey density and non-trophic interaction strengths, and attributed it to a balance between predation rates and satiation of competing predators. That is, they argued that the effects of interference at low and high prey densities are respectively hidden by high prey depletion and diminished by predator satiation. Interference should thus be strongest at intermediate resource levels. We observed similar albeit weaker patterns for intraspecific predator pairs but found very little evidence for it in interspecific pairs.

In sum, we revealed that the dependence of non-trophic interaction strengths on temperature, and prey density was assemblage specific. This suggests that the processes underlying non-trophic interaction strengths are more complex than those driving the overall interaction strengths.

Conclusions

Overall, we found that predator identity, prey density, and temperature all affect trophic and non-trophic interaction strengths. The magnitude of responses varied strongly across predator pairs and temperatures. Based on the current evidence, we lack strong support for a general mechanism underlying the dependence of interaction strengths on species traits and environmental conditions, although all studies of freshwater vertebrate and invertebrate predators so far found negative non-trophic interaction strengths that can help stabilize freshwater food webs. Our results thus highlight the need for further studies required to elucidate the mechanisms linking individual properties and environmental drivers to interaction strengths in freshwater food webs.

Authors' Contributions

LV, DSB and AS conceived the experiment and conducted data analyses. LV, AK, MB, IK, MF, BY, MP and PK conducted the experiment. LV wrote the first draft of the manuscript. DSB, MB, AK and AS provided comments and additional revisions of the text.

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Table 1. *Summary of all most parsimonious models of interaction strengths (ANOVA, type III). Model types given as subheadings; see main text for details. df = degrees of freedom, resid. df = residual degrees of freedom. Significant values (P < 0.05) in bold.*

	df	**resid. df**	**F**	**P-value**
Predator assemblage				
temperature	1	308	42.0	**< 0.001**
prey density	1	308	133.1	**< 0.001**
predator assemblage	5	308	34.6	**< 0.001**
predator assemblage x temperature	5	308	2.63	**0.02**
Interaction type				
temperature	1	316	33.5	**< 0.001**
prey density	1	316	102.1	**< 0.001**
interaction type	1	316	68.1	**< 0.001**
interaction type x temperature	1	316	5.9	**0.01**

Table 2. *Summary of all most parsimonious models of non-trophic interaction strengths (ANOVA, type III). Model types given as subheadings; see main text for details. Symbols as in Table 1.*

	df	**resid. df**	**F**	**P-value**
Predator assemblage				
temperature	1	308	0.19	0.66
prey density	1	308	15.9	**< 0.001**
predator assemblage	5	308	14.2	**< 0.001**
predator assemblage x temperature	5	308	5.22	**< 0.001**
Interaction type				
temperature	1	316	0.1	0.77
prey density	1	316	9.62	**0.002**
interaction type	1	316	1.37	**0.24**
interaction type x temperature	1	316	12.8	**< 0.001**

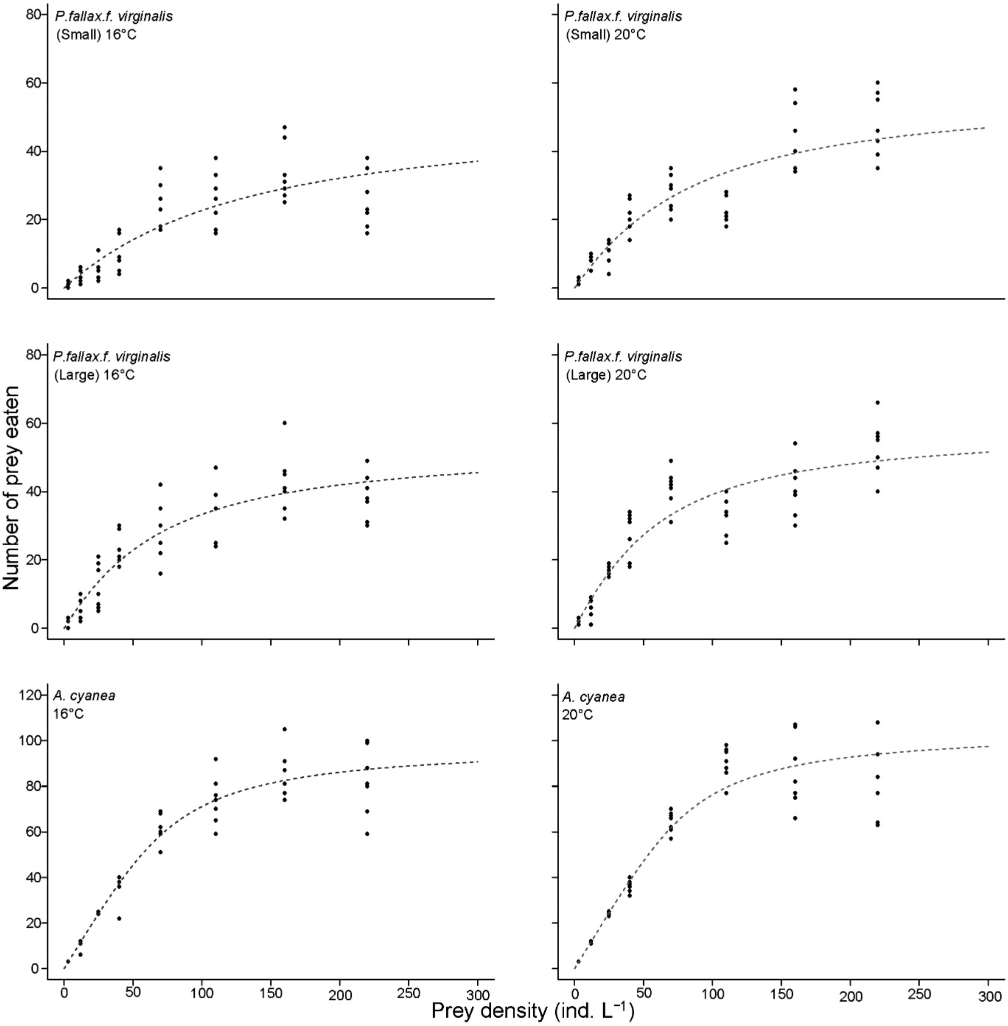

Figure 1. *Temperature-dependent functional responses in the single-predator experiment. Individual replicates (points) overlaid by the model prediction (dashed line).*

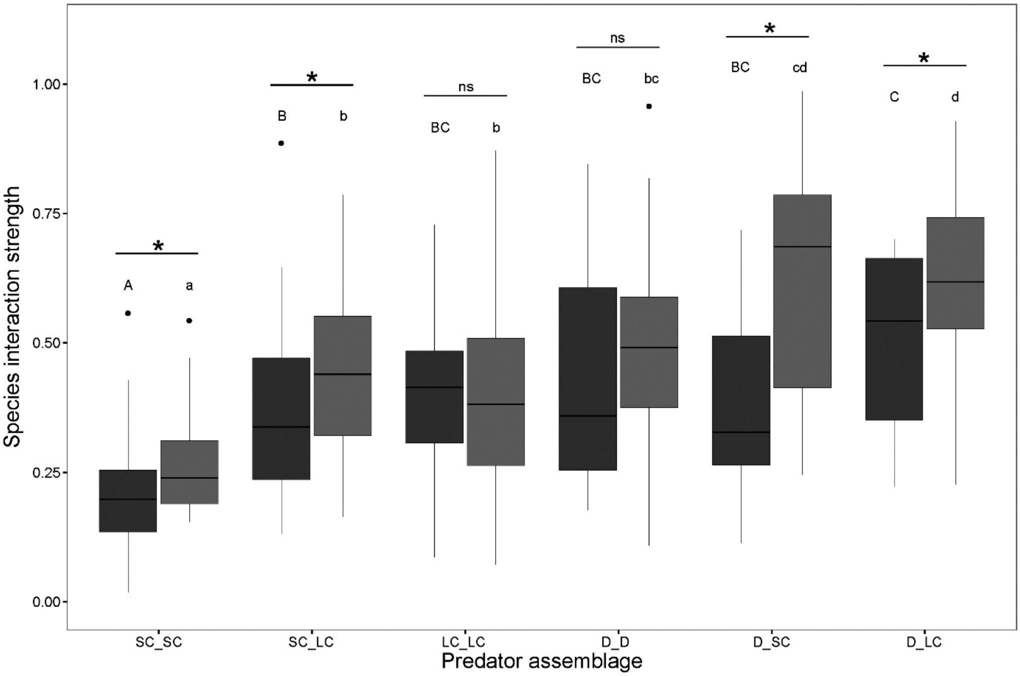

Figure 2. *Interaction strengths for all predator assemblages in the multiple-predator experiment. Data pooled across all prey densities used in Experiment 2. Black = 16 °C, grey = 20 °C. Different letters denote significant (P < 0.05) differences between assemblages at the given temperature (16 °C = capital letters, 20 °C = small letters). Significant differences between temperatures within each predator assemblage (horizontal bars) marked by asterisk, non-significant ones by 'ns'.*

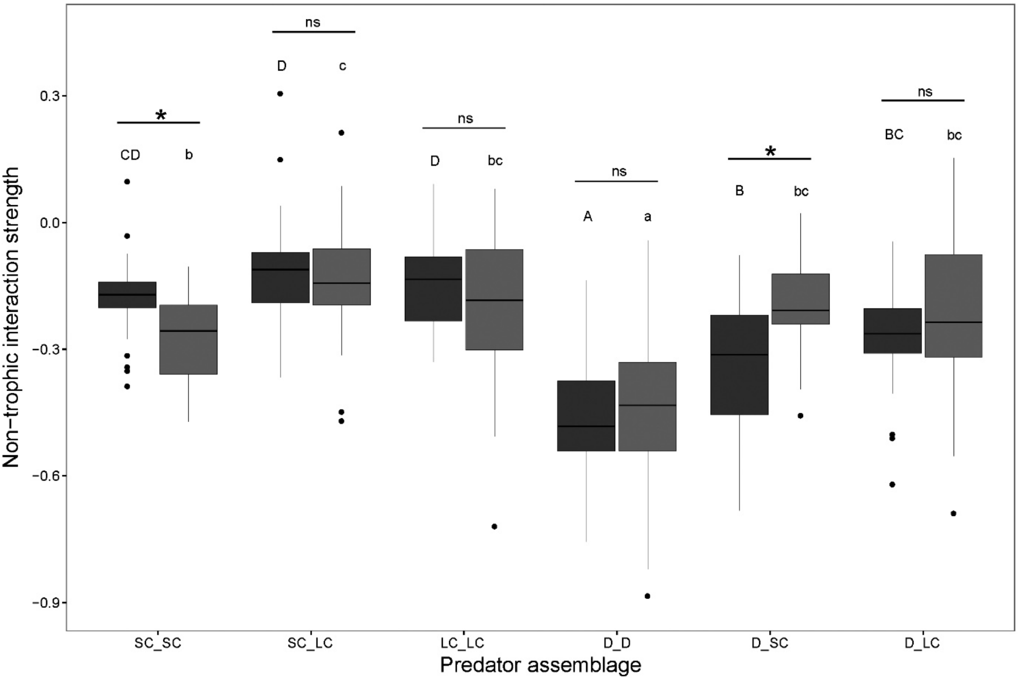

Figure 3. *Estimated non-trophic interaction strengths for all predator assemblages in the multiple predator experiment. Symbols as in Fig. 2.*

SUPPLEMENTARY INFORMATION

Table S1. *Logistic regression of the proportion of prey eaten by each predator at 16°C and 20°C. Value, standard error and P–value of the linear coefficient P1.*

Predators	**16 °C**			**20 °C**		
	P_1	**SE**	**P-value**	**P_1**	**SE**	**P-value**
Dragonfly	-2.43	0.28	< 0.001	-2.23	0.27	< 0.001
Large crayfish	-3.05	0.33	< 0.001	-2.92	0.32	< 0.001
Small crayfish	-1.53	0.43	< 0.001	-3.01	0.33	< 0.001

Table S2. *Comparison of the functional response models for all three predators. dAICc = difference in AICc value from the most parsimonious model (in bold); df = degrees of freedom; weight = Akaike weight.*

	Dragonfly			**Large crayfish**			**Small crayfish**		
	dAICc	df	weight	dAICc	df	weight	dAICc	df	weight
Temperature-dependent *a* and *h*	**0.0**	**4**	**0.53**	0.5	4	0.42	1.4	4	0.34
Only a temperature dependent	1.6	3	0.24	**0.0**	**3**	**0.56**	**0.0**	**3**	**0.66**
Only *h* temperature dependent	1.7	3	0.23	7.7	3	0.012	28.8	3	< 0.001
a and *h* temperature independent	16.9	2	< 0.001	27.2	2	< 0.001	90.2	2	< 0.001

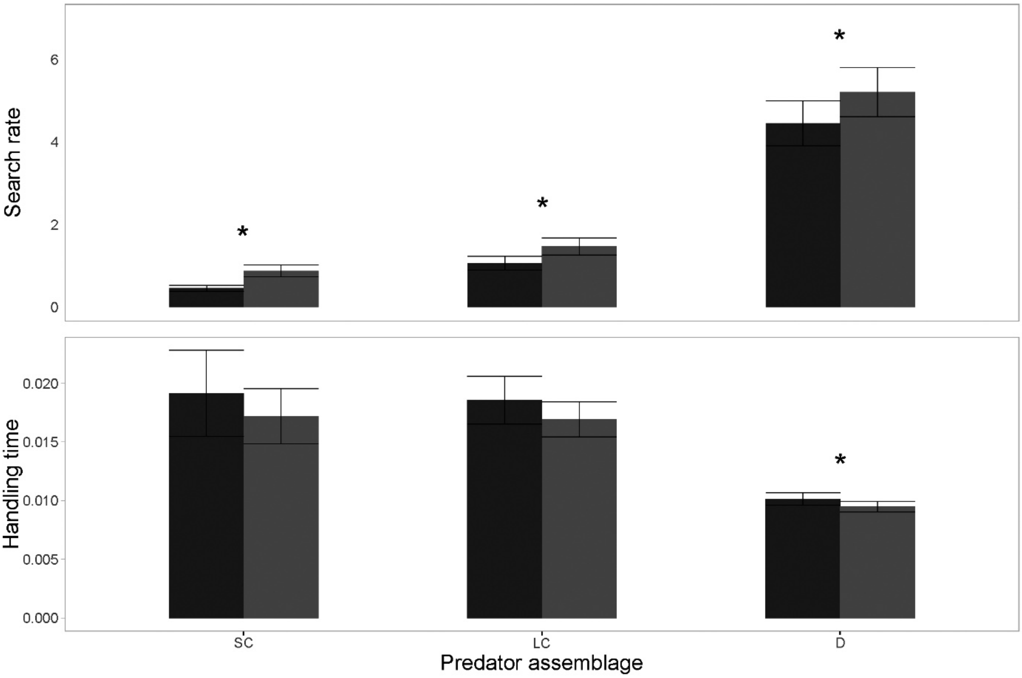

Figure S3. *Temperature-dependent search rate and handling time in single predator experiment. Black = 16 °C, grey = 20 °C; data shown as mean ± 95% CI. Significant differences between temperatures marked by asterisk.*

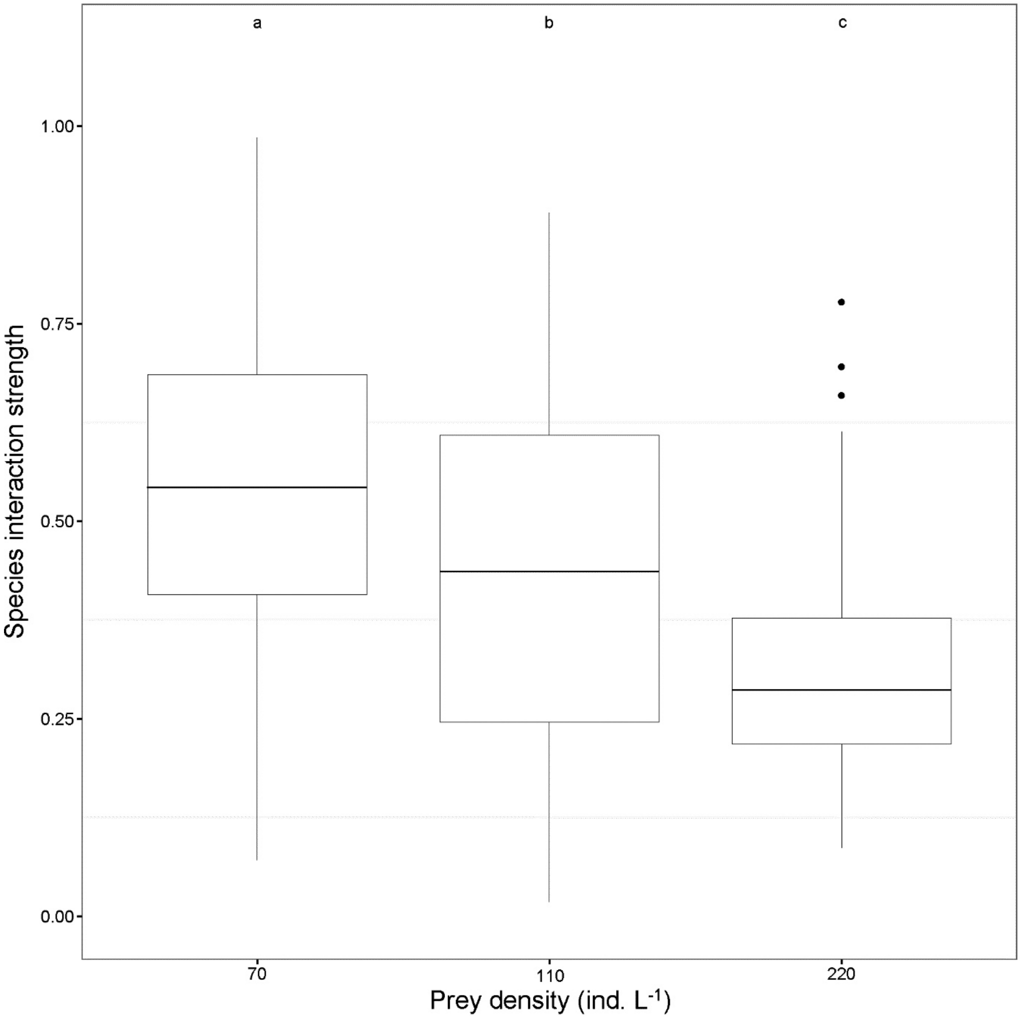

Figure S4. *Dependence of interaction strengths on prey density in the multiple predator experiment. Data pooled across all predator assemblages and temperatures. Significant pairwise differences marked by different letters.*

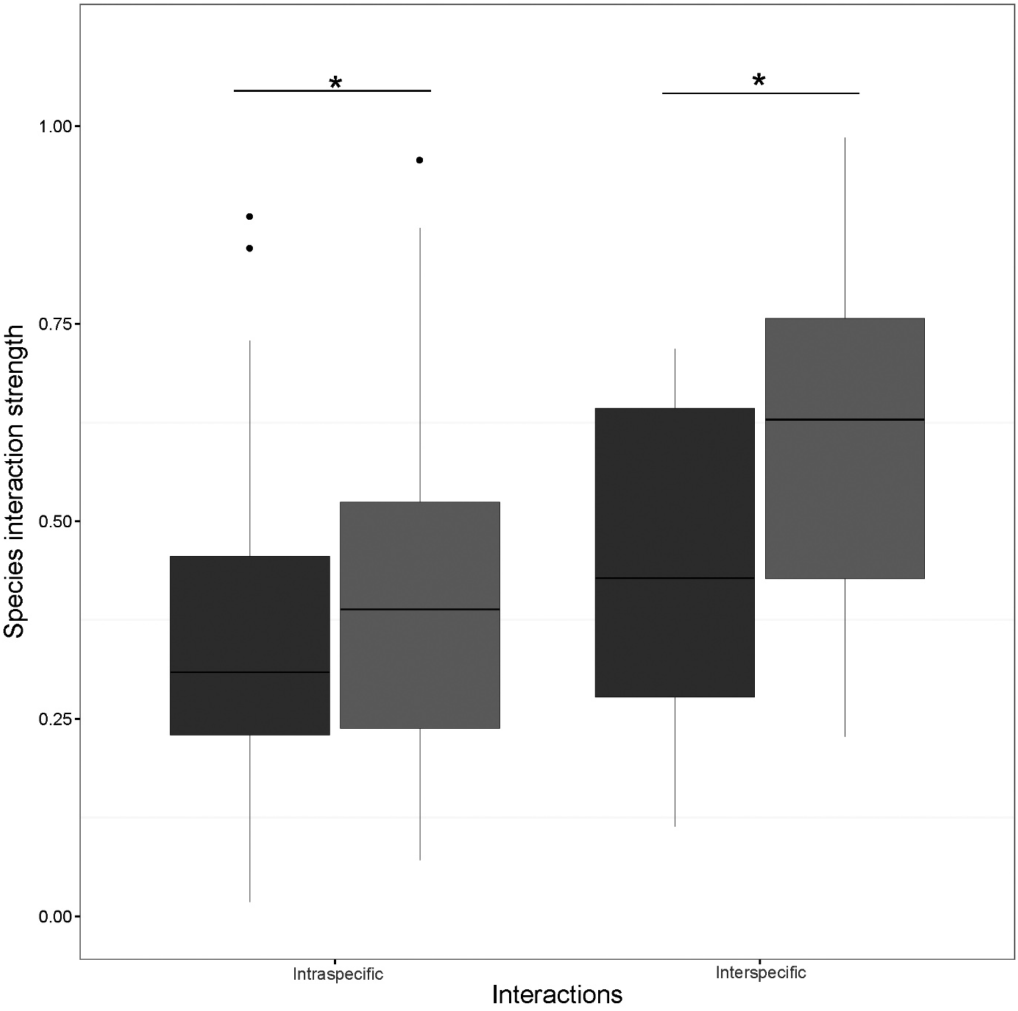

Figure S5. *Dependence of inter- and intraspecific interaction strengths on temperature (black = 16 °C, grey = 20 °C) in the multiple predators experiment. Data pooled across all prey densities. Significant differences between temperatures marked by asterisk.*

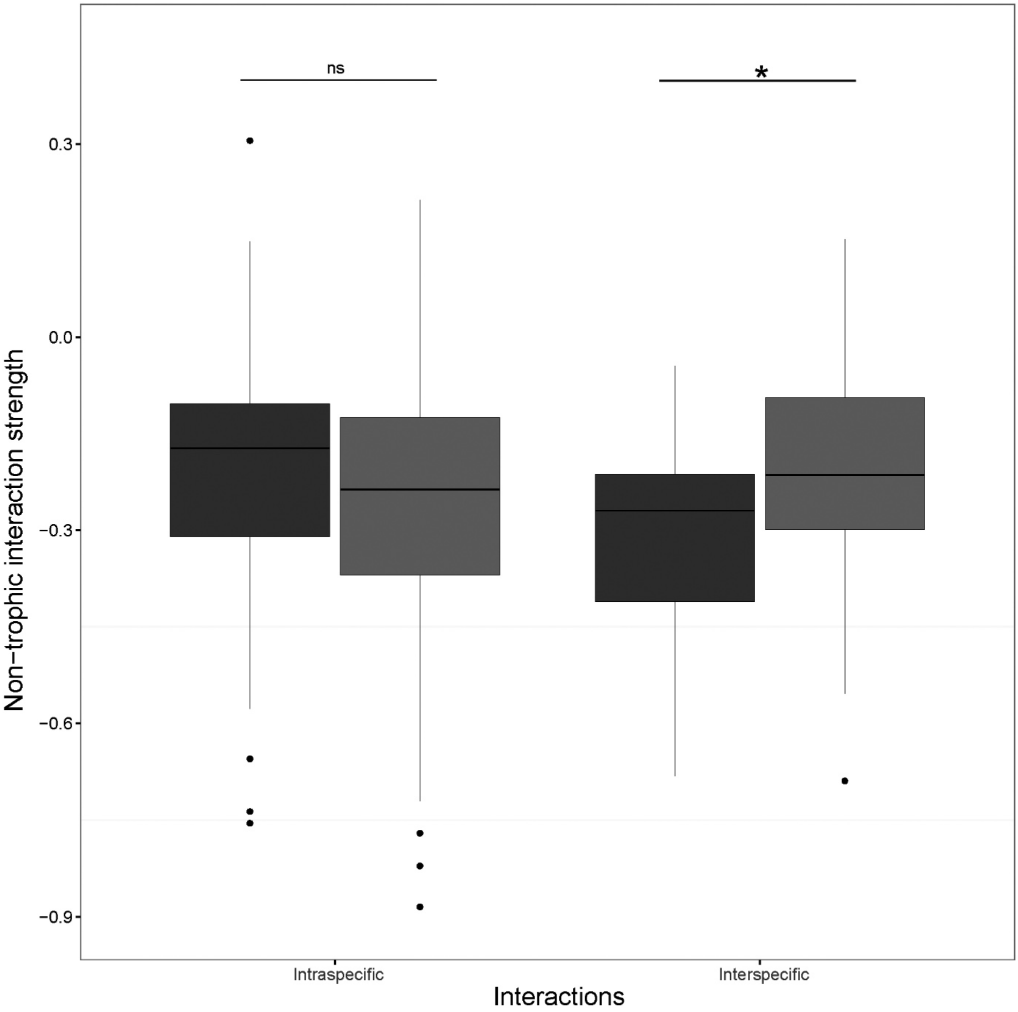

Figure S6. *Dependence of inter- and intraspecific non-trophic interaction strengths on temperature in the multiple predators experiment. Symbols as in Fig. S5 ('ns' = not significant). Data pooled across all densities.*

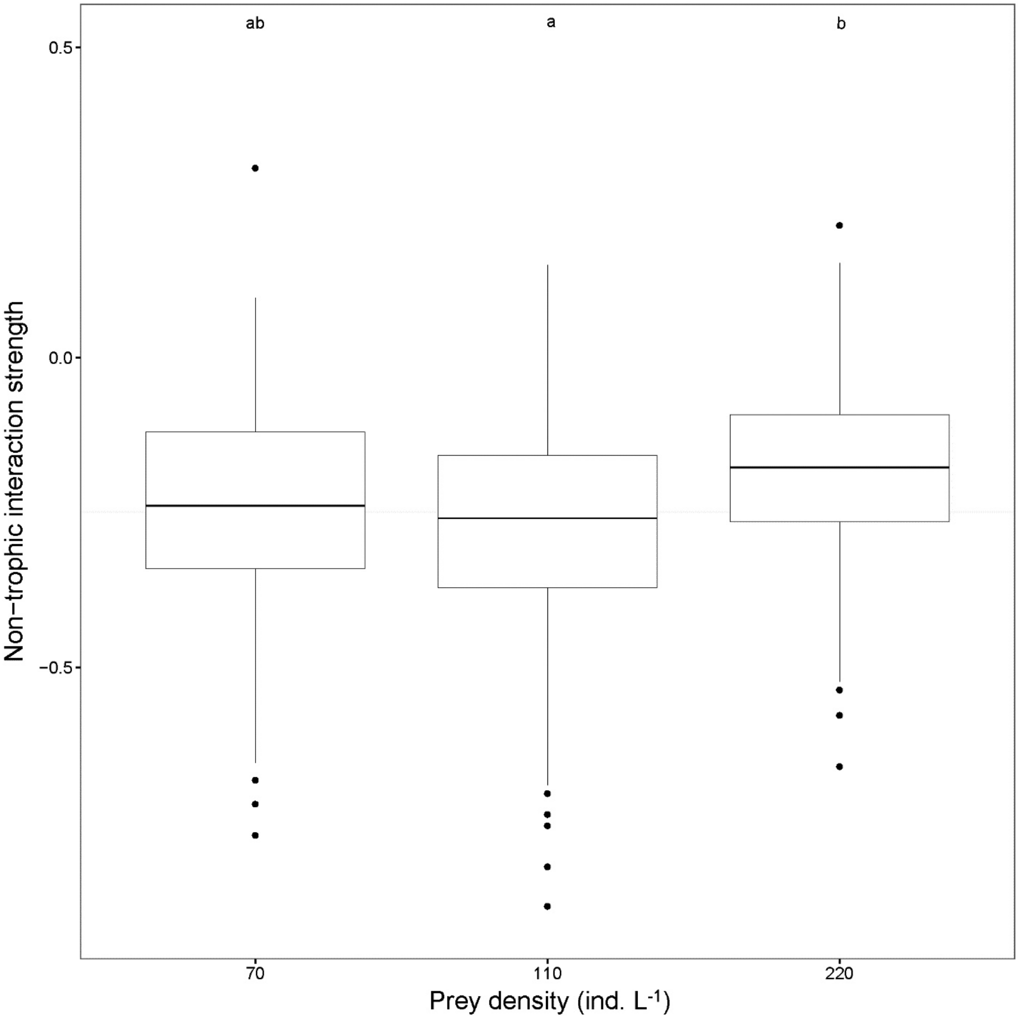

Figure S7. *Dependence of estimated non-trophic interaction strengths on prey density. Data pooled across predator assemblages and temperatures. Significant pairwise differences marked by different letters.*

CHAPTER 7

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

GENERAL DISCUSSION

Nowadays organisms face many environmental drivers and their multiplicative effects (Harley et al., 2006; Neves and Angermeier, 1990, 2013). Crayfish are prominent freshwater invertebrates, playing key roles in freshwater trophic webs and helping to maintain ecosystem functioning (Momot, 1995; Twardochleb et al., 2013). With human escalated environmental changes, crayfish populations are being influenced in various ways. The goal of this study was to assess crayfish as a model group of aquatic invertebrates for studying the effect of biotic and abiotic factors that are shifting in their magnitude (or are expected to do so in the future; e.g. temperature, droughts, nutrient enrichment, biotic interactions) or are unknown in the context – given mainly by the species intercontinental translocations that expose them to new challenges (e.g. assessing tolerance of non-North American crayfish to the crayfish plague pathogen; evaluating marbled crayfish salinity tolerance since the species is supposed to occur in Euro–Asian inland seas or at least in their surroundings in the future).

Propagule pressure, together with other biotic and abiotic factors, strongly influence population establishment in new ecosystems (Catford et al., 2009). In our first study lasting for 6.5 months (Chapter 2), we used four species of highly invasive warm-water crayfish that are common in the pet trade (Chucholl, 2013; Patoka et al., 2014). Specifically, we used the red swamp crayfish, marbled crayfish, yabby and redclaw *Cherax quadricarinatus*, evaluating their survival and foraging activity under conditions simulating winter temperatures in lentic waters of the temperate zone. We revealed low winter mortality in the red swamp crayfish. This result is supported by theoretical studies modelling the future distribution of this crayfish in Europe (Capinha et al., 2012; Larson and Olden, 2012), suggesting the European continent as a suitable place for its establishment. Their findings are in agreeance with recent dispersal of red swamp crayfish. Following their introduction to Spain in 1973 (Holdich, 2002), red swamp crayfish now occupy 16 European territories (sensu Holdich et al. (2009) centred in the southern and south-western Europe (Kouba et al., 2014; Weiperth et al., 2015). Indeed, the distribution of the species suggests a role of low winter temperatures. Red swamp crayfish successfully cope with low temperatures in southern Germany (Chucholl, 2011) and occur even in further localities of more continental parts of the Europe. However these locations often have elevated temperature regimes due to the presence of thermal springs (Petutschnig, 2008; Weiperth et al., 2015). With respect to winter survival it is necessary to mention that there are more factors determining population success. For example, the temperature during the whole season is also important, since low temperatures could reduce reproduction success in terms of egg development or cause insufficient vegetation growth for the survival of juveniles (Holdich and Crandall, 2002). Even so, confirmed survival of the species at 2.5 °C for three months during the coldest phase of the experiment is highly alarming, highlighting the issue of its potential spread to colder regions. Red swamp crayfish is a typical example of a highly invasive taxon impacting indigenous crayfish species as well as entire ecosystems (Souty-Grosset et al., 2016 and references therein).

Considering the native range and recent distribution of yabby in Europe, covering Spain and Italy (Kouba et al., 2014; Scalici et al., 2009), the discovery of its substantial resistance to low water temperatures was surprising (Chapter 2). Although winter mortality was higher compared to red swamp crayfish, the yabby still exhibited a good ability to survive. Considering our results, we expect that the establishment of new populations of yabby in the temperate zone of Europe is thus possible. Our results are in opposition to Capinha et al. (2012) and Scalici et al. (2009) who predict Italy and Spain are not suitable areas in terms of climatic conditions. Capinha and Anastácio (2011) argue that the mean annual temperature and mean temperature of the coldest quarter seems to be the best predictor of future distribution. Observed foraging of the red swamp crayfish and yabby even at the lowest temperature (2.5 °C), where at least one specimen fed, were also surprising. Croll and Watts (2004) studying the effect of temperature on feed consumption and nutrient absorption reported that the red swamp crayfish were generally lethargic at 8 °C and food consumption was minimal and remained low even at 14 °C. Winter food intake, likely occurring in both aforementioned species, might have adverse effects on local communities although its extent could be much reduced by low temperatures compared to foraging in the vegetation season. Though capable of withstanding a wide range of water temperatures, yabby does not grow at temperatures below 15 °C when it falls into a state of partial hibernation (Withnall, 2000). Finding this substantial resistance of yabby to low water temperatures opens an array of topics, including the assessment of its life history at both recently known and new localities, and assessing the impacts on European freshwater communities. Following aquaculture-mediated introductions out of its native mainly in Australia and Tasmania (Elvey et al., 1997), it poses a threat to endemic crayfish and occupied ecosystems (Coughran and Daly, 2012), thanks to its highly plastic life–history and environmental tolerance (Beatty et al., 2005). Thus, it might also become problematic in the European context.

Marbled crayfish suffered massive mortality with only a single specimen surviving experimental conditions. Unfortunately, its parthenogenetic nature makes the marbled crayfish are particularly problematic, since even a single specimen has an ability to establish a new population. As It is the only decapod that reproduces via obligatory apomictic parthenogenesis, producing genetically uniform offspring (Martin et al., 2007; Scholtz et al., 2003). Marbled crayfish are characterised by their early maturation (Seitz et al., 2005), ability to reproduce throughout the whole year under favourable conditions (Seitz et al., 2005; Vogt and Tolley, 2004), and their high competitiveness for food and shelters (Jimenez and Faulkes, 2011). This taxon was first discovered in the German aquarium trade in the mid-1990s, from where it further dispersed (Scholtz et al., 2003). At the beginning of the new millennium, reports began emerging on the occurrence of single specimens in the wild, followed by confirmation of established populations in Germany and Slovakia in 2010 (Chucholl and Pfeiffer, 2010; Janský and Mutkovič, 2010). Although the establishment success in some countries is not clear, it further appeared in Croatia, the Czech Republic, Hungary, Italy, the Netherlands, Sweden, and Ukraine (Bohman et al., 2013; Kotovska et al., 2016; Lőkkös et al., 2016; Marzano et al., 2009; Novitsky and Son, 2016; Patoka et al., 2016; Samardžić et al., 2014; Soes and Koese, 2010; Vojkovská et al., 2014; Weiperth et al., 2015). Non-European destinations are represented by Madagscar (Jones et al., 2009) and Japan (Kawai et al., 2016). Since it is the most common pet traded species available in North America, it might further spread out of its presumed native range in Florida and Georgia (cf. with the range of the closest relative slough crayfish *P. fallax*; Taylor et al. (2007)). Its competitive pressure might have devastating consequences for many crayfish counterparts in this biodiversity hotspot (Crandall and Buhay, 2008). The ability of marbled crayfish to carry the crayfish plague pathogen, which is particularly problematic for crayfish not originating from North America, has been confirmed both in the aquarium trade (Mrugała et al., 2015) and in the field (Keller et al., 2014). Our confirmation of an ability to overwinter during temperate winters is particularly alarming and is in line with the distribution mentioned above. On the contrary, the redclaw does not represent a threat to European temperate aquatic ecosystems at least in the near future which is in accordance with the distribution model of Larson and Olden (2012).

Long-term drought could devastate freshwater fauna (Carpenter et al., 1992; Erwin, 2009). In order to remain in contact with water or stay in a sufficiently humid environment in dry conditions, the ability to withstand desiccation by dwelling in the hyporheic zone, particularly through vertical burrowing, is crucial (Bond et al., 2008; Collas et al., 2014; Poznańska et al., 2015). We evaluated the ability of three European indigenous crayfish species: noble crayfish, narrow-clawed crayfish *Astacus leptodactylus*, stone crayfish *Austropotamobius torrentium* and five non-indigenous crayfish species currently present in Europe: red swamp crayfish, marbled crayfish, signal crayfish, spiny-cheek crayfish and yabby, to survive and construct vertical burrows in a humid sandy-clay substrate under a simulated one-week drought (Chapter 3). A certain level of burrowing activity can be found among crayfish species (Berrill and Chenoweth, 1982; Harvey et al., 2014; Horwitz and Richardson, 1986), however, studies focussing on this life history trait usually do not distinguish the direction in which the burrows are constructed. Also a simple excavation under objects (e.g. stones and logs and in clay banks) is considered a form of burrowing. We argue that burrowing in vertical direction is a key component in crayfish survival under severe long-term droughts, which is common in crayfish specialists e.g. primary and secondary burrowers (Hobbs, 1972, 1942). Considering desiccation resistance and burrowing abilities, the red swamp crayfish was the most tolerant species in our experiment. Moreover, only the red swamp crayfish close their burrow entrance with a mud plug (Barbaresi and Gherardi, 2000; Correia and Ferreira, 1995). In addition, females created deeper burrows than males. Red swamp crayfish females use burrows for egg incubation. Females with eggs are not usually submerged in the ground water due to low availability of dissolved oxygen, with oxygen diffusing directly from the burrow atmosphere while eggs are fanned by swimmeret movements (Huner et al., 1991). Thus, deeper burrows constructed by females in our experiment suggest the possible importance of having better access to the water.

Other species in this study exhibit a combined strategy in terms of increasing desiccation capacity (yabby, marbled crayfish and signal crayfish) and burrowing (marbled crayfish and to a lesser extent also yabby and spiny-cheek crayfish). All of our studied indigenous species had the lowest success in terms of both desiccation and burrowing. We consider desiccation capacity and burrowing activity as a crucial trait, whose importance will increase with ongoing and predicted weather fluctuations (Sutton and Hodson, 2005; Underwood, 2015). Furthermore, relevant information comparing crayfish burrowing activity is rare (Magoulick and Kobza, 2003; Richman et al., 2015), which adds a novel component to our study.

Eastern Europe comprises the entire, or at least significant parts of, the native range for indigenous crayfish species of the genus *Astacus*, particularly for the thick-clawed crayfish *Astacus pachypus* (Kouba et al., 2014). This region has been largely overlooked by astacologists and considered relatively safe from the adverse impacts of gradually expanding non-indigenous crayfish species (Holdich et al., 2009; Perdikaris et al., 2012). The discovery of two distant marbled crayfish populations in Dnepropetrovsk and Odessa, Ukraine in 2015 drastically changed this assumption (Novitsky and Son, 2016). Pet trade surveys provided extended lists on NICS, often of North American origin, both in the Ukraine (Kotovska et al., 2016) and Lower Volga region, Russian Federation (Vodovsky et al., 2017). This raised concerns about non-indigenous crayfish species as these might potentially negatively impair ecosystems of Azov, Black and Caspian Seas as well as their tributaries. These are inhabited by indigenous crayfish species and as a whole are unique ecosystems. Therefore, our study goal (Chapter 4) was to investigated survival, growth, and reproduction of marbled crayfish in a range of relevant salinities. This information is important for the implementation of potential management measures regarding the spread of this species in Eastern Europe, but also in estuaries elsewhere.

Salinity can be considered as a physiological barrier for freshwater organisms. However, certain animals, typically those with close marine ancestors, have the ability to withstand saline conditions (Croghan, 1976), though the time spent in the saline environment is limited (Anger, 2003; Holdich et al., 1997). We provide the first insight into salinity tolerance of the potentially very problematic marbled crayfish. We performed a 155-day experiment using marbled crayfish exposed to five different salinities (6, 9, 12, 15, 18 ppt) and a freshwater control, evaluating its survival, growth and reproduction (Chapter 4). Most crayfish species are able to survive in saline environments from a few days to a few months, while the effects of salinity on physiological processes differ among crayfish species and families (Alcorlo et al., 2008; Holdich et al., 1997; Jones, 1989). According to the available literature, at least some crayfish are able to reproduce under environmental conditions of elevated salinity. Besides salinity level, the ability of crayfish to reproduce depends on salinity fluctuation in a given ecosystem (Anson and Rouse, 1994; Holdich et al., 1997; Jones, 1989). Compared to these cases, marbled crayfish exhibited lower survival, growth and no reproduction even in the lowest salinity (6 ppt) tested. Furthermore, increasing salinity contributed to high direct mortality often apparent during moulting, probably due to osmotic stress and the imbalance of ion composition (Bissattini et al., 2015; Wheatly and Gannon, 1995). The closest known relative of the marbled crayfish, the slough crayfish and the Everglades crayfish *Procambarus alleni* sometimes live in sympatry in freshwaters (Hendrix and Loftus, 2000; Martin et al., 2010), but it seems that salinity is an important factor separating these two species in brackish conditions (Hendrix and Loftus, 2000). Everglades crayfish can inhabit saline environments in a range of 0–18 ppt (Hendrix and Loftus, 2000), but we are not aware of any study evaluating salinity tolerance in slough crayfish. This is in line with outputs of our study on marbled crayfish. Nevertheless, the ability to withstand saline environments for more than 80 days suggests that the species might inhabit watersheds in the vicinity and gradually adapt to more saline conditions using brackish waters as a biological corridor. This might promote its spread to coastal areas and estuaries and thus enable colonisation of different river basins. Also, different water compositions could either reduce or enhance physiological or survival conditions of marbled crayfish, depending on pH, and the composition of cations and anions (Bailey and James, 2000; Frey, 1993). Our study highlights the need for further studies since salinity stress and salinity fluctuations may be amplified by other environmental conditions such as temperature, oxygen, and pH (Gilles and Pequeux, 1983).

Non-indigenous organisms bring not only themselves but also their illnesses or parasites to new ecosystems, which can have negative consequences for indigenous species (Linz et al., 2007; Perrault et al., 2003). Crayfish plague is one of the worst threats to crayfish not originating from North America, as it is prominent primarily in declining populations of European astacids (Unestam and Weiss, 1970). The causative agent in the crayfish plague, *A. astaci*, is listed as among the worst invasive species in Europe as well as globally (Hulme et al., 2010; Lowe et al., 2000). Following confirmation of yabby resistance to low winter temperatures, suggesting its potential to settle in Europe (Chapter 2), we investigated its resistance toward three different genotypes (representing genotype groups A, B and E, see Svoboda et al. (2017) for details) of crayfish plague present in Central European waters (Chapter 5). We included highly susceptible, similarly aged noble crayfish in the experimental design to enable a comparison. We exposed both species to two different dosses of zoospores (10 and 100 spores ml^{-1}). This is the first study comparing virulence of the more known *A. astaci* genotypes on yabby. We confirmed the elevated resistance of yabby to crayfish plague in comparison with noble crayfish where all individuals died in all treatments during the experiment, reflecting the concentration of the initial *A. astaci* inoculum. While in genotype A survival among yabby individuals was observed after exposure, total mortality was noted after infection with the two more virulent strains (B and E). Depending on the pathogen virulence, this may lead to chronic infection status or delayed mortalities in yabby populations. Thus it is possible that yabby might, under certain conditions, contribute to crayfish plague spread in Europe (Mrugała et al., 2016). Together, the overwintering abilities (Veselý et al., 2015), burrowing activity (Kouba et al., 2016) and ability to succeed in interactions with other non-indigenous crayfish species already present in Europe (Fořt et al., 2015), paints yabby as a species having the potential to negatively influence indigenous astacofauna and freshwater ecosystems.

Stability and functioning of ecosystems is affected by global changes which can be difficult to predict (Hooper et al., 2005; Tilman et al., 1997). Nowadays, we still have limited information how multiple environmental drivers may influence organisms and their interactions in complex systems involving multiple predator species (Sentis et al., 2016). We combined an experimental and modelling approach to investigate temperature, and resource availability on the strength of trophic and non-trophic interactions in multiple predator communities (Chapter 6). We used marbled crayfish at two size classes and larvae of dragonfly *Aeshna cyanea* as a model predator organism. We used a full-factorial design to test intra and interspecific predator combinations. Common carp *Cyprinus carpio* larvae were used as prey. Temperature influences the speed of biochemical reactions and thus drives biological rates and interactions (Brown et al., 2004). We found that warming increased the search rate of all three predators, while the handling time decreased only in the dragonfly larvae. Our results are consistent with a previous meta-analysis of Rall et al. (2012). Interaction strengths are not randomly distributed: food webs are often characterized by many weak and a few strong consumer-resource interactions (Berlow et al., 2004). This pattern is thought to increase food-web stability and prevent species invasions, population oscillations and other disturbances affecting ecosystem stability and functioning (Berlow et al., 2004; Kokkoris et al., 1999). On longer timescales, stronger interactions can lead to amplified population oscillations and population extinctions (Binzer et al., 2012). In terms of non-trophic interaction strength, the interference among predators should dampen population oscillations and stabilize food webs (Brose et al., 2006; Rall et al., 2008) due to weaker trophic interactions (Sentis et al., 2016). Though it is generally known how warming and enrichment effects could affect interaction strength, we have still limited insight into these processes. Furthermore, the effects of these drivers on non-trophic interaction strength are virtually unknown (Arditi et al., 2004; Kéfi et al., 2012; Rall et al., 2008; Sentis et al., 2016). Our results suggest that the effects of prey density on trophic and non-trophic interaction strengths is always independent of predator identity, interaction type and temperature. This suggests that prey density acts as an independent factor in shaping species interactions. The effects of temperature on both trophic and non-trophic interactions always depends on predator identity or interaction type which reveals complex effects of this abiotic factor. The effects of temperature on trophic and non-trophic interactions is weaker in intraspecific predator assemblages compared to interspecific predator assemblages. This suggests that species-rich communities might be more sensitive to climate change than species-poor communities, which could accelerate the detrimental effects of climate change on biodiversity. Statistical analyses showed that trophic and non-trophic interactions are influenced by the same environmental factors and their interactions (i.e. the fixed effects of the most parsimonious models were the same for trophic and non-trophic interactions). Nevertheless, temperature tends to increase both trophic and non-trophic interaction strength (although it effects depended on interaction type) whereas the effects of prey density were different for trophic and non-trophic interactions. Trophic interaction strength decreased with prey density whereas non-trophic interaction had a unimodal relationship with prey density. Sentis et al., (2016) found a similar result in a different set of species which suggest that these effects of prey density could be general. Nevertheless, future studies are needed to confirm the generality of such a pattern. Overall, our results highlight the need for further studies required to elucidate the mechanisms linking individual properties and environmental drivers to interaction strengths in freshwater food webs.

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ENGLISH SUMMARY

Crayfish in changing biotic and abiotic conditions

Abiotic and biotic factors directly or indirectly influence physiology, behaviour, ecology and the distribution of organisms. Particularly in todays fluctuating and fast changing environmental conditions, organisms are facing new challenges resulting from abiotic and biotic factors varying in their magnitudes and/or when appearing in new contexts. The latter appear to be related to human activities, e.g. intercontinental introductions of species, which results in previously unthinkable situations. As a consequence, we are dealing with new issues like: How warm water organisms from one continent will cope with cold climates of others, or what will be the impact of a novel translocated pathogen on potentially susceptible hosts present there. Crayfish play a central role in aquatic ecosystems, making them a perfect model for studying the effects of changing abiotic and biotic factors on aquatic biota. This thesis provides unique results in terms of the ability of crayfish to cope with a series of these abiotic and biotic factors. We believe these results are of interest for a broad range of potential readers including those involved in biological invasions and conservation management, providing baselines for further studies and opening new questions.

Successful overwintering is key factor facilitating establishment and spread of non-indigenous species. We provide experimental evidence of substantial resistance of three out of four warm water crayfish species to the low water temperatures tested in our study. To sum up, red swamp crayfish together with yabby demonstrated high survival in experimental conditions and exhibited feeding activity even during the coldest part of the experiment. Although suffering substantial mortality, a single specimen of marbled crayfish survived. Even this finding is highly alarming considering its reproduction mode (obligatory apomictic parthenogenesis), especially when combined with other life history traits. Considering the native range and recent distribution of the Australian yabby in Europe, our results highlight the need for closer consideration of the environmental plasticity of this species, while redclaw is expected not to pose a risk for the European temperate zone at least in the near future. It should be noted that the mentioned species have been broadly available in pet markets of many European countries, opening an issue of animal escapes or deliberate introductions. The future will show us whether recent legislation measures applied at the EU level will help save the situation.

Drought is an important factor for freshwater organisms determining their success in a given ecosystem. Considering their survival, organisms must cope with this situation in different ways. Among these strategies are an elevated desiccation capacity and burrowing ability. We evaluated the ability of three European native and five non-native crayfish to survive and burrow in a humid sandy-clayey substrate under a simulated one-week drought. Survival of non-native species was substantially higher, being absolute in the red swamp crayfish and yabby. Red swamp crayfish and marbled crayfish constructed bigger and deeper burrows than yabby and spiny-cheek crayfish. Three native species (noble crayfish, narrow-clawed crayfish and stone crayfish) suffered extensive mortalities. The native species and signal crayfish exhibited no ability to burrow under drought conditions. In the context of predicted weather fluctuations, the ability to withstand desiccation through constructing vertical burrows into the hyporheic zone under drought conditions might play a significant role in the success of particular crayfish species, as well as a wide range of further hyporheic-dwelling aquatic organisms in general.

Salinity is an important abiotic factor responsible for key processes in aquatic animals. Marbled crayfish are a successful emerging invader with high ecological plasticity, capable of

colonization new habitats not only in Europe. It is also established in the wild of Ukraine and widely pet-traded in Eastern Europe. If released, it might pose a risk to populations of native crayfish that also inhabit saline ecosystems of the Black, Azov and Caspian Sea. In recognition of a gap in our knowledge, we conducted the first study on salinity tolerance in marbled crayfish. In summary, marbled crayfish are probably unable to invade saline ecosystems due to its low survival, reduced growth and inhibited reproduction. Nevertheless, it showed an ability to withstand even the highest salinity tested (18‰) for an extended period of time, opening a possibility for its spread to other freshwaters via this corridor. Also, a short generation time might lead to local adaptations to saline environments in the future.

Yabby generally demonstrated an ability to withstand simulated low winter temperatures of Central Europe winter, opening the question of its potential establishment in the region. To successfully do so, yabby would have to deal with present strains (A, B, and E) of *Aphanomyces astaci*, a causative agent of crayfish plague, a devastating disease to all crayfish not originating from North America. Our comparative study revealed the ability to survive (strain A) or delay the effects (strains B and E) of crayfish plague compared to native noble crayfish. Our result, together with the environmental plasticity of yabby, highlights a high potential for its establishment in temperate Europe. As a prominent invader, it may cause negative impacts on entire ecosystems and contribute to the spread of crayfish plague in Europe.

Nowadays, organisms face multiple global drivers influencing the strength of links in food webs. Altogether, we found that predator identity, prey density, and temperature all affect trophic and non-trophic interaction strengths. Effects of prey density were always independent of temperature and predator identity in trophic as well as non-trophic interaction strength. On the other hand, the magnitude of responses in trophic as well as non-trophic interaction strengths varied strongly across predator pairs and temperatures. Based on the current evidence, we could not find strong support for a general mechanism underlying the dependence of interaction strengths on species traits and environmental conditions. Therefore, other studies dealing with the effects of global drivers on trophic as well as non-trophic interaction strengths are needed.

CZECH SUMMARY

Raci v měnících se biotických a abiotických podmínkách

Abiotické a biotické faktory přímo či nepřímo ovlivňují fyziologii, chování, ekologii a distribuci organizmů. Fluktuace, rychlé změny a rozsah abiotických a biotických faktorů jsou výzvou, jíž musí zvláště v dnešní době organizmy čelit. Biotické faktory mohou být zjevně spojeny s lidskou činností. Jako příklad lze uvést mezikontinentální introdukce druhů, což nezřídka vede k nečekaným situacím. Jako důsledek těchto introdukcí musíme přemýšlet nad otázkami, jak některé teplovodní druhy organizmů budou schopny čelit chladnějšímu klimatu či jaký vliv bude mít nově přenesený patogen na potenciální hostitele vyskytující se v oblasti introdukce. Raci hrají klíčovou roli ve sladkovodních ekosystémech. Tento fakt z nich dělá vhodný model pro studium vlivu měnících se abiotických a biotických podmínek na vodní biotu. Tato práce poskytuje unikátní výsledky ve smyslu schopnosti raků odolávat či se adaptovat na dané abiotické a biotické podmínky prostředí. Věříme, že výsledky této práce budou zajímavé pro širokou čtenářskou obec, zahrnující i ty, kteří se zabývají biologickými invazemi a ochranou přírody. Tato práce navíc otevírá nové otázky a může posloužit jako startovací bod pro budoucí studie.

Úspěšné přezimování je klíčový faktor usnadňující uchycení a šíření nepůvodních druhů. Naše výsledky poskytují důkaz o značné odolnosti vůči nízké teplotě u třech ze čtyř druhů nepůvodních druhů raků testovaných v našem experimentu. Celkově vzato, rak červený společně s australským rakem *Cherax destructor* vykazovali vysokou míru přežití v experimentálních podmínkách, stejně jako schopnost konzumovat potravu i v nejchladnější fázi experimentu. Ačkoliv rak mramorovaný trpěl vysokou mortalitou v průběhu experimentu, jeden jedinec přežil. Tento výsledek je díky jeho reprodukční strategii (apomiktická partenogeneze) v kombinaci s dalšími znaky jeho životní strategie alarmující. S ohledem na původní areál raka *C. destructor* a jeho současný výskyt v Evropě se jeví námi pozorované přezimování jako klíčové, zdůrazňující potřebu bližšího poznání skutečné plasticity tohoto druhu. Na druhou stranu, druhý testovaný australský rak *C. quadricarinatus* nebude v nejbližší budoucnosti představovat riziko pro temperátní zónu Evropy. V neposlední řadě je nutné zmínit, že vyjmenované druhy jsou snadno dostupné v sítích akvaristických obchodů v řadě evropských zemí, což otevírá otázku problému úniku či úmyslného vypuštění do volných vod. Pouze budoucnost ukáže, jestli přijímané legislativní změny v rámci Evropské Unie dokážou těmto situacím zabránit.

Sucho je významný faktor pro sladkovodní organizmy, determinující jejich úspěšné přežití v daném ekosystému. Sladkovodní organizmy se musí se suchem vypořádat s pomocí několika možných strategií. Jednou z nich je zvýšená odolnost proti vysychání a schopnost norovat. V dalším z experimentů jsme porovnávali schopnosti přežít a norovat v písčito-jílovém substrátu u třech původních a pěti nepůvodních druhů raků, vystavených týdennímu simulovanému suchu. Přežití u nepůvodních druhů bylo vyšší ve srovnání s původními druhy, výrazně zejména ve srovnání s rakem červeným a *C. destructor*. Rak červený a mramorovaný tvořili větší a hlubší nory než *C. destructor* a rak pruhovaný. Tři původní druhy (rak říční, rak bahenní a rak kamenáč) charakterizovala vysoká mortalita. Původní druhy a rak signální neprokázali schopnost tvořit vertikální nory během simulovaného sucha. V kontextu predikce fluktuace klimatických podmínek na Zemi může být schopnost přečkat vysychání díky schopnosti vytvářet vertikální nory zasahující do hyporheické zóny považována za klíčovou vlastnost determinující přežití v dané lokalitě jak pro raky, tak i pro ostatní biotu schopnou tvořit nory a pronikat do hyporheické zóny.

Salinita je významný abiotický faktor u vodních organizmů zodpovědný za klíčové pochody. Rak mramorovaný je velice úspěšný invazivní druh s vysokou ekologickou plasticitou, schopný kolonizovat nové ekosystémy nejen v Evropě. Tento druh se úspěšně usadil na Ukrajině s tím, že ve východní Evropě je hojně obchodovaný akvaristický druh. Pokud by se tento druh dále rozšířil, mohl by představovat riziko pro původní druhy raků, kteří jsou také schopni obývat slanovodní ekosystémy Černého, Azovského a Kaspického moře. Provedli jsme studii zabývající se tolerancí raka mramorovaného vůči zvýšené salinitě vody, neboť tato nebyla u zmiňovaného druhu prozatím zjišťována. Celkově vzato rak mramorovaný pravděpodobně není schopen kolonizovat ekosystémy se zvýšenou salinitou díky jeho nízkému přežívání, růstu a neschopnosti se rozmnožit ve slané vodě. Tato studie nicméně prokázala jeho schopnost vydržet i největší testovanou salinitu (18‰) po značnou dobu. Tento výsledek otevírá možnost využití ekosystémů se zvýšenou salinitou jako biokoridoru pro šíření do okolních sladkovodních ekosystémů. Také krátká generační doba tohoto druhu možná povede k jeho budoucím lokální adaptaci na podmínky se zvýšenou salinitou.

Zjištění, že rak *C. destructor* je schopný přečkat simulované zimní podmínky střední Evropy otevírá zásadní otázku, zdali je tento druh schopný založit vitální populaci v této části světa. Pokud by se tak mělo stát, musel by si *C. destructor* poradit s račím morem *Aphanomyces astaci*, konkrétně s kmeny A, B a E. Račí mor je patrně nejhorší račí nemoc postihující veškeré druhy raků, které nepochází ze Severní Ameriky. Ve srovnání s rakem říčním naše studie odhalila schopnost druhu *C. destructor* přežít (kmen A) nebo zpomalit působení této nemoci (kmeny B a E) račího moru. Toto zjištění společně s environmentální plasticitou druhu zdůrazňují jeho vysoký potenciál k založení populací v temperátní zóně Evropy. *Cherax destructor* by mohl mít jako významný invazivní druh negativní vliv na celý ekosystém, a navíc by mohl jako chronický přenašeč přispět k dalšímu šíření račího moru.

V současné době čelí organizmy vícenásobnému vlivu globálních hybatelů ovlivňujících sílu spojení mezi jednotlivými skupinami v potravním řetězci. Celkově vzato, naše poslední studie odhalila, že identita predátora, hustota kořisti a teplota ovlivňují sílu jak trofických, tak i netrofických interakcí v ekosystému. Efekt hustoty kořisti byl vždy nezávislý na teplotě i na identitě predátora u trofických i netrofických interakcí. Naopak síla odezvy u trofických i netrofických interakcí silně závisela na identitě predátora a teplotě. Na základě současných studií postrádáme dostatek důkazů pro podporu obecného mechanizmu podtrhující závislost síly interakce na vlastnostech organizmů a podmínkách prostředí. Proto jsou nezbytné další studie, které se zabývají efektem globálních hybatelů na tropické a netrofické interakce.

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LIST OF PUBLICATION

Peer reviewed journals and their IF

Veselý, L, Hrbek, V, Kozák, P, Buřič, M, Sousa, R, Kouba, A., 2017. Salinity tolerance of marbled crayfish *Procambarus fallax* f. *virginalis*. Knowl. Manag. Aquat. Ecosyst. 418, 21. (IF 2016 = 1.217)

Buřič, M., Fořt, M., Bláha, **M., Veselý, L.**, Kouba, A., 2016. Crayfish bury their own exuviae – a newly discovered behavioral pattern in decapods. SpringerPlus 5, 1674. (IF 2015 = 0.982)

Kouba, A., Tíkal, J., Císař, P., **Veselý, L.**, Fořt, M., Příborský, J., Patoka, J., Buřič, M., 2016. The significance of droughts for hyporheic dwellers: evidence from freshwater crayfish. Sci. Rep. 6, 26569. (IF 2014 = 5.228)

Mrugała, A., **Veselý, L.**, Petrusek, A., Viljamaa-Dirks, S., Kouba, A., 2016. May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe? Aquat. Invasions 11, 459–468. (IF 2015 = 1.955)

Veselý, L., Buřič, M., Kouba, A., 2015. Hardy exotics species in temperate zone: Can „warm water" crayfish invaders establish regardless of low temperatures? Sci. Rep. 5, 16340. (IF 2014 = 5.578)

International conferences

Veselý, L., Boukal, D., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., Prchal, M., Kozák, P., Kouba, A., Sentis, A., 2017. Temperature, prey availability and predator diversity jointly influence surplus killing in a freshwater food web. In: 5th meeting of Fresh Blood for FreshWater. 9–13 April, 2017, České Budějovice, Czech Republic.

Mrugała, A., Kozubíková-Balcarová, E., Chucholl, C., Kawaii, T., Kouba, A., Svoboda, J., **Veselý, L.**, Viljamaa-Dirks, S., Petrusek, A., 2016. The crayfish plague pathogen, *Aphanomyces astaci*: pathways, vectors and potential consequences of its spread in the introduced ranges. In: 21st Symposium of International Association of Astacology (IAA 21), 5–8 September, 2016, Madrid, Spain, p. 20.

Veselý, L., Bláha, M., Buřič, M., Fořt, M., Pešek, V., Kozák, P., Ruokonen, T.J., Ercoli, F., Kouba, A., 2016. Crayfish role in a canyon-shaped reservoir: case study from the Nýrsko, Czech Republic. In: 21st Symposium of International Association of Astacology (IAA 21), 5–8 September, 2016, Madrid, Spain, p. 28.

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Fořt, M., Kouba, A., Buřič, M., **Veselý, L.**, Kozák, P., 2015. Aggressive interspecific interaction in non-native crayfish species. In: Abstract volume, European Crayfish conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 62.

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Veselý, L., Sentis, A., Kuklina, I., Buřič, M., Fořt, M., Yazicioglu, B., Prchal, M., Boukal, D., Kouba, A., 2015 Effect of temperature and nutrient enrichment on prey-predator complex system. In: Abstract volume, European Crayfish Conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 17.

Veselý, L., Kouba, A., 2014. Surviving ornamental crayfish in Mid-European winter. In: Abstract volume, 8th International Crustacean Congress (ICC-8). 18–23 August, 2014, Frankfurt am Main, Germany, p. 216.

TRAINING AND SUPERVISION PLAN DURING STUDY

Name	Lukáš Veselý
Research department	2013–2017 – Laboratory of Ethology of Fish and Crayfish of FFPW
Supervisor	Antonín Kouba, Ph.D.
Period	30th October 2013 until 14th September 2017

Ph.D. courses	**Year**
Basic of scientific communication	2014
Applied hydrobiology	2014
Biostatistics	2014
Modelling for ecologists in R	2014
Pond aquaculture	2015
Ichthyology and fish systematics	2015
English language	2017

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2014 2015 2016 2017

International conferences	**Year**
Veselý, L., Boukal, D., Buřič, M., Kuklina, I., Fořt, M., Yazicioglu, B., Prchal, M., Kozák, P., Kouba, A., Sentis, A., 2017. Temperature, prey availability and predator diversity jointly influence surplus killing in a freshwater food web. In: 5th meeting of Fresh Blood for FreshWater. 9–13 April, 2017, České Budějovice, Czech Republic.	2017
Mrugała, A., Kozubíková-Balcarová, E., Chucholl, C., Kawaii, T., Kouba, A., Svoboda, J., **Veselý, L.**, Viljamaa-Dirks, S., Petrusek, A., 2016. The crayfish plague pathogen, *Aphanomyces astaci*: pathways, vectors and potential consequences of its spread in the introduced ranges. In: 21st Symposium of International Association of Astacology (IAA 21), 5–8 September, 2016, Madrid, Spain, p. 20.	2016
Veselý, L., Bláha, M., Buřič, M., Fořt, M., Pešek, V., Kozák, P., Ruokonen, T.J., Ercoli, F., Kouba, A., 2016. Crayfish role in a canyon-shaped reservoir: case study from the Nýrsko, Czech Republic. In: 21st Symposium of International Association of Astacology (IAA 21), 5–8 September, 2016, Madrid, Spain, p. 28.	2016
Buřič, M., **Veselý, L.**, Kouba, A., 2015. Molting and growth of adult signal crayfish *Pacifastacus leniusculus* (Dana 1852): Effective investment due to seasonal morphological changes? In: Abstract volume, European Crayfish conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 61.	2015
Fořt, M., Kouba, A., Buřič, M., **Veselý, L.**, Kozák, P., 2015. Aggressive interspecific interaction in non-native crayfish species. In: Abstract volume, European Crayfish Conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 62.	2015
Kouba, A., Tíkal, J., Císař, P., **Veselý, L.**, Fořt, M., Příborský, J., Buřič, M., 2015. The impact of simulated drought on vertical burrowing activity in eight crayfish species. In: Abstract volume, European Crayfish Conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 15.	2015
Mrugala, A., **Veselý, L.**, Petrusek, A., Kouba, A., 2015. Will low winter temperatures and *Aphanomyces astaci* limit *Cherax destructor* survival in central Europe? In: Abstract volume, European Crayfish Conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 25.	2015

Veselý, L., Sentis, A., Kuklina, I., Buřič, M., Fořt, M., Yazicioglu, B., Prchal, M., Boukal, D., Kouba, A., 2015 Effect of temperature and nutrient enrichment on prey-predator complex system. In: Abstract volume, European Crayfish Conference: Research and Management, 9–12 April, 2015, Landau, Germany, p. 17.	2015
Veselý, L., Kouba, A., 2014. Surviving ornamental crayfish in Mid-European winter. In: Abstract volume, 8th International Crustacean Congress (ICC-8). 18–23 August, 2014, Frankfurt am Main, Germany, p. 216.	2014

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Dr. Fabio Ercoli, University of Jyväskylä, Finland (1 month, Learning stable isotope analysis and data processing).	2015
Dr. Fabio Ercoli, University of Jyväskylä, Finland (2 month, Learning advance techniques of stable isotope analysis and data processing).	2015
Dr. Timo J. Ruokonen, University of Jyväskylä, Finland (2 month, Analysing and processing my own samples, discussion about new project).	2016

Pedagogical activities	**Year**
Leading of project entitled Catch me if you can: problematic trapping of *Orconectus limosus* at Summer school	2016
Announcing the project entitled Using different fractionalization factors for crayfish and their application for trophic webs modelling at Summer school	2017
Lecturing of students of bachelor study, discipline Fishery at USB FFPW in a range of 40 teaching hours	2014–2016
Training of two Ph.D in basics of R Language in a range of 50 teaching hours	2014–2016

CURRICULUM VITAE

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RESEARCH INTEREST

- Global changes, stable isotopes, trophic interactions, freshwater ecology, crayfish
- Biotic and abiotic factors affecting organisms
- Mesocosmos as well as broad scale ecosystem studies

EDUCATION

2013 – present	Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic
2010 – 2013	Dipl.-Ing. in Fishery, University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Water, Fisheries
2010 – 2014	M.Sc., in Ecology University of South Bohemia in České Budějovice, Faculty of Science, Department of Ecosystem Biology
2006 – 2010	B.Sc., in Biology, University of South Bohemia in České Budějovice, Faculty of Science, Department of Ecosystem Biology

Completed courses

Modelling for ecologists in R, Biostatistics, Applied hydrobiology, Pond aquaculture, Ichthyology and fish systematics, Basics of scientific communication

Training

14. 4. – 20 . 4. 2014	Stable isotopes in biology and ecology

RESEARCH STAY AND COLABORATION

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1. 10. – 30. 11. 2015	Dr. Fabio Ercoli, University of Jyväskylä, Finland
2. 10. – 30. 11. 2016	Dr. Timo J. Ruokonen, University of Jyväskylä, Finland