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NEW ALIEN CRAYFISH SPECIES IN CENTRAL EUROPE

Introduction pathways, life histories, and ecological impacts



DISSERTATION

zur Erlangung des Doktorgrades *Dr. rer. nat.*
der Fakultät für Naturwissenschaften der Universität Ulm

vorgelegt von

Christoph Chucholl

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Cover picture: *Orconectes immunis* male (blue color morph) (photo courtesy of Dr. H. Bellmann)

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Part 2 – Publications and manuscripts

Chapter 1: The pet trade as a pathway for alien crayfish introductions into Central Europe

- Chucholl, C. (in press). Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. *Biol. Inv.*..... 59
- Chucholl, C. *et al.* (in press). The clones are coming – strong increase in Marmorcrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. *Aquat. Invas.* 93

Chapter 2: Understanding invasion success: life-history traits of New alien crayfish in Central Europe

- Chucholl, C. (2011a). Population ecology of an alien “warm water” crayfish (*Procambarus clarkii*) in a new cold habitat. *Knowl. Managt. Aquatic Ecosyst.* 401: art. no. 29. 107
- Chucholl, C. (2012). Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae). *Knowl. Managt. Aquatic Ecosyst.* 404: art. no. 04. 131

Chapter 3: Ecological role and impact of New alien crayfish, both from an intra-guild and synecological perspective

- Chucholl, C. *et al.* (2008). Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. *Fund. App. Lim./Arch. Hydrobiol.* 172: 27–36. 157
- Chucholl, C. (submitted). Feeding ecology and ecological impact of an alien “warm water” omnivore in temperate lakes. 177

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- Chucholl, C., Daudey, T. (2008). First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). *Aquat. Invas.* 3: 105–107. 219
- Chucholl, C., Pfeiffer, M. (2010). First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquat. Invas.* 5: 405–412. 223
- Chucholl, C. (2011b). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquat. Invas.* 6: 109–113. 231
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“The careful study of one of the commonest and most insignificant of animals, leads us, step by step, from every-day knowledge to the widest generalizations and the most difficult problems of zoology; and, indeed, of biological science in general”

Thomas H. Huxley (1879), in: *The Crayfish*

Part 1
–
SUMMARY

Introduction

Invasive alien species—a global menace

Biological invasions represent a significant component of global environmental change (Sala *et al.* 2000; Lockwood *et al.* 2007; McGeoch *et al.* 2010). The spread of species into new habitats has always been a part of the natural dynamics of ecosystems. However, in recent decades, the magnitude and frequency of human-mediated biological invasions have dramatically increased (Hulme 2009). Invasive alien species (IAS) now constitute a leading global threat to biodiversity (CBD 2000; Sala *et al.* 2000; McGeoch *et al.* 2010). IAS negatively affect all levels of biodiversity (*i.e.*, genetic diversity, species diversity, and ecosystem diversity) and alter ecosystem processes (Sala *et al.* 2000; Lockwood *et al.* 2007).

IAS often decrease native species abundance and richness *via* competition, predation, and spread of disease (Gaertner *et al.* 2009; Lee 2011; Vilà *et al.* 2011). For instance, non-native Nile perch (*Lates niloticus*) has contributed to the extinction of more than 200 endemic fish species in Lake Victoria, Africa (Lowe *et al.* 2000), while non-native rat species (*Rattus exulans*, *Rattus norvegicus*, and *Rattus rattus*) have eliminated at least 50 bird species on approximately 40 islands worldwide (Lee 2011). Moreover, IAS can threaten close native relatives through hybridization, resulting in the elimination of unique genotypes and the obscuring of species boundaries. This was demonstrated for non-native Rainbow trout (*Oncorhynchus mykiss*) and native Apache trout (*Oncorhynchus apache*) in the American Southwest (Primack 2011). Deleterious ecosystem-wide effects may occur, with IAS eliminating keystone species or altering key processes, food web structures, or physical habitat properties (Lockwood *et al.* 2007; Michelan *et al.* 2010). For instance, *Caulerpa taxifolia*, a seaweed native to the Indian Ocean, displaces native hard-substrate algae and seagrasses in the Mediterranean Sea. These algae and seagrasses provide key habitats and serve as spawning grounds and nurseries for many native marine species. Thus, an ecologically diverse and rich native community is replaced by a species-poor community (Galil 2011). Finally, IAS decrease inter-habitat diversity and inter-ecosystem diversity within and across continents (Lockwood *et al.* 2007; Winter *et al.* 2010). Generalist species, such as Wild boar (*Sus scrofa*), European shore crab (*Carcinus maenas*), and Water hyacinth (*Eichhornia crassipes*), are successfully invading different habitats and ecosystems worldwide (Lowe *et al.* 2000), ultimately leading to a global homogenization of biota (Lockwood *et al.* 2007; Winter *et al.* 2010).

In addition to their influence on biodiversity, IAS can also have a considerable economic impact, in terms of costs of damage and control measures (COM 2008; Kettunen *et al.*

2008). Costs of damage mainly occur in the agricultural, forestry, and fisheries sectors, but may also relate to human health, *e.g.*, treatment costs for IAS-induced asthma. Based on the documented costs of damage and control measures, Kettunen *et al.* (2008) recently estimated the annual costs of IAS in Europe as €12.7 billion, with costs related to terrestrial IAS forming the major part. However, this figure is most likely an underestimate, because it does not include the costs related to biodiversity loss. In comparison, the annual costs related to economic and ecological impacts of IAS in the Great Lakes basin, North America, were estimated as \$5.7 billion (approximately €4.5 billion) (Pimentel 2005).

Overall, IAS pose a significant threat to biodiversity and adversely affect human livelihoods and economies. Thus, their prevention, control, and eradication represent a priority of environmental management (Sala *et al.* 2000; McGeoch *et al.* 2010; CBD 2011). Many IAS act as “endangering” species, in that they constitute a major threat to native species (Primack 2011). This is often on top of other endangering drivers, such as habitat loss and fragmentation, overharvesting, or pollution (D’Antonio *et al.* 2001). While these drivers can theoretically be turned off within a reasonable time, and have potentially reversible effects, IAS may represent a long-term threat that cannot be removed (D’Antonio *et al.* 2001; Primack 2011). In 2010, the International Year of Biodiversity, IAS were recognized as the second biggest threat to global biodiversity (CBD 2011). The new strategic plan of the Convention on Biological Diversity for 2011–2020 (the “Aichi Biodiversity Targets”) explicitly and for the first time proposes that the signatory states should control or eradicate priority IAS and manage invasion pathways to prevent their introduction and establishment (CBD 2011). Nevertheless, the threat from IAS is growing because of increasing global trade and travel, which facilitates the day-by-day introduction of alien species through various pathways (Hulme 2009; McGeoch *et al.* 2010).

“Invasive” matters

When considering the consequences and risks of introduced species it is crucial to distinguish between an alien species *per se* (*i.e.*, a species that has been introduced beyond its natural range by way of human-mediated pathways; Hulme 2009) and an invasive alien species, specifically, an alien species that spreads widely and causes (or is likely to cause) ecological or economic damage (Lockwood *et al.* 2007).

However, before doing so, it is necessary to clarify terms, because the terminology used in the literature on invasion ecology varies considerably (*e.g.*, Kinzelbach *et al.* 2001; Lockwood *et al.* 2007). For instance, “non-indigenous”, “alien”, “exotic”, and “neozoan/ neo-

phyte” are all used to describe a species that is not native to a particular location (summarized in Lockwood *et al.* 2007). For consistency throughout this thesis, I have used the term “alien” to describe a non-native organism, unless a term is used as a part of a preexisting abbreviation.

The differentiation between alien species *per se* and IAS is essential for acknowledging the fact that only some alien species become IAS, and therefore represent a risk to native species and ecosystems (Williamson 1996; Lockwood *et al.* 2007; DAISIE 2012). In fact, a large number of alien species fail to undergo the three obligatory, sequential invasion stages that eventually lead to an IAS: (1) introduction; (2) establishment; and (3) spread/impact (Lockwood *et al.* 2007). Initially, an organism must be picked up from its native range and transported to a new area. This can either happen intentionally, such as in the case of exotic pets (Jenkins 2011), or inadvertently, such as by way of ships’ ballast tanks (Carlton 2011). The alien organisms must then escape/be released from its captivity/ contained environment into nature, referred to as introduction. A newly introduced organism must be able to cope with the new abiotic and biotic environment, and needs to acquire sufficient resources for growth and reproduction in order to become established. Upon establishment, the alien organism requires suitable nearby habitats or dispersal vectors/hosts to spread widely and eventually become invasive. Although biological invasions constitute a continuous process, these invasion stages represent identifiable steps and are thus adopted as a conceptual model throughout this thesis (Lockwood *et al.* 2007).

The invasion stages basically represent a set of ecological barriers, or filters. The empirical “tens rule” of Williamson (1996) states that on average only 10% of alien species are able to successfully traverse an invasion stage. For example, of the 10 961 alien species introduced into Europe, only 11% and 13% are known to have an ecological or economic impact, respectively, *i.e.*, have become invasive (COM 2008; DAISIE 2012).

The invasion success of an alien species is mainly a product of its invasive potential (*i.e.*, its “invasiveness”), the propagule pressure (*i.e.*, the number of independent introduction events plus the number of individuals released per introduction event), and the invasibility of the recipient community (Lockwood *et al.* 2007; Fridley 2011; Rejmánek 2011). Since the formation of invasion biology as a scientific discipline in the 1950s, understanding and forecasting the invasion success of alien species has been a primary objective or the “holy grail” (Richardson 2011). Given the rise in daily introduction events caused by a steady increase in global connectedness, this objective is now more important than ever.

Crustaceans—successful invaders

Crustaceans (Metazoa, Euarthropoda) represent one of the most successful taxonomic groups of aquatic IAS worldwide and constitute more than half of the IAS recorded from European inland waters (Holdich and Pöckl 2007; Karatayev *et al.* 2009; Hänfling *et al.* 2011). Invasive crustaceans include entomostracans (*e.g.*, Cladocera and Copepoda) and malacostracans, with Amphipoda, Mysida, and Decapoda being the most prominent groups (reviewed by Holdich and Pöckl 2007; Hänfling *et al.* 2011; Ricciardi 2011).

Most crustaceans invading European inland waters originate from the Ponto-Caspian basin, North America, Asia, or within Europe, and were introduced: (1) *via* ship transport as ballast water; (2) for aquaculture or fisheries purposes; or (3) through canal systems that connect formerly isolated watersheds (Holdich and Pöckl 2007; Hänfling *et al.* 2011). Among others, several mysids (*e.g.*, *Hemimysis anomala*, *Katamysis warpachowskyi*, and *Limnomysis benedeni*), and numerous amphipods (*e.g.*, *Chelicorophium curvispinum*, *Dikerogammarus* spp., and *Echinogammarus* spp.), and decapods (*e.g.*, *Eriocheir sinensis* and freshwater crayfish), have become highly successful invaders, and are now dominant in many European rivers and lakes (Kley and Maier 2006; Holdich and Pöckl 2007; Wittmann 2007; Hänfling *et al.* 2011).

Many crustacean invaders play vital functional roles in freshwater habitats. Severe ecological and economic impacts of several species have been documented (Dick *et al.* 2002; Rodríguez *et al.* 2005; Holdich and Pöckl 2007; Hänfling *et al.* 2011). For instance, the Chinese mitten crab, *E. sinensis*, frequently forms mass occurrences during annual spawning migration, causing damage to stream banks through excessive burrowing and interference with fisheries (reviewed by Holdich and Pöckl 2007; Dittel and Epifanio 2009). Invasive amphipods often displace native amphipods through competition and intraguild predation, especially from disturbed habitats (Kinzler *et al.* 2009; Kley *et al.* 2009), and can strongly influence whole macroinvertebrate communities. The large Ponto-Caspian amphipod *Dikerogammarus villosus*, for example, is a rather unspecialized omnivore with exceptional predatory capabilities, in contrast to its native counterparts, which are primarily herbivorous and detritivorous shredders (Dick *et al.* 2002; Mayer *et al.* 2008, 2009). *Dikerogammarus villosus* consequently inhabits a higher trophic level than do native amphipods, and can rapidly eliminate other macroinvertebrates. This has given rise to its common name “killer shrimp” (Dick *et al.* 2002; Van Riel *et al.* 2006; Ricciardi 2011).

The case of alien crayfish in Europe

Non-indigenous crayfish species (NICS) in Central Europe represent one of the most extensive and dramatic, yet often neglected, case examples of introduction pathways, invasion mechanisms, and consequences of IAS.

The indigenous crayfish species (ICS) of Europe belong to the possibly polyphyletic Astacidae (Crustacea, Decapoda, Astacida), of which three to four species naturally occur in Central Europe. These are the Noble crayfish (*Astacus astacus*), the Stone crayfish (*Austropotamobius torrentium*), and the White-clawed crayfish (which is thought to represent a species complex with two species—*Austropotamobius pallipes* and *Austropotamobius italicus*) (Souty-Grosset *et al.* 2006; Füreder 2009). However, NICS currently outnumber ICS throughout most parts of Central Europe. Indeed, in some areas, the number of NICS found in the wild is up to three-fold higher than the number of ICS (Holdich *et al.* 2009; Chucholl and Dehus 2011). The vast majority of NICS originate from North America (Table 1), where crayfish have undergone a stronger species radiation than in Eurasia during the last 90 million years, and where approximately 460 recent species occur (Crandall and Buhay 2008; Breinholt *et al.* 2009).

In addition to frequently being more competitive than European ICS, the NICS of North American origin are also latent carriers of a further IAS—*Aphanomyces astaci*, the causative agent of crayfish plague (Souty-Grosset *et al.* 2006; Kozubíková *et al.* 2009; Reynolds and Souty-Grosset 2012). *Aphanomyces astaci* is a fungus-like oomycete (Chromalveolata, Stramenopiles, Peronosporomycetes), which parasitizes the cuticle of crayfish and has repeatedly been introduced into Europe with infected North American crayfish. The immune defense system of North American crayfish species is generally able to cope with the hyphae of *A. astaci* by encapsulating their growth zones, leading to stable parasite–host equilibrium upon infection. By contrast, the immune defense system of European ICS is unable to halt hyphal growth, and this inevitably results in death of the host within 10 days of initial infection (Souty-Grosset *et al.* 2006; Reynolds and Souty-Grosset 2012, and citations therein). Indeed, crayfish plague is one of the most devastating wildlife diseases known, and *A. astaci* is listed among the “100 of the world’s worst IAS” (Lowe *et al.* 2000).

NICS and crayfish plague constitute an ever-increasing threat to ICS, and have been among the leading causes of ICS population declines during the last 140 years (Souty-Grosset *et al.* 2006; Füreder 2009; Holdich *et al.* 2009). Many ICS have been driven to local or regional extinction, and the remaining relict populations are highly fragmented and increasingly threatened by spreading NICS (Holdich *et al.* 2009; Chucholl and Dehus 2011; Reynolds and

Souty-Grosset 2012). All ICS are of major conservational concern, and are included in national or international red lists of threatened species (Souty-Grosset *et al.* 2006; Füreder 2009; Reynolds and Souty-Grosset 2012). The White-clawed crayfish, for example, is considered to be close to extinction in Germany and Austria (red list category one), with invasive NICS and crayfish plague being by far the most pressing threats (Petutschnig 2010; Chucholl and Dehus 2011).

Apart from the extirpation of ICS, NICS can also cause ecological havoc (Nyström 1999, 2002; Reynolds and Souty-Grosset 2012). Crayfish are omnivores that interact with virtually all trophic levels in freshwater ecosystems, and have frequently been recognized as keystone species (reviewed by Momot 1995 and Nyström 2002). NICS can eliminate plant or animal species from ecosystems through direct consumption, predation, or competition, and may have severe indirect impacts by degrading key habitats or resources for other species. For instance, the invasion of a shallow lake on the Iberian Peninsula by the Red swamp crayfish (*Procambarus clarkii*) led to a switch from a clear water state to a turbid one, followed by a collapse of trophic chains dependent on macrophytes. Consequently, biodiversity was severely reduced, including 71% losses in macroinvertebrate genera, 83% losses in amphibian species and 75% losses in duck species (Rodríguez *et al.* 2005).

New versus Old alien crayfish

Since 1880, at least 12 NICS have been introduced into European waters *via* various pathways (summarized in Table 1). Holdich *et al.* (2009) coined the terms *New* and *Old* to categorize NICS, according to their time of introduction and introduction pathway.

Old NICS are alien crayfish species that were introduced into Europe prior to 1975. Initially, all of these *Old* NICS were deliberately stocked into European waters, mainly to support the production or harvest of crayfish for human consumption. Secondary introductions of many species were common and still occur today, despite the fact that they are now illegal (Souty-Grosset *et al.* 2006; Füreder 2009; Chucholl and Dehus 2011). A major motivation for the initial introductions was to compensate for the dramatic loss of native Noble crayfish populations, which occurred from the 1860s onwards. During the 19th century, Noble crayfish formed an integral part of inland fisheries, before stocks collapsed because of habitat degradation and crayfish plague. Crayfish plague was first recorded in Northern Italy during late 1859, and it subsequently spread rapidly throughout the whole of Europe (Souty-Grosset *et al.* 2006; Reynolds and Souty-Grosset 2012). Moreover, *Old* NICS were promoted as “ecological homologs” to ICS, with no consideration for the strong ecological distinctions among

individual crayfish species. Most *Old* NICS rapidly became invasive, and all species of North American origin subsequently turned out to be carriers of crayfish plague. Thus, their introduction unintentionally created an additional, persistent, and deadly threat to the remaining ICS stocks (Souty-Grosset *et al.* 2006; Reynolds and Souty-Grosset 2012, and citations therein). In this respect, the early introductions of *Old* NICS fell prey to the “Frankenstein effect” (Gherardi 2006), in analogy to the story of Shelley (1818), in which good intentions to improve ultimately led to a catastrophic end result (*i.e.*, a monster).

New NICS are alien crayfish species that were introduced after 1980. In terms of introduction pathways, the group is much more diverse than *Old* NICS (Table 1). *New* NICS include species that have reached European inland waters possibly *via* aquaculture (*Orconectes virilis* and *Procambarus acutus/Procambarus zonangulus*), illegal pond stocking (*Orconectes juvenilis*), as fishing bait (probably *Orconectes immunis*) or, most recently, as aquarium species (*Procambarus clarkii*, *Procambarus alleni*, Marmorkrebs, *Cherax destructor*, and *Cherax quadricarinatus*).

Table 1. Presumed introduction pathways, NICS status, invasion stage, current distribution, and origin of the NICS recorded from freshwater habitats in Central Europe. Introduction pathway abbreviations are as follows: *A*, aquarium trade; *C*, consumption trade; *FB*, fishing bait; and *S*, deliberate stocking/aquaculture. The terms *Old* and *New* were coined by Holdich *et al.* (2009), based on initial introduction history and time. Current distribution abbreviations are as follows: *W*, widespread; *R*, regional, spreading; *L*, localized; *O*, present, few occurrences; *F*, failed introduction; and *P*, present, no further details. Key references: 1, Souty-Grosset *et al.* (2006); 2, Pekny (2003); 3, Jaklič and Vrezec (2011) and Zompro (2011); 4, Chucholl (2012); 5, Chucholl and Daudey (2008); 6, Soes and Koese (2010); 7, Chucholl (2011a); 8, Chucholl *et al.* (in press).

Species	Introduction pathway(s)	NICS status	Invasion stage	Central European distribution	Origin	Key references
<i>Astacus leptodactylus</i>	S, C	<i>Old</i>	invasive	<i>W</i>	Eastern Europe	1
<i>Orconectes limosus</i>	S	<i>Old</i>	invasive	<i>W</i>	North America	1
<i>Pacifastacus leniusculus</i>	S	<i>Old</i>	invasive	<i>W</i>	North America	1
<i>Cherax destructor</i>	A	<i>New</i>	introduced	<i>F</i>	Australia	2
<i>Cherax quadricarinatus</i>	A	<i>New</i>	established	<i>L</i>	Australia	3
<i>Orconectes immunis</i>	<i>FB</i>	<i>New</i>	invasive	<i>R</i>	North America	4
<i>Orconectes juvenilis</i>	S	<i>New</i>	unknown	<i>P</i>	North America	5
<i>Orconectes virilis</i>	unknown	<i>New</i>	invasive	<i>R</i>	North America	6
<i>Procambarus acutus/P. zonangulus</i>	unknown	<i>New</i>	established	<i>L</i>	North America	6
<i>Procambarus alleni</i>	A	<i>New</i>	unknown	<i>P</i>	North America	1
<i>Procambarus clarkii</i> *	A, C, S	<i>New</i>	invasive	<i>R</i>	North America	7
Marmorkrebs (<i>P. fallax</i> f. <i>virginalis</i>)	A	<i>New</i>	invasive	<i>O</i>	North America	8

* see page 10 for the classification as *New* NICS

Aims of this thesis

The overall objective of this PhD project was to further our understanding of the drivers, mechanisms, and consequences of biological invasions in general, and crayfish invasions in particular, by gaining insights into the introduction pathways, life histories, and ecological roles and impacts of *New NICS*.

I focused my research on *New NICS* for the following reasons: (1) the introduction of *New NICS* remains an ongoing process (*e.g.*, Marmorkrebs); (2) many *New NICS* are apparently highly successful invaders (*e.g.*, *O. immunis*); (3) *New NICS* have considerable potential to cause ecological and economic damage (*e.g.*, *P. clarkii*); and (4) the introduction pathways and ecology of *New NICS* are understudied topics.

Specifically, I explored three research topics. These are presented in three separate chapters, with each chapter summarizing two publications.

Chapter 1) *Invaders for sale: the pet trade as a pathway for New NICS introductions into Central Europe*

Chapter 2) *Understanding invasion success: life-history traits of New NICS in Central Europe*

Chapter 3) *Ecological role and impact of New NICS, both from an intra-guild and synecological perspective*

In addition to furthering our understanding of biological invasions, I hope that the three chapters will inform stakeholders, authorities, and environmental managers about the risks and problems associated with, and the prevention and management of, *New NICS*.

Furthermore, the Appendix includes three short notes that, notwithstanding their focus on *New NICS*, were beyond the narrower scope of this thesis. The research themes of these short notes are: (1) clarification of the species identity of a recently discovered *New NICS* in eastern France (Chucholl and Daudey 2008); (2) the first evidence for an established Marmorkrebs population in Germany (Chucholl and Pfeiffer 2010); and (3) insights into the local distribution and invasion history of *P. clarkii* in southern Germany (Chucholl 2011b).

Main study species

I focused my PhD research on three *New* NICS in Central Europe, namely the Calico crayfish, Red swamp crayfish, and Marmorkrebs, all of which are representatives of the North American Cambaridae (Crustacea, Decapoda, Astacida).

Calico crayfish (*Orconectes immunis* Hagen, 1870)



Orconectes immunis originates from northeastern and central North America, where it is widely distributed in 26 US states and three Canadian provinces (Hobbs 1989). The species is a recent invader of the Upper Rhine plain, where it has built up high population densities and is rapidly spreading (Gelmar *et al.* 2006; Chucholl and Dehus 2011). *Orconectes immunis* was presumably introduced as live fishing bait by Canadian soldiers, who had been stationed at an airbase near the two localities where the species was first discovered (Gelmar *et al.* 2006). Prior to my PhD research, very little was known about the ecology of *O. immunis* and its interactions with resident biota, except for the displacement of its invasive predecessor, the *Old* NICS *Orconectes limosus*, from preferred habitats (Chucholl 2006).

Red swamp crayfish [*Procambarus clarkii* (Girard, 1852)]



Procambarus clarkii is a native of the southern USA and northeastern Mexico. It is one of the most important freshwater decapods farmed for human consumption, and is established on every continent except Australia and Antarctica (Hobbs 1989; Gherardi 2006, and citations therein). In Europe, *P. clarkii* was deliberately introduced into Spain in 1973, to create an

additional income for inland fisheries (Souty-Grosset *et al.* 2006). It is therefore technically an *Old* NICS; however, most, if not all, of the Central European populations are considerably younger, and originate from different introduction pathways, such as aquaria or escapes from garden ponds (Dehus *et al.* 1999; Soes and van Eekelen 2006; Dümpelmann *et al.* 2009). Hence, throughout this thesis, I have considered the species as a *New* NICS in Central Europe.

Procambarus clarkii is widely regarded as one of the worst invasive crayfish species worldwide, and its ecology in warmer temperature regimes is well understood (Gherardi 2006; Souty-Grosset *et al.* 2006; Capinha *et al.* 2011). Prior to my PhD research, however, the species' life history and impact at higher latitudes were largely unknown.

Marmorkrebs [*Procambarus fallax* f. *virginalis* (Hagen, 1870)]



The Marmorkrebs is an interesting species for three reasons. (1) It is the only known decapod crustacean to obligatorily propagate by apomictic parthenogenesis—only females exist, and these produce genetically identical offspring (Martin *et al.* 2007). (2) No indigenous population has yet been recorded; the species suddenly occurred in the German pet trade in the mid-1990s, and all existing records worldwide most likely trace back to this occurrence (summarized in CABI 2011). (3) Marmorkrebs were never present in the food trade (except for Madagascar; Jones *et al.* 2009) or aquaculture, which leaves little doubt that all records of free-living individuals in Europe originate from introductions *via* aquaria (Souty-Grosset *et al.* 2006; CABI 2011).

Scientists have puzzled for almost a decade about the phylogenetic position and status of Marmorkrebs (CABI 2011). Most recently, Martin *et al.* (2010) showed that this enigmatic crayfish is a parthenogenetic lineage of *P. fallax* and proposed the tentative scientific name *Procambarus fallax* f. *virginalis*. However, *forma* (f.) is not approved by the International Code of Zoological Nomenclature (Martin *et al.* 2010). Given the provisional nature of this scientific designation, I have preferentially used the unique common name Marmorkrebs throughout this thesis.

Summary of chapters

Chapter 1

Biological invasions are sequential, multi-staged processes that involve the importation/transportation, introduction, establishment, and spread of alien organisms. An early and crucial stage of the invasion process is introduction, *i.e.*, the deliberate or accidental release of an alien organism from a contained environment into nature. The prevention of new introductions is often cited as a primary objective (Hulme *et al.* 2009; Justo-Hanani *et al.* 2010; CBD 2011), yet the underlying mechanisms and determinants of introductions remain understudied topics (D'Antonio *et al.* 2001; Duggan *et al.* 2006).

A novel, and at present most likely the major, pathway for *New NICS* introductions into Central Europe is the trade of live ornamental crayfish (Table 1; Holdich *et al.* 2009), which has grown rapidly in the last decade (Lukhaup and Pekny 2009). Hence, a key objective of this PhD project was to assess the trade of ornamental crayfish, and to identify the determinants of crayfish introductions from aquaria (**Chucholl in press**). Based on related research on aquarium fish species, which has shown clear links between the ecological (*e.g.*, size) and economic (*e.g.*, popularity) traits of a species and its likelihood of being recorded as introduced or established (Duggan *et al.* 2006; Duggan 2011), I hypothesized that *NICS* introduced from aquaria share similar ecological and economic traits. Specifically, I expected these species to be: (1) widely available and inexpensive in the aquarium trade; and (2) typically larger than aquarium crayfish species that have not been introduced.

To test this hypothesis, I gathered two datasets on crayfish species in the pet trade, and used multiple binary logistic regression analysis to predict the introduction status of a species based on its availability, price, and size (**Chucholl in press**). The first dataset provided information on the crayfish species imported into Germany between 2005 and 2009 and on the general availability of these species. The second dataset provided more objective information on the availability and price of crayfish species offered through online shops in 2009. In addition, I used an invasiveness-screening tool (FI-ISK) to assess the potential invasiveness of the crayfish species offered online (Cefas 2008; Tricarico *et al.* 2009). I focused on: (1) the German pet trade because this is most likely the main pathway of *NICS* imports into Europe (Pekny and Lukhaup 2005); and (2) the online pet trade because this is more easily accessible than conventional pet stores, and also facilitates the broad-scale spread of the offered species. Thus, the scope of my research was extended to neighboring countries, because pets are often sold across borders (Faulkes 2010; Peay *et al.* 2010; Soes and Koese 2010).

In total, 120 NICS were available as ornamental aquarium species. The vast majority (105) of these species originate from North or Central America. Thus, they are suspected to be vectors of crayfish plague. Despite the large number of imported species, only 20 were found to be common or very common in the trade. The import rate since 2005 was estimated to be 7 new species per year, mostly originating from North America. In 2009, 16 online shops offered at least 37 NICS, of which 6 represented high-risk species (FI-ISK score >16). Among these 6 high-risk NICS, 5 were introduced from aquaria into nature, namely *C. destructor*, *C. quadricarinatus*, Marmorcrebs, *P. alleni*, and *P. clarkii*. Multiple binary logistic regression analysis of the 2 datasets concordantly revealed that the availability and size of species were significant predictors of the likelihood of being recorded as introduced from aquaria (**Chucholl in press**, Table 3, Figure 5).

My initial hypothesis that NICS introduced from aquaria share similar ecological and economic traits was supported by all data, except for pricing, which had no significant effect on the introduction status. The major determinants of crayfish introductions from aquaria are size and availability of species, with large species (total body length >13 cm) that are widely available through the aquarium trade being likely to be introduced into nature. The size of species may influence the likelihood of deliberate release in two ways: (1) large species may outgrow their aquaria; and (2) larger species may rapidly overpopulate their aquaria because they produce considerably more offspring than do smaller species. The size of species was found to correlate with potential invasiveness (FI-ISK score), and therefore release events are likely to be associated with high-risk species. Thus, NICS originating from aquarium introductions are probably equally “dangerous” invaders as many *Old* NICS are, which have been intentionally pre-selected for large size, high fecundity and environmental tolerance (Holdich and Gherardi 1999; Lindqvist and Huner 1999; Holdich *et al.* 2009).

I anticipated that species availability would affect the likelihood of being introduced from aquaria, because a greater availability/popularity is potentially related to a higher number of release events and, thus, higher propagule pressure (Duggan *et al.* 2006; Lockwood *et al.* 2007). The propagule pressure is a composite measure of the number of independent release events (propagule number) plus the number of individuals released in any one event (propagule size), and has been shown to be a major determinant of the establishment success of alien species (Lockwood *et al.* 2005; Duggan *et al.* 2006).

The significance of species popularity for the likelihood of becoming introduced and established is well illustrated by the case of Marmorcrebs. I compiled a synopsis of the current records of this species in Europe, which revealed an alarming increase in established

populations during the last three years (**Chucholl *et al.* in press**). Given the probable lag between initial introduction and detection, and the fact that most populations are large, it is likely that the currently observed trend of increasing population numbers is a result of introductions that happened several years ago—presumably around the time that Marmorkrebs became popular in home aquaria (**Chucholl *et al.* in press**, Figure 3). The proliferation of Marmorkrebs as aquarium pets probably increased the propagule pressure, and therefore the likelihood of introduction and establishment in nature (Lockwood *et al.* 2005; Duggan *et al.* 2006). Specifically, great popularity as an aquarium pet is potentially related to a high propagule number, which serves to overcome negative forces that are spatially structured, such as unsuitable habitat at the site of introduction (Lockwood *et al.* 2005; Duggan *et al.* 2006). For instance, Marmorkrebs apparently failed to establish in cool, rapid-flowing brooks, but eventually invaded lentic habitats (**Chucholl *et al.* in press**, Table 1). As a consequence of the high propagule number, the current Marmorkrebs distribution in Europe is highly scattered and disjunct, with each population forming a potential node for further active spread of the species (**Chucholl *et al.* in press**, Figure 2).

In conclusion, I revealed that the trade of live ornamental crayfish constitutes an actual risk. The continued propagation of crayfish as pets, which relates to substantial propagule pressure, is likely to lead to a further increase in the number of established *New* NICS populations over time. My findings indicate an urgent need for the development of proactive strategies for risk mitigation. This requirement is of particular interest because, to date, neither Germany nor the European Union has established a regulatory framework for the import, trade, and keeping of NICS (Genovesi 2007; Hulme *et al.* 2009; Shine *et al.* 2009).

Chapter 2

The second objective of this PhD project was to investigate the life histories of *New* NICS in Central Europe, to further our understanding of their invasiveness. In this regard, I analyzed the population ecology of *P. clarkii* and *O. immunis* (**Chucholl 2011a** and **Chucholl 2012**, respectively). *Procambarus clarkii* was of particular interest because it is usually considered as a “warm-water” species, but has recently been shown to be able to thrive in colder climates at higher latitudes. By contrast, *O. immunis* is native to temperate zones, but has a largely unknown life history in Europe, despite being a highly successful invader. On the basis of related research that has frequently linked successful aquatic IAS to *r*-selected life histories (Kley and Meier 2006; Füreder and Pöckl 2007; Grabowski *et al.* 2007), I hypothesized that *P. clarkii* and *O. immunis* exhibit *r*-selected life-history traits.

To assess the life histories of *P. clarkii* and *O. immunis*, I monitored their population dynamics in typical habitats, using a standardized trapping protocol. The life history of *P. clarkii* was investigated in two mesotrophic gravel pit lakes along the Danube River, from 2007 to 2009. The life history of *O. immunis* was studied in a backwater habitat adjacent to the Rhine River, during 2007. Life-history parameters of the study populations, including growth rate, asymptotic size, longevity, mean lifetime, and mortality, were estimated from the obtained size-frequency data, using Von Bertalanffy's growth function (VBGF). The reproductive cycle was tracked using the gonadosomatic index (gonad weight in relation to total body weight), reproductive traits, and reproductive form. Mature cambarid crayfish show a cyclic dimorphism between a reproductively active (I) and a reproductively inactive form (II). The form usually changes with every molt, *i.e.*, an individual in form I molts into form II and *vice versa*. In each of the life-history studies, I used the temporal variation in the proportion of form I individuals within the total catch to assess the reproductive cycle. In the case of *O. immunis*, I also used this temporal variation to delineate the molting pattern of the sampled population.

The growth rate (k , curvature parameter of the VBGF, *i.e.*, the rate at which the asymptotic size is approached) of *P. clarkii* along the Danube River was estimated to be slower ($0.45\text{--}0.49\text{ year}^{-1}$) than at lower latitudes, whereas the longevity (6.1–6.6 years), mean lifetime (3.5–4.0 years), and asymptotic size (75–80 mm carapace length, CL) were considerably greater. Reproduction was univoltine, and occurred from August to the onset of winter. A small proportion of females carried eggs throughout the winter. This is in contrast to the multi-voltine life cycle of the species at lower latitudes, with year-round breeding and several reproductive peaks per year (**Chucholl 2011a**, Figure 9).

The growth rate (k) of *O. immunis* was estimated to be $1.15\text{--}1.22\text{ year}^{-1}$, and was combined with short longevity (2.5 years) and mean lifetime (2.1 years), and a small asymptotic CL (50 mm). The adult population molted up to four times during the summer months, with the non-breeding form (II) lasting for a remarkably short period of time (**Chucholl 2012**, Figure 2). Most females of the study population had probably laid their eggs in autumn and carried them throughout the winter. This autumnal egg laying is in distinct contrast to other *Orconectes* species, such as the Old NICS *O. limosus*, which typically lay their eggs in spring (Souty-Grosset *et al.* 2006). *Orconectes immunis* juveniles hatch as early as March, and may attain sexual maturity by the end of their first summer. This rapid maturation probably represents a unique life-history trait, because no other ICS or NICS in Central Europe is known to mature within its first summer.

In summary, my findings suggest that *O. immunis* exhibits a strongly *r*-selected life history. This contrasts markedly with the life histories of ICS, which are more *K*-selected (*i.e.*, slower growth rate, longer lifetime, and lower fecundity; summarized in **Chucholl 2012**, Table 4). Similarly, I found that *P. clarkii* exhibits *r*-selected life-history traits, such as a very high fecundity; however, compared to its life history at lower latitudes, the species also adopted some *K*-selected life-history traits, such as a slow growth rate and high longevity (**Chucholl 2011a**, Figure 8). This remarkable life-history plasticity highlights the need to assess an invader's effective life history across different environmental gradients (Bufford and Daehler 2011). With regard to population management, the *r*-selected life histories of *P. clarkii* and *O. immunis* imply a high resilience against control efforts limited in time (such as removal by trapping), because the populations can rapidly recover (Gherardi *et al.* 2011).

Chapter 3

Finally, I approached the question of the ecological role and impact of *New* NICS. Related studies have shown that invasive crayfish tend to displace resident crayfish species (Capelli and Munjal 1982; Hill and Lodge 1994; Gherardi and Daniels 2004), and that invasive crayfish are capable of altering entire communities (Nyström 1999, 2002). Thus, I hypothesized that *New* NICS would have a profound ecological impact, not only on other crayfish species, but also on littoral communities. To test this hypothesis, I explored: (1) the observed species displacement between the *Old* NICS *O. limosus* and the *New* NICS *O. immunis* (**Chucholl *et al.* 2008**); and (2) the ecological role and impact of *P. clarkii* in temperate lakes in southern Germany (**Chucholl submitted**).

Coincidental with the arrival of *O. immunis* in the Upper Rhine plain, resident populations of its invasive predecessor, *O. limosus* have declined. Direct aggressive interactions between different crayfish species are considered to constitute an important mechanism for species replacement. Thus, by means of laboratory experiments, I studied aggressive interactions and competition for shelter between these two NICS. Agonistic behavior was monitored in heterospecific 1:1 combinations and analyzed using a well-established ethogram of crayfish fight dynamics. In addition, intraspecific shelter affinity and interspecific shelter competition experiments were performed to assess whether the results of the direct aggressive interaction experiments were consistent with those obtained when the two species compete for a limited resource (shelter). Most of these laboratory experiments were originally performed during my diploma study (Chucholl 2006), but were extended for the purposes of my PhD project (**Chucholl *et al.* 2008**). The initial hypothesis that *O. immunis* is aggressively dominant over

O. limosus was clearly supported. Indeed, even 4 mm smaller *O. immunis* were dominant over larger *O. limosus*. *Orconectes immunis* was also highly superior in terms of competition for shelter (**Chucholl et al. 2008**).

Further to the laboratory experiments, I revisited this species displacement in a follow-up field study. I compared the reproductive biology of each species in a mixed population during the spring of 2007 (included in **Chucholl 2012**, chapter 2). In comparison with *O. limosus*, *O. immunis* exhibited an earlier hatching period (**Chucholl 2012**, Figure 5). Moreover, young-of-the-year *O. immunis* may attain sexual maturity by the end of their first summer, which is much sooner than *O. limosus* juveniles mature. This early hatching period and rapid maturation rate most likely provide *O. immunis* with an additional competitive advantage over *O. limosus*.

Overall, *O. immunis* displaces its invasive congener, *O. limosus*, from preferred habitats, probably because of dominance in direct interactions and a faster life cycle. Based on these findings, I propose that *O. immunis* has the potential to out-compete ICS, beyond being a carrier of the causative agent of crayfish plague (Maiwald et al. 2009). Therefore, *O. immunis* poses a serious threat to the remaining, endangered ICS stocks.

I analyzed the ecological role and impact of *P. clarkii* by linking two complementary approaches (**Chucholl submitted**): (1) investigation of the feeding ecology using stomach-content analyses; and (2) evaluation of the impact of *P. clarkii* on a typical littoral community of temperate lakes by using an *in situ* enclosure experiment.

The diet of *P. clarkii* was assessed from the stomach contents of active crayfish captured from a small mesotrophic gravel pit lake during June 2007 and September 2007. To reduce the biases produced by a single measure of the stomach content, two response variables were used to calculate a relative importance index (*RI*) for each prey item and food category. The *RI* of each prey item was compared with the relative abundance of the prey item in the sampled littoral zone, and a prey electivity index was calculated.

Using this approach, I clearly demonstrated that *P. clarkii* is a polytrophic omnivore, which feeds on macrophytes, detritus, and macroinvertebrates (**Chucholl submitted**, Figure 1). The number of consumed small and agile prey negatively correlated with the crayfish size, suggesting an ontogenetic shift in diet. A remarkable phenomenon was the occurrence of terrestrial insects, such as Hymenoptera and Scolytinae, in the stomachs of large crayfish. These insects were most likely gleaned from the water surface or from nearby terrestrial habitats. The high occurrence of macroinvertebrates in the diet of *P. clarkii* emphasizes the importance of crayfish as functional predators (Momot 1995). By selectively preying upon preferred prey

taxa, *P. clarkii* can eliminate sensitive macroinvertebrates (e.g., Gastropods) from invaded communities (see below). Overall, *P. clarkii* greatly enhances the connectance (fraction of all possible links that are realized) of the food web, and accesses all energy pools and trophic pathways. The ability to feed on virtually all organic matter enables *P. clarkii* to build up and maintain the high population densities typical for invasive crayfish species.

The five-week *in situ* enclosure experiment was performed in a different mesotrophic lake during the summer of 2008. The experimental design included three treatments differing in stocked crayfish density (0 crayfish m⁻², 2.5 crayfish m⁻², or 5 crayfish m⁻²), with each treatment replicated five times. Prior to addition of the crayfish, each of the 0.9 m × 0.9 m × 0.9 m enclosures was stocked with the basal levels of a typical littoral food web, *i.e.*, detritus (conditioned tree leaves), primary producers (three macrophyte species), primary benthic consumers (three aquatic snails species), and benthic planktivores (*Dreissena polymorpha*). To distinguish between the macrophyte loss resulting from consumption by crayfish and the macrophyte loss resulting from uprooting by crayfish, macrophyte fragments floating in the enclosures were collected, weighed, and re-anchored throughout the experiment.

As expected, crayfish had a significant effect on the final macrophyte biomass. However, the impact mechanisms and outcomes differed markedly among the macrophyte species (**Chucholl submitted**, Figures 2–4). *Procambarus clarkii* led to a depletion of the native *Myriophyllum spicatum* (mostly resulting from uprooting) and *Chara* sp. (mostly resulting from consumption), but had no significant effect on the alien *Elodea nuttallii*. Thus, it induced a marked shift in the relative abundance of these macrophyte species. The crayfish also had a dramatic, density-dependent effect on the final abundance of the three aquatic snail species, and reduced the detritus biomass (**Chucholl submitted**, Figures 5 and 6).

In summary, the stomach-content analysis and the *in situ* enclosure experiment concordantly suggest that *P. clarkii* is a keystone species, which can profoundly alter recipient communities *via* direct trophic links and non-consumptive destruction. At high crayfish densities, susceptible macroinvertebrate and macrophyte species, such as aquatic snails and *M. spicatum*, were completely eliminated. Surprisingly, other IAS, such as *D. polymorpha* and *E. nuttallii*, were not affected, and may even have been indirectly facilitated by *P. clarkii*. In particular, *E. nuttallii* appeared to benefit from *P. clarkii*, which, in turn, may also have benefited from *E. nuttallii*. *Elodea nuttallii* constitutes an important food resource, and provides abundant shelter for smaller crayfish. The two species may therefore show an indirect positive mutualism, consistent with an invasional meltdown scenario (Simberloff and Von Holle 1999; Von Holle 2011).

General conclusions

While many invasions from aquaria are considered rather benign (Duggan 2011), *New* NICS introductions from aquaria mark a distinct exception: (1) release events are likely to be associated with high-risk species, which are likely to become invasive (**Chucholl in press**); (2) *New* NICS pose a serious threat to endangered ICS (**Chucholl 2012**); and (3) *New* NICS can cause severe ecological damage (**Chucholl submitted**). The prevention of new introductions is therefore imperative (CBD 2011), especially because the eradication of NICS is rarely successful and relies on brute-force chemical methods (Holdich *et al.* 1999; Sandodden and Johnsen 2010; Gherardi *et al.* 2011). Species availability is a major determinant of the likelihood of becoming introduced from aquaria (**Chucholl in press**). Any efforts focused on mitigating the risk of harmful aquarium introductions should therefore aim to drastically reduce the availability of high-risk species. For instance, a regulatory framework for the import, trade, and keeping of NICS could greatly mitigate the risk of high-risk crayfish releases (Souty-Grosset and Reynolds 2009; Justo-Hanani *et al.* 2010).

The invasion success of alien species has often been linked to species-level traits, such as life-history strategies, plasticity, or dietary generalism (reviewed by Bufford and Daehler 2011 and Rejmánek 2011). However, a consistent determinant criterion for invasiveness has remained elusive, leading to the recent suggestion of propagule pressure as a key element of establishment success (Lockwood *et al.* 2005). The findings presented in this thesis suggest that all of the above-cited elements are likely to play a role in *New* NICS invasiveness.

Propagule pressure from the pet trade is most likely the major driver of establishment success of Marmorcrebs in Central Europe (**Chucholl *et al.* in press**). This may also apply to *P. clarkii*, which is similarly popular in aquaria. However, propagule pressure is unlikely to support the invasion success of *O. immunis*, because this species was presumably released only once and is unlikely to be introduced from aquaria on the basis of its rather small size and rare availability. In chapter 2, I demonstrated that *O. immunis* exhibits a strongly *r*-selected life history, which is a highly recurrent, but not exclusively predictive or consistent, trait among successful IAS (Kley and Maier 2006; Füreder and Pöckl 2007; Bufford and Daehler 2011). Specifically, I found that *O. immunis* has a high fecundity and the fastest recorded life cycle among the crayfish species present in Central Europe (summarized in **Chucholl 2012**, Table 4), characteristics that most likely promote its invasiveness. *r*-selected species allocate much of their resources towards growth and reproduction, and the resulting fast life cycle and high reproductive output are considered beneficial throughout all stages of the invasion process (Lindqvist and Huner 1999; Bufford and Daehler 2011). In addition to

displaying a very high fecundity, *P. clarkii* exhibits remarkable life-history plasticity (**Chucholl 2011a**), which probably supports its surprising invasiveness in cold climates at higher latitudes, well beyond its indigenous sub-tropical range (Lindqvist and Huner 1999; Capinha *et al.* 2011; Rejmánek 2011). Moreover, omnivory, as shown in the present thesis for *O. immunis* and *P. clarkii* (**Chucholl 2012, Chucholl submitted**), generally adds further to the invasiveness of NICS, because this trait allows for a high flexibility in the allocation of nutrients required for growth and reproduction (Bufford and Daehler 2011; Rejmánek 2011).

Omnivorous NICS do not fit neatly into trophic-chain models, and therefore it is difficult to predict or generalize their impacts on recipient communities. However, a predominant feature among the ecological impacts of NICS is the depletion of aquatic snails and large, single-stemmed macrophytes (summarized in **Chucholl submitted**, Table 3), which I further corroborated in my present research. A novel aspect, in contrast, was that high densities of *P. clarkii* are likely to result in an *Elodea*-dominated littoral community. This suggestion is consistent with the situation found at the study lake, where *E. nuttallii* and *P. clarkii* have co-existed at high densities since the early 2000s. This coexistence between an abundant macrophyte species and *P. clarkii* differs from the impact of *P. clarkii* on the Iberian Peninsula, where the invasion of *P. clarkii* into macrophyte-dominated habitats has resulted in the rapid collapse of macrophytes (Rodríguez *et al.* 2005). The fact that *E. nuttallii* is itself an IAS, together with the observation that *P. clarkii* and *E. nuttallii* may show an indirect positive mutualism, demonstrate that interactions between IAS can produce unpredictable outcomes. This further emphasizes the need to consider interactions between IAS when assessing the impact of IAS on recipient communities (Simberloff and Von Holle 1999; Von Holle 2011).

In conclusion, my present findings suggest that: (1) the pet trade is a major pathway of NICS introductions by generating substantial propagule pressure; (2) *r*-selected life histories and life-history plasticity support the invasiveness of *O. immunis* and *P. clarkii*, respectively; and (3) *O. immunis* and *P. clarkii* are keystone species, which can have severe ecological impacts (Figure 1). New introductions are likely to be associated with high-risk species, and therefore introduction pathway management is urgently required. Moreover, well-established *New* NICS should be subjected to control and containment efforts, particularly where they endanger sensitive ecosystems or ICS populations.

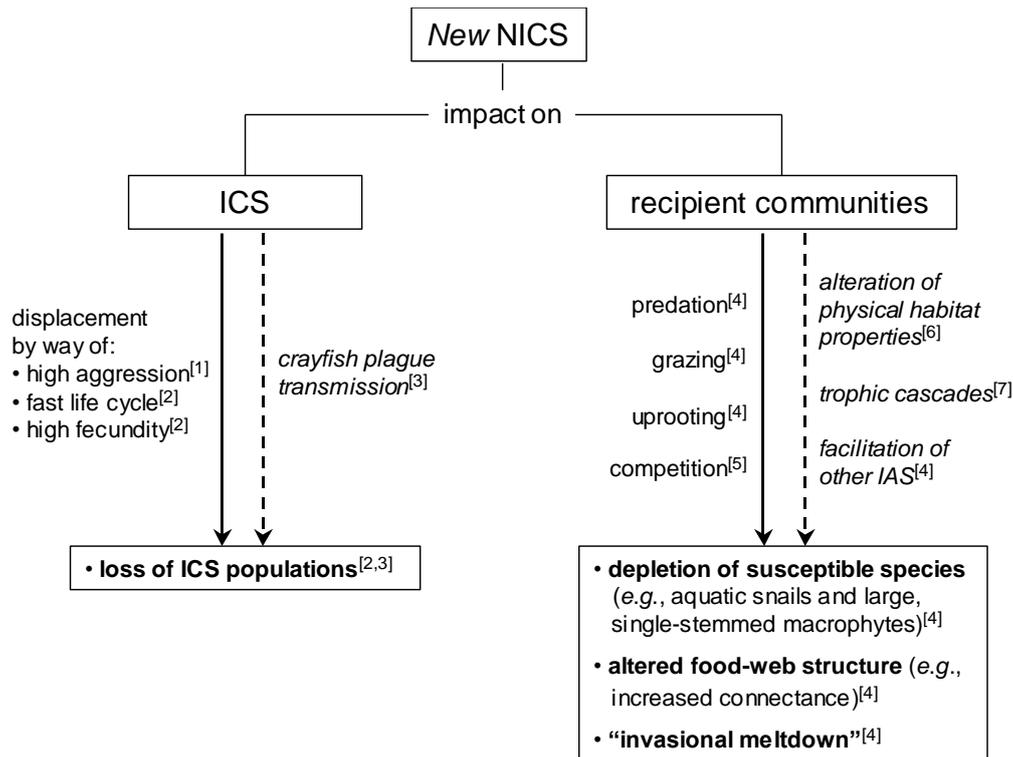


Figure 1. Potential impacts of *New NICS* on ICS populations and recipient communities in Central Europe. Direct impact mechanisms are depicted with solid arrows and in normal font; indirect impact mechanisms are depicted with dashed arrows and in italic font. Superscript numbers in brackets indicate key references: 1, Chucholl *et al.* (2008); 2, Chucholl (2012); 3, Chucholl (2011b) and Maiwald *et al.* (2009); 4, Chucholl (submitted); 5, Hirsch and Fischer (2008); 6, Statzner *et al.* (2000); and 7, Rodríguez *et al.* (2005).

References

- Breinholt, J., Pérez-Losada, M., Crandall, K.A., 2009. The timing of the diversification of the Freshwater Crayfishes. *Crustacean Issues* 18: 343–356.
- Bufford, J.L., Daehler, C.C., 2011. Life history strategies. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 437–441.
- Capelli, G.M., Munjal, B., 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. *Journal of Crustacean Biology* 2: 486–492.
- CABI, 2011. *Procambarus fallax f. virginialis* (Marmorokrebs) [original text by C. Chucholl]. In: *Invasive Species Compendium*. Wallingford, UK: CABI. <http://www.cabi.org/isc>.

- Capinha, C., Leung, B., Anastacio, P., 2011. Predicting worldwide invasiveness for four major problematic decapods: an evaluation of using different calibration sets. *Ecography* 34: 448–459.
- Carlton, J.T., 2011. Ballast. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 43–49.
- CBD, 2000. Global strategy on invasive alien species. Convention on Biological Diversity, UNEP/CBD/SBTTA/6/INF/9: 1-52. UNEP, Montreal, Canada Publ Internet. <http://www.cbd.int/programmes/cross-cutting/alien/documents.aspx> [Accessed 22 December 2008]
- CBD, 2011. Aichi Biodiversity Targets. Convention on Biological Diversity. <http://www.cbd.int/sp/targets/>. [Accessed 19 December 2011]
- Chucholl, C., 2006. Konkurrenz zwischen zwei Neozoen: Verdrängungs-Mechanismen zwischen Kamberkrebs (*Orconectes limosus*, Crustacea: Decapoda) und Kalikokrebs (*O. immunis*). Diploma thesis, University of Ulm, Germany, 97 p.
- Chucholl, C., 2011b. Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquatic Invasions* 6: 109–113.
- Chucholl, C., Daudey, T., 2008. First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). *Aquatic Invasions* 3: 105–107.
- Chucholl, C., Pfeiffer, M., 2010. First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquatic Invasions* 5: 405–412.
- Chucholl, C., Dehus, P., 2011. Flusskrebse in Baden-Württemberg, Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen, Germany, 92 p.

- COM, 2008. Towards an EU strategy on invasive species. Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions. Brussels, 789 p.
- Crandall, K.A., Buhay, J.E., 2011. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in freshwater. *Hydrobiologia* 595: 295–301.
- D’Antonio, C., Meyerson, L.A., Denslow, J., 2001. Exotic species and conservation – Research needs. In: Soulé, M.E., Orians, G.H., (Eds.), Conservation biology: research priorities for the next decade. Island press, Washington, Covelo, London, pp. 59–80.
- DAISIE, 2012. European Invasive Alien Species Gateway. Available from: <http://www.europe-aliens.org/> [accessed 1st February 2012].
- Dehus, P., Phillipson, S., Bohl, E., Oidtmann, B., Keller, M., Lechleiter, S., 1999. German conservation strategies for native crayfish species with regard to alien species. *Custacean Issues* 11: 149–159.
- Dick, J.T.A., Platvoet, D., Kelly, D.W., 2002. Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1078–1084.
- Dittel, A.I., Epifanio, C.E., 2009. Invasion biology of the Chinese mitten crab *Eriocheir sinensis*: A brief review. *Journal of Experimental Marine Biology and Ecology* 374: 79–92.
- Duggan, I., 2011. Aquaria. In: Simberloff, D., Rejmánek, M., (Eds.), Encyclopedia of Biological Invasions, University of California Press, Los Angeles, pp. 32–35.
- Duggan, I., Rixon, C.A.M., MacIsaac, H.J., 2006. Popularity and Propagule Pressure: Determinants of Introduction and Establishment of Aquarium Fish. *Biological Invasions* 8: 377–382.

- Dümpelmann, C., Bonacker, F., Häckl, M., 2009. Erstnachweis des Roten Amerikanischen Sumpfkrebse *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia* 67: 39–47.
- Faulkes, Z., 2010. The spread of the parthenogenetic marbled crayfish, Marmorkrebs (*Procambarus* sp.), in the North American pet trade. *Aquatic Invasions* 5: 447–450.
- Fridley, J.D., 2011. Invasibility of Communities and Ecosystems. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 356–360.
- Füreder, L. (Ed.), 2009. Flusskrebse: Biologie, Ökologie, Gefährdung. Veröffentlichungen des Naturmuseums Südtirol, 6, Folio-Verlag, Bozen, Wien, 144 p.
- Füreder, L., Pöckl, M., 2007. Ecological traits of aquatic NIS invading Austrian fresh waters. In: Gherardi, F., (Ed.), *Biological invaders in inland waters: profiles, distribution and threats*, Springer, Dordrecht, pp. 233–257.
- Gaertner, M., Den Bree, A., Hui, C., Richardson, D.M., 2009. Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. *Progress in Physical Geography* 33: 319–338.
- Galil, B.S., 2011. Mediterranean Sea: Invasions. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 452–458.
- Gelmar, C., Pätzold, F., Grabow, K., Martens, A., 2006. Der Kalikokrebs *Orconectes immunis* am nördlichen Oberrhein: ein neuer amerikanischer Flusskrebs breitet sich rasch in Mitteleuropa aus (Crustacea: Cambaridae). *Lauterbornia* 56: 15–25.
- Genovesi, P., 2007. Towards a European strategy to halt biological invasions in inland waters. In: Gherardi, F., (Ed.), *Biological invaders in inland waters: profiles, distribution and threats*, Springer, Dordrecht, pp. 627–637.

- Gherardi, F., 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* 39: 175–191.
- Gherardi, F., Daniels, W.H., 2004: Agonism and shelter competition between invasive and indigenous crayfish species. *Canadian Journal of Zoology* 82: 1923–1932.
- Gherardi, F., Aquiloni, L., Diéguez-Urbeondo, J., Tricarico, E., 2011. Managing invasive crayfish: is there a hope? *Aquatic Sciences*, doi 10.1007/s00027-011-0181-z.
- Grabowski, M., Bacela, K., Konopacka, A., 2007. How to be an invasive gammarid – comparison of life history traits. *Hydrobiologia* 590: 75–84.
- Hänfling, B., Edwards, F., Gherardi, F., 2011. Invasive alien Crustacea: dispersal, establishment, impact and control. *BioControl* 56: 573–595.
- Hill, A.M., Lodge, D.M., 1994: Diel changes in resource demand: Competition and predation in species replacement among crayfish. *Ecology* 75: 2118–2126.
- Hirsch, P.E., Fischer, P., 2008. Interactions between native juvenile burbot (*Lota lota*) and the invasive spinycheek crayfish (*Orconectes limosus*) in a large European lake. *Canadian Journal of Fisheries and Aquatic Sciences* 65: 2636–2643.
- Hobbs, H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology* 480, 236 p.
- Holdich, D.M., Gherardi, F., 1999. Native and alien crayfish in Europe: An introduction. *Crustacean Issues* 11: 3–12.
- Holdich, D.M., Pöckl, M., 2007. Invasive crustaceans in European inland waters. In: Gherardi, F., (Ed.), *Biological invaders in inland waters: profiles, distribution and threats*, Springer, Dordrecht, pp. 29–75.

- Holdich, D.M., Reynolds J.D., Souty-Grosset C., Sibley P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 11: 394–395.
- Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46: 10–18.
- Hulme, P.E., Pysek, P., Nentwig, W., Vilà, M., 2009. Will Threat of Biological Invasions Unite the European Union? *Science* 324: 40–41.
- Jaklič, M., Vrezec, A., 2011. The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae). *Crustaceana* 84: 651–665.
- Jenkins, P.T., 2011. Pet trade. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 539–543.
- Jones, J.P.G., Rasamy, J.R., Harvey, A., Toon, A., Oidtmann, B., Randrianarison, M.H., Raminosoa, N., Ravoahangimalala, O.R., 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions* 11: 1475–1482.
- Justo-Hanani, R., Dayan, T., Tal, A., 2010. The role of regulatory decision-making on non-indigenous species introductions. *Biological Invasions* 12: 2815–2824.
- Karatayev, A.Y., Burlakova, L.E., Padilla, D.K., Mastitsky, S.E., Olenin, S., 2009. Invaders are not a random selection of species. *Biological Invasions* 11: 2009–2019.
- Kettunen, M., Genovesi, P., Gollasch, S., Pagad, S., Starfinger, U. ten Brink, P., Shine, C. 2008. Technical support to EU strategy on invasive species (IAS) - Assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission). Institute for European Environmental Policy (IEEP), Brussels, Belgium. 44 p. + Annexes.

- Kinzelbach, R., Geiter, O. Homma, S., 2001. Bestandsaufnahme und Bewertung von Neozoen in Deutschland. Untersuchung der Wirkung von Biologie und Genetik ausgewählter Neozoen auf Ökosysteme und Vergleich mit den potentiellen Effekten gentechnisch veränderter Organismen. Umweltbundesamt, Berlin, 290 p.
- Kinzler, W., Kley, A., Mayer, G., Waloszek, D., Maier, G., 2009. Mutual predation and cannibalism within several freshwater gammarids: *Dikerogammarus villosus* versus one native and three invasives. *Aquatic Ecology* 43: 457–464.
- Kley, A., Maier, G., 2006. Reproductive characteristics of invasive gammarids in the Rhine-Main-Danube catchment, South Germany. *Limnologica* 36: 79–90.
- Kley, A., Kinzler, W., Schank, Y., Mayer, G., Waolszek, D., Maier, G., 2009. Influence of substrate preference and complexity on co-existence of two non-native gammarideans (Crustacea: Amphipoda). *Aquatic Ecology* 43: 1047–1059.
- Kozubíková, E., Filipová, L., Kozák, P., Ďuriš, Z., Martín, M.P., Diéguez-Uribeondo, J., Oidtmann, B., Petrusek, A., 2009. Prevalence of the crayfish plague pathogen *Aphanomyces astaci* in invasive American crayfishes in the Czech Republic. *Conservation Biology* 23: 1204–1213.
- Lee, W.G., 2011. Islands. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 391–395.
- Lindqvist, O.V., Huner, J.V., 1999. Life history characteristics of crayfish: what makes them good colonizers? *Crustacean Issues* 11: 23–30.
- Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20: 223–228.
- Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2007. *Invasion Ecology*, Blackwell Publishing, Oxford, 304 p.

- Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12 p. First published as special lift-out in *Aliens* 12, December 2000. Updated and reprinted version: November 2004.
- Lukhaup, C., Pekny, R., 2009. Flusskrebse in der Aquaristik. In: Füreder, L., (Ed.) Flusskrebse: Biologie, Ökologie, Gefährdung. Veröffentlichungen des Naturmuseums Südtirol, 6, Folio Verlag, Bozen, Wien, pp 129–132.
- Maiwald, T., Vralstad, T., Jarausch, W., Schulz, H.K., Smietana, P., Schulz, R., 2009. Kamberkreb und Edelkreb zwischen Koexistenz und Krebspest, Abstracts volume 4, Internationales Flusskrebbsforum, Gersfeld/ Röhn, Germany, 43–48.
- Martin, P., Kohlmann, K., Scholtz, G., 2007. The parthenogenetic Marmorkreb (marbled crayfish) produces genetically uniform offspring. *Naturwissenschaften* 94: 843–846.
- Martin, P., Dorn, N., Kawai, T., van der Heiden, C., Scholtz, G., 2010. The enigmatic Marmorkreb (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contributions to Zoology* 79: 107–118.
- Mayer, G., Maier, G., Maas, A., Waloszek, D., 2008. Mouthparts of the Ponto-Caspian invader *Dikerogammarus villosus* (Amphipoda: Pontogammaridae). *Journal of Crustacean Biology* 28: 1–15.
- Mayer, G., Maier, G., Maas, A., Waloszek, D., 2009. Mouthpart morphology of *Gammarus roeselii* compared to a successful invader, *Dikerogammarus villosus* (Amphipoda). *Journal of Crustacean Biology* 29: 161–174.
- McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J., Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity and Distributions* 16: 95–108.

- Michelan, T.S., Thomaz, S.M., Mormul, R.P., Carvalho, P., 2010. Effects of an exotic invasive macrophyte (tropical signalgrass) on native plant community composition, species richness and functional diversity. *Freshwater Biology* 55: 1315–1326.
- Momot, W.T., 1995. Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science* 3: 33–63.
- Nyström, P., 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. *Crustacean Issues* 11: 63–85.
- Nyström, P., 2002. Ecology. In: Holdich, D.M. (Ed.), *Biology of Freshwater Crayfish*, Blackwell Scientific Press, Oxford, pp. 192–224.
- Peay, S., Holdich, D.M., Brickland, J., 2010. Risk assessments of non-indigenous crayfish in Great Britain. *Freshwater Crayfish* 17. (in press)
- Pekny, R., 2003. Flusskrebse aus aller Welt - Mögliche Invasoren in Europa. Internationales Flusskrebbsforum, Augsburg 2003, Tagungsband: 27–34.
- Pekny, R., Lukhaup, C., 2005. Aquarienkrebse in Europa - eine rasante Entwicklung! 2. Internationale Flusskrebstagung, Baden 2005, Tagungsband: 78–94.
- Petutschnig, J., 2010. Rote Liste der Flusskrebse (Decapoda) Österreichs. *Forum Flusskrebse* 14: 27–32.
- Pimentel, D., 2005. Aquatic Nuisance Species in the New York State Canal and Hudson River Systems and the Great Lakes Basin: An Economic and Environmental Assessment. *Environmental Management* 35: 692–702.
- Primak, R.B., 2011. Endangered and threatened species. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 189–193.

- Rejmánek, M., 2011. Invasiveness. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 379–385.
- Reynolds, J., Souty-Grosset, C. (Eds.), 2012. *Management of freshwater biodiversity. Crayfish as Bioindicators*. Cambridge University Press, Cambridge, 374 p.
- Ricciardi, A., 2011. Crustaceans (Others). In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 135–137.
- Richardson, D.M., (Ed.) 2011. *Fifty years of Invasion Ecology: The Legacy of Charles Elton*. Wiley-Blackwell, West Sussex, 432 p.
- Rodríguez, C.F., Bécares, E., Fernández-Aláez, M., Fernández-Aláez C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions* 7: 75–85.
- Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, B.H., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity scenario for the year 2100. *Science* 287: 1770–1774.
- Sandodden, R., Johnsen, S.I., 2010. Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquatic Invasions* 5: 75–81.
- Simberloff, D., Von Holle, B., 1999. Positive interactions of non-indigenous species: Invasional meltdown? *Biological Invasions* 1: 21–32.
- Shelley, M.W., 1818. *Frankenstein, or the Modern Prometheus*. In: Butler, M. (Ed.) 1994. Oxford, Oxford University press.

- Shine, C., Kettunen, M., ten Brink, P., Genovesi, P., Gollasch, S., 2009. Technical support to EU strategy on invasive species (IAS) – Recommendations on policy options to control the negative impacts of IAS on biodiversity in Europe and the EU. Final report for the European Commission. Institute for European Environmental Policy (IEEP), Brussels, Belgium, 35 p.
- Soes, M., van Eekelen, R., 2006. Rivierkrefeten, een oprukkend probleem? *De Levede Natur* 107: 56–59.
- Soes, M., Koese, B., 2010. Invasive crayfish in the Netherlands: a preliminary risk analysis. Interim report, Bureau Waardenburg bv, Stichting EIS-Nederland, Invasive Alien Species Team, Waardenburg, 69 p.
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P. (Eds.), 2006. Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris, 192 p.
- Souty-Grosset, C., Reynolds, J.D., 2009. Current ideas on methodological approaches in European crayfish conservation and restocking procedures. *Knowledge and Management of Aquatic Ecosystems* 394-395, 01. doi:10.1051/kmae/2009021
- Statzner, B., Fièvet, E., Campagne, J. Y., Morel, R., Herouin, E., 2000. Crayfish as geomorphic agents and ecosystem engineers: Biological behaviour affects sand and gravel erosion in experimental streams. *Limnology and Oceanography* 45: 1030–1040.
- Tricarico, E., Vilizzi, L., Gherardi, F., Copp, G.H., 2009. Calibration of FI-ISK, an Invasiveness Screening Tool for Nonnative Freshwater Invertebrates. *Risk Analysis* 30: 285–292.
- Van Riel, M.C., Van der Velde, G.V.D., Rajagopal, S., Marguillier, S., Dehairs, F., Vaate, A.B., 2006. Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia* 565: 39–58.

- Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P., 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14: 702–708.
- Von Holle, B., 2011. Invasional Meltdown. In: Simberloff, D., Rejmánek, M., (Eds.), *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, pp. 360-364.
- Williamson, M.H., 1996. *Biological Invasions*. London: Chapman and Hall, 256 p.
- Winter, M., Kühn, I., La Sorte, F.A., Schweiger, O., Nentwig, W., Klotz, S., 2010. The role of non-native plants and vertebrates in defining patterns of compositional dissimilarity within and across continents. *Global Ecology and Biogeography* 19: 332–342.
- Wittmann, K.J., 2007. Continued massive invasion of Mysidae in the Rhine and Danube river systems, with first records of the order Mysidacea (Crustacea: Malacostraca: Peracarida) for Switzerland. *Revue Suisse de Zoologie* 114: 65–86.
- Zompro, O., 2011. Ein neu eingeschleppter Flusskrebs aus Australien – *Cherax quadricarinatus*, der Rotscherenkrebs. *AKFS-aktuell* 27: 27–29.

Teil 1
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ZUSAMMENFASSUNG

Einleitung

Invasive gebietsfremde Arten – ein globales Problem

Biologische Invasionen sind ein wesentlicher Bestandteil des globalen Umweltwandels (Sala *et al.* 2000; Lockwood *et al.* 2007; McGeoch *et al.* 2010). Die Ausbreitung von Arten in neue Lebensräume gehört seit jeher zur natürlichen Dynamik von Ökosystemen. In den letzten Jahrzehnten haben aber das Ausmaß und die Häufigkeit von biologischen Invasionen durch menschliche Einflussnahme dramatisch zugenommen (Hulme 2009). Invasive gebietsfremde Arten (engl.: invasive alien species; nachfolgend IAS) stellen mittlerweile eine der weltweit größten Gefährdungsursachen für die biologische Vielfalt dar (CBD 2000; Sala *et al.* 2000; McGeoch *et al.* 2010). IAS gefährden alle Ebenen der Biodiversität, von der genetischen Diversität und Artenvielfalt bis zur Diversität von Ökosystemen, und können ökosystemare Prozesse verändern (Sala *et al.* 2000; Lockwood *et al.* 2007).

Häufig verdrängen oder eliminieren IAS natürlich vorkommende Arten durch Konkurrenz, Prädation oder Krankheitsübertragung (Gaertner *et al.* 2009; Lee 2011; Vilà *et al.* 2011). Gebietsfremde Nilbarsche (*Lates niloticus*) haben beispielsweise im Viktoriasee zum Aussterben von 200 endemischen Buntbarscharten beigetragen (Lowe *et al.* 2000), während gebietsfremde Ratten (*Rattus exulans*, *Rattus norvegicus* und *Rattus rattus*) auf etwa 40 Inseln weltweit mindestens 50 Vogelarten ausgerottet haben (Lee 2011). Darüber hinaus können IAS durch Hybridisierung mit nah verwandten indigenen Arten einzigartige Genotypen eliminieren und zum Verwischen von Artgrenzen führen. Dies wurde zum Beispiel im Fall der gebietsfremden Regenbogenforelle (*Oncorhynchus mykiss*) und der autochthonen Apacheforelle (*Oncorhynchus apache*) im amerikanischen Südwesten gezeigt (Primack 2011). Negative Auswirkungen auf gesamte Ökosysteme können auftreten, wenn IAS ökologische Schlüsselarten eliminieren, oder ökosystemare Prozesse, die Struktur von Nahrungsnetzen, oder physikalische Habitateigenschaften verändern (Lockwood *et al.* 2007; Michelan *et al.* 2010). Im Mittelmeer werden beispielsweise indigene Makroalgen und Seegräser durch die gebietsfremde „Killeralge“ *Caulerpa taxifolia* verdrängt. Mit den indigene Algen- und Seegraswiesen gehen ökologische Schlüsselfunktionen verloren, da diese Biozönosen Lebensraum, Laichhabitats und Jungtierstuben für zahlreiche andere marine Arten darstellen (Galil 2011). Schließlich verringern IAS die Diversität von Lebensräumen und Ökosystemen innerhalb und über Kontinente hinweg (Lockwood *et al.* 2007; Michelan *et al.* 2010; Winter *et al.* 2010). Generalisten, wie die Europäische Strandkrabbe (*Carcinus maenas*) oder Wildschweine (*Sus scrofa*), sind weltweit in verschiedenen Lebensräumen und

Ökosystemen invasiv (Lowe *et al.* 2000), wodurch es zu einer globalen Angleichung und Homogenisierung der Biota kommt (Lockwood *et al.* 2007; Winter *et al.* 2010).

Neben ökologischen Schäden, können IAS auch erhebliche ökonomische Schäden verursachen, resultierend aus direkten finanziellen Schäden und den Kosten für Kontrollmaßnahmen (COM 2008; Kettunen *et al.* 2008). Direkte finanzielle Schäden entstehen überwiegend in den Agrar-, Forst- und Fischerei-Sektoren, können aber auch Kosten im Gesundheitswesen umfassen, etwa wenn IAS bei Menschen Allergien oder Asthma verursachen. Basierend auf dokumentierten direkten finanziellen Schäden und Kosten für Kontrollmaßnahmen, haben Kettunen *et al.* (2008) die jährlichen Gesamtkosten, die durch IAS in der EU entstehen, kürzlich auf 12,7 Milliarden € geschätzt. Diese Schätzung beinhaltet allerdings nicht die Kosten des durch IAS verursachten Biodiversität-Verlusts. Die Kosten unter Berücksichtigung des ökonomischen und ökologischen Schadens wurden zum Vergleich alleine im Einzugsgebiet der Großen Seen in Nordamerika auf jährlich 5,7 Milliarden US \$ (etwa 4,5 Milliarden €) geschätzt (Pimentel 2005).

Insgesamt stellen IAS eine erhebliche Bedrohung für die biologische Vielfalt dar und können Lebensgrundlagen für Menschen zerstören. Prävention, Kontrolle und Eliminierung von IAS sind daher prioritäre Zielsetzungen im Umweltmanagement (Sala *et al.* 2000; McGeoch *et al.* 2010; CBD 2011). Viele IAS gefährden indigene Arten, oft zusätzlich zu anderen Gefährdungsursachen, wie Verlust und Fragmentierung von Lebensräumen, Übernutzung oder Umweltverschmutzung. Während diese Gefährdungsursachen theoretisch beseitigt werden können und ihre Folgen potentiell reversibel sind, könnten sich IAS als langfristige, irreversible Bedrohung herausstellen (D'Antonio *et al.* 2001; Primack 2011). Im Internationalen Jahr der biologischen Vielfalt, 2010, wurden IAS als die zweitgrößte Gefährdungsursache für die weltweite Biodiversität eingestuft (CBD 2011). Der neue strategische Plan des Übereinkommens über die biologische Vielfalt für die Jahre 2011–2020 (die „Aichi-Ziele“) ruft die Unterzeichnerstaaten zum ersten Mal ausdrücklich dazu auf, prioritäre IAS zu bekämpfen und zu kontrollieren, und ihre weitere Einschleppung zu verhindern (CBD 2011). Zunehmende globale Verkehrs- und Handelsströme erhöhen allerdings die Wahrscheinlichkeit der Verschleppung von gebietsfremden Arten (Hulme 2009; McGeoch *et al.* 2010).

Gebietsfremd vs. invasiv

Bei der Betrachtung der Folgen und Risiken der Verschleppung von Arten ist es notwendig, zwischen gebietsfremden Arten *per se* (d.h. Arten, die durch menschliche Einflussnahme über ihr natürliches Verbreitungsgebiet hinaus verbreitet wurden; Hulme 2009) und invasiven gebietsfremden Arten, d.h. solchen gebietsfremden Arten, die sich rasch ausbreiten und ein erhebliches ökologisches und/oder ökonomisches Schadenspotenzial aufweisen (Lockwood *et al.* 2007), zu differenzieren.

Vorab ist es allerdings sinnvoll Begriffe festzulegen, da die Terminologie in der Invasionsbiologie beträchtlich variiert (siehe Kinzelbach *et al.* 2001; Lockwood *et al.* 2007). Beispielsweise werden die Begriffe „nicht-heimisch“¹, „exotisch“, „gebietsfremd“, „allochthon“ und „Neozoe/Neophyte“ verwendet, um einen Organismus zu bezeichnen, der in einem bestimmten Gebiet nicht natürlich vorkommt (zusammengefasst in Lockwood *et al.* 2007). In der vorliegenden Arbeit habe ich einen nicht natürlich vorkommenden Organismus durchgängig als „gebietsfremd“ bezeichnet. Ausnahmen sind Begriffe als Teil von englischen Abkürzungen, die ich stringent in meinen englischsprachigen Publikationen verwendet habe. Zur besseren Verständlichkeit habe ich diese hier beibehalten.

Die Unterscheidung zwischen gebietsfremden Arten *per se* und IAS ist essenziell, da nur wenige gebietsfremde Arten zu IAS werden und folglich indigene Arten und Ökosysteme gefährden (Williamson 1996; Lockwood *et al.* 2007; DAISIE 2012). Tatsächlich schafft es ein großer Teil der gebietsfremden Arten nicht die drei obligatorischen, aufeinanderfolgenden Invasionsstadien zu durchlaufen an deren Ende eine invasive Art steht: (1) Einfuhr/Freisetzung, (2) Etablierung und (3) Ausbreitung/Impact (Lockwood *et al.* 2007). Zunächst muss ein Organismus in seinem natürlichen Verbreitungsgebiet von einem Transportvektor aufgenommen und in ein neues Gebiet verbracht werden. Dieser Transport kann absichtlich passieren, etwa für den Zoohandel (Jenkins 2011), oder unabsichtlich, zum Beispiel im Ballastwasser von Schiffen (Carlton 2011). Anschließend muss der Organismus von der abgeschlossenen Umgebung/Gefangenschaft in die freie Natur gelangen, was als Freisetzung oder Einfuhr (engl.: introduction) bezeichnet wird. Ein freigesetzter Organismus muss mit der neuen abiotischen und biotischen Umgebung zurecht kommen und ausreichend Ressourcen für Wachstum und Reproduktion vorfinden, um eine Population aufzubauen und sich zu etablieren. Einmal fest etabliert, benötigt der Organismus geeignete Habitate in der Nähe, respektive einen Ausbreitungsvektor/Wirt, um sich weiter ausbreiten zu können und invasiv

¹ Der Begriff „heimisch“ und damit indirekt auch das Antonym „nicht-heimisch“, ist in der BRD durch das BNatSchG (§ 7, Abs. 2) unglücklich belegt, was die Terminologie im Deutschen zusätzlich erschwert.

zu werden. Obwohl biologische Invasionen ein kontinuierlicher Prozess sind, stellen diese drei Invasionsstadien leicht erkennbare, distinkte Stufen dar und wurden daher in der vorliegenden Arbeit als konzeptionelles Modell benutzt (Lockwood *et al.* 2007).

Die Invasionsstadien können als eine Abfolge von ökologischen Hürden, oder Filtern, verstanden werden. Nach der empirischen Zehner-Regel von Williamson (1996) durchlaufen durchschnittlich nur jeweils 10% gebietsfremder Arten ein Invasionsstadium erfolgreich. Von den 10 961 gebietsfremden Arten in Europa ist beispielsweise nur von 11% bzw. 13% bekannt, dass sie einen ökologischen oder ökonomischen Schaden verursachen, d.h. dass sie invasiv geworden sind (COM 2008; DAISIE 2012).

Der Invasionserfolg einer gebietsfremden Art ist abhängig von ihrer Invasivität (d.h. dem Potenzial invasiv zu werden), dem Freisetzungs-Druck (engl.: propagule pressure; bezeichnet die Anzahl unabhängiger Freisetzungseignisse plus die Anzahl an freigesetzten Individuen pro Freisetzungseignis) und der Anfälligkeit der aufnehmenden Gemeinschaft für biologische Invasionen (engl.: Invasibility) (Lockwood *et al.* 2007; Fridley 2011; Rejmánek 2011). Das Verständnis und die Vorhersage des Invasionserfolgs einer gebietsfremden Art ist eine zentrale Fragestellung, oder der „heilige Gral“, der Invasionsbiologie (Richardson 2011). Angesichts der zunehmenden Verschleppung von Organismen infolge der wachsenden Globalisierung ist dieses Ziel heute wichtiger denn je.

Crustaceen – erfolgreiche Invasoren

Krebstiere (Metazoa, Euarthropoda, Crustacea) sind eine der erfolgreichsten invasiven Tiergruppen in aquatischen Lebensräumen weltweit und stellen mehr als die Hälfte der IAS in europäischen Binnengewässern (Holdich und Pöckl 2007; Karatayev *et al.* 2009; Hänfling *et al.* 2011). Invasive Crustaceen umfassen Vertreter der Entomostraca (z.B. Cladocera und Copepoda) und Malacostraca, zu denen mit zahlreichen Flohkrebse (Amphipoda), Schwebegarnelen (Mysida) und Zehnfußkrebse (Decapoda) die wichtigsten invasiven Arten gehören (zusammengefasst in Holdich und Pöckl 2007; Hänfling *et al.* 2011; Ricciardi 2011).

Die meisten invasiven Crustaceen in europäischen Binnengewässern stammen aus der Pontokaspis, Nordamerika, Asien oder Regionen innerhalb Europas. Die wichtigsten Einfuhrvektoren sind (1) Schiffe (*via* Ballastwasser), (2) Aquakultur/Fischerei und (3) Kanalsysteme, die ursprünglich voneinander isolierte Flusseinzugsgebiete verbinden (zusammengefasst in Holdich und Pöckl 2007; Hänfling *et al.* 2011). Mehrere Mysiden (*Hemimysis anomala*, *Katamysis warpachowskyi* und *Limnomysis benedeni*) und zahlreiche Amphipoden (z.B. *Chelicorophium curvispinum*, *Dikerogammarus* spp. und *Echinogammarus* spp.) und

Dekapoden (z.B. *Eriocheir sinensis* und gebietsfremde Flusskrebse) wurden sehr erfolgreiche Invasoren und sind heute in vielen europäischen Flüssen und Seen numerisch dominant (Kley und Maier 2006; Holdich und Pöckl 2007; Wittmann 2007; Hänfling *et al.* 2011).

Viele invasive Crustaceen nehmen wichtige ökologische Funktionen in Süßwasserhabitaten ein. Von mehreren Arten sind negative ökologische und/oder ökonomische Auswirkungen belegt (Dick *et al.* 2002; Rodríguez *et al.* 2005; Holdich und Pöckl 2007; Hänfling *et al.* 2011). Massenvorkommen der Chinesischen Wollhandkrabbe (*E. sinensis*) während der jährlichen Fortpflanzungswanderung verursachen beispielsweise Schäden an Flussufern durch exzessive Grabaktivität und eine Behinderung der Fischerei (zusammengefasst in Holdich und Pöckl 2007; Dittel und Epifanio 2009). Invasive Flohkrebse verdrängen oft autochthone Flohkrebse durch Konkurrenz und „Intraguild predation“, vor allem von degradierten Habitaten (Kinzler *et al.* 2009; Kley *et al.* 2009), und können die gesamte Makroinvertebraten-Zönose stark beeinflussen. Der großwüchsige pontokaspische Flohkrebs *Dikerogammarus villosus* ist beispielsweise ein relativ unspezialisierter Omnivore mit großem räuberischen Potenzial, im Gegensatz zu den indigenen Flohkrebsen, die sich als Zerkleinerer (Shredder) primär von Detritus und pflanzlichen Ressourcen ernähren (Dick *et al.* 2002; Mayer *et al.* 2008; Mayer *et al.* 2009). *Dikerogammarus villosus* nimmt daher eine höhere trophische Ebene ein als die indigenen Arten und kann andere Makroinvertebraten rasch eliminieren. Dies führte im englischen Sprachraum zu der Bezeichnung „killer shrimp“ (Dick *et al.* 2002; Van Riel *et al.* 2006; Ricciardi 2011).

Gebietsfremde Flusskrebse in Europa

Gebietsfremde Flusskrebsarten (engl.: non-indigenous crayfish species; nachfolgend NICS) in Mitteleuropa stellen eines der umfangreichsten und dramatischsten, zugleich aber oft vernachlässigten, Fallbeispiele für Einfuhrvektoren, Invasions-Mechanismen und Folgen von IAS dar.

Die indigenen Flusskrebsarten (engl.: indigenous crayfish species; nachfolgend ICS) gehören zu den möglicherweise polyphyletischen Astacidae (Crustacea, Decapoda, Astacida), von denen drei bis vier Arten natürlicherweise in Mitteleuropa vorkommen. Diese sind der Edelkrebs (*Astacus astacus*), der Steinkrebs (*Austropotamobius torrentium*) und der Dohlenkrebs (der einen Artkomplex mit wahrscheinlich zwei Arten darstellt: *Austropotamobius pallipes* und *Austropotamobius italicus*) (Souty-Grosset *et al.* 2006; Füreder 2009). Gegenwärtig kommen in Mitteleuropa allerdings mehr NICS als ICS vor. Tatsächlich ist in einigen Regionen die Zahl gebietsfremder Flusskrebsarten um bis das

dreifache höher als die der indigenen Arten (Holdich *et al.* 2009; Chucholl und Dehus 2011). Fast alle NICS stammen aus Nordamerika (Tabelle 1), wo Flusskrebse in den letzten 90 Millionen Jahren eine wesentlich stärkere Radiation durchgemacht haben als in Eurasien und wo etwa 460 rezente Arten vorkommen (Crandall und Buhay 2008; Breinholt *et al.* 2009).

Nordamerikanische NICS sind den ICS häufig in direkter Konkurrenz überlegen und zudem latente Carrier einer weiteren invasiven Art: *Aphanomyces astaci*, dem Erreger der Krebspest (Souty-Grosset *et al.* 2006; Kozubíková *et al.* 2009; Reynolds und Souty-Grosset 2012). *Aphanomyces astaci* ist ein pilzähnlicher Oomycete (Chromalveolata, Stramenopiles, Peronosporomycetes) der in der Kutikula von Flusskrebsen parasitiert und wiederholt mit nordamerikanischen Flusskrebsen nach Europa verschleppt wurde. Das Immunsystem von nordamerikanischen Flusskrebsen reagiert in der Regel schnell auf den Parasiten und kapselt die Wachstumszonen der Krebspest-Hyphen ab, wodurch es zu einem stabilen Parasit-Wirt-Gleichgewicht kommt. Das Immunsystem von europäischen Flusskrebsen ist dagegen nicht in der Lage das Hyphen-Wachstum zu stoppen, was innerhalb von zehn Tagen nach der Infektion unweigerlich zum Tod des Wirts führt (Souty-Grosset *et al.* 2006; Reynolds und Souty-Grosset 2012, und Zitate darin). Tatsächlich ist die Krebspest eine der verheerendsten bekannten Tierseuchen und *A. astaci* ist unter den „100 der weltweit schlimmsten IAS“ gelistet (Lowe *et al.* 2000).

NICS und Krebspest sind eine beständig wachsende Gefährdung für ICS und eine der Hauptursachen für die seit 140 Jahren anhaltenden Bestandrückgänge der ICS (Souty-Grosset *et al.* 2006; Füreder 2009; Holdich *et al.* 2009). Viele ICS sind lokal oder regional ausgestorben. Die verbliebenen Reliktpopulationen sind stark fragmentiert und zunehmend durch invasive NICS bedroht (Holdich *et al.* 2009; Chucholl und Dehus 2011; Reynolds und Souty-Grosset 2012). Der Arterhalt der indigenen Flusskrebse ist in Mitteleuropa gefährdet und alle ICS sind in nationalen oder internationalen Roten Listen gefährdeter Arten aufgeführt (Souty-Grosset *et al.* 2006; Füreder 2009; Reynolds und Souty-Grosset 2012). Der Dohlenkrebs gilt zum Beispiel in Deutschland und Österreich als vom Aussterben bedroht (Rote Liste Kategorie 1), wobei invasive NICS und Krebspest die schwerwiegendsten Gefährdungsursachen darstellen (Petutschnig 2010; Chucholl und Dehus 2011).

Neben der Verdrängung von ICS, können NICS auch verheerende ökologische Schäden verursachen (Nyström 1999, 2002; Reynolds und Souty-Grosset 2012). Flusskrebse sind Allesfresser, die mit praktisch allen trophischen Ebenen interagieren und wurden regelmäßig als ökologische Schlüsselarten identifiziert (zusammengefasst in Momot 1995 und Nyström 2002). NICS können andere Tier- und Pflanzenarten durch direkte trophische

Interaktionen und Konkurrenz aus Ökosystemen eliminieren und können weitreichende indirekte Effekte verursachen, indem sie Schlüsselhabitats oder -ressourcen für andere Arten degradieren. Die Invasion des Roten Sumpfkrebse (*Procambarus clarkii*) in einem Flachwassersee auf der Iberischen Halbinsel führte beispielsweise zu einer Verschiebung des natürlichen Klarwasserzustands in einen trüben Zustand, gefolgt von einem Kollaps Makrophyten-basierter Nahrungsketten. In der Folge wurde die Biodiversität des Gewässers massiv reduziert, wobei 71% der Makroinvertebraten-Gattungen, 83% der Amphibien-Arten und 75% der Enten-Arten völlig verschwanden (Rodríguez *et al.* 2005).

Neue vs. Alte gebietsfremde Flusskrebse

Seit 1880 wurden mindestens 12 NICS in europäische Gewässer eingeführt (zusammengefasst in Tabelle 1). Holdich *et al.* (2009) teilten diese nach Einfuhrzeitraum und Einfuhrvektor in *Alte* und *Neue* NICS ein.

Alte NICS sind Arten, die vor 1975 nach Europa eingeführt wurden. Ursprünglich wurden alle *Alten* NICS absichtlich in europäischen Gewässern angesiedelt, hauptsächlich um die fischereiliche Nutzung und Produktion von Flusskrebsen zu fördern. Sekundäre Verschleppungen kamen regelmäßig vor und passieren auch heute noch, obwohl das Aussetzen dieser Arten inzwischen meist illegal ist (Souty-Grosset *et al.* 2006; Füreder 2009; Chucholl und Dehus 2011). Durch die Ansiedlung der *Alten* NICS wurde primär versucht die seit den 1860ern auftretenden dramatischen Bestandseinbrüche des indigenen Edelkrebse zu kompensieren. Der kommerzielle Fang von Edelkrebsen bildete im 19. Jahrhundert einen integralen Bestandteil der Binnenfischerei, bis die Bestände durch Lebensraumverschlechterung und das Wüten der Krebspest kollabierten (Souty-Grosset *et al.* 2006; Reynolds und Souty-Grosset 2012). Die *Alten* NICS wurden ferner als „ökologischer Ersatz“ für die ICS propagiert, wobei deutliche ökologische Unterschiede zwischen den individuellen Arten ignoriert wurden. Die meisten *Alten* NICS wurden rasch invasiv und alle nordamerikanischen Arten stellten sich hinterher als Carrier der Krebspest heraus. Sie wurden zu einer zusätzlichen, dauerhaften und tödlichen Bedrohung für die verbleibenden ICS Bestände (Souty-Grosset *et al.* 2006; Reynolds und Souty-Grosset 2012 und Zitate darin). Der erhoffte wirtschaftliche Erfolg blieb in Mitteleuropa zudem aus (Souty-Grosset *et al.* 2006). Die Einfuhren der *Alten* NICS fielen dem „Frankenstein-Effekt“ zum Opfer, in Analogie zu der Geschichte von Shelly (1818), in der ursprünglich gute Intentionen, etwas zu verbessern in einem katastrophalen Endergebnis, respektive einem Monster, resultierten.

Neue NICS sind gebietsfremde Flusskrebsarten, die nach 1980 nach Europa eingeführt wurden. In Bezug auf die Einfuhrvektoren ist diese Gruppe wesentlich diverser als die *Alten* NICS (Tabelle 1). *Neue* NICS umfassen Arten, die möglicherweise durch die Aquakultur (*Orconectes virilis* und *Procambarus acutus/Procambarus zonangulus*), die Nutzung als Angelköder (wahrscheinlich *Orconectes immunis*), den illegalen Besitz von Teichen (*Orconectes juvenilis*), oder, in jüngerer Zeit, den Aquarienhandel in das Freiland gelangten (*Procambarus clarkii*, *Procambarus alleni*, Marmorkrebs, *Cherax destructor* und *Cherax quadricarinatus*).

Tabelle 1. Wahrscheinliche Einfuhrvektoren, NICS Status, Invasionsstadium, aktuelle Verbreitung und Herkunft der NICS in Mitteleuropa. Die Abkürzungen für die Einfuhrvektoren sind wie folgt: *A* = Aquarienhandel, *C* = Speisehandel, *FB* = Nutzung als Angelköder, und *S* = absichtlicher Besitz/Aquakultur. Die Begriffe *Alte* und *Neue* NICS wurden von Holdich *et al.* (2009) geprägt, basierend auf dem Einfuhrzeitraum und Einfuhrvektor. Die Abkürzungen für die mitteleuropäische Verbreitung sind wie folgt: *W* = weitverbreitet, *R* = regional, in Ausbreitung begriffen, *L* = lokal, *O* = vorhanden, wenige Vorkommen, *F* = gescheiterte Freisetzung und *P* = vorhanden, keine weiteren Informationen. Literaturnachweis: 1 = Souty-Grosset *et al.* (2006), 2 = Pekny (2003), 3 = Jaklič und Vrezec (2011) und Zompro (2011), 4 = Chucholl (2012), 5 = Chucholl und Daudey (2008), 6 = Soes und Koese (2010), 7 = Chucholl (2011a), 8 = Chucholl *et al.* (im Druck).

Art	Einfuhrvektor(en)	NICS Status	Invasionsstadium	Mittel-europäische Verbreitung	Herkunft	Literaturnachweis
<i>Astacus leptodactylus</i>	S, C	Alt	invasiv	W	Osteuropa	1
<i>Orconectes limosus</i>	S	Alt	invasiv	W	Nordamerika	1
<i>Pacifastacus leniusculus</i>	S	Alt	invasiv	W	Nordamerika	1
<i>Cherax destructor</i>	A	Neu	freigesetzt	F	Australien	2
<i>Cherax quadricarinatus</i>	A	Neu	etabliert	L	Australien	3
<i>Orconectes immunis</i>	FB	Neu	invasiv	R	Nordamerika	4
<i>Orconectes juvenilis</i>	S	Neu	unklar	P	Nordamerika	5
<i>Orconectes virilis</i>	unklar	Neu	invasiv	R	Nordamerika	6
<i>Procambarus acutus/P. zonangulus</i>	unklar	Neu	etabliert	L	Nordamerika	6
<i>Procambarus alleni</i>	A	Neu	unklar	P	Nordamerika	1
<i>Procambarus clarkii</i> *	A, C, S	Neu	invasiv	R	Nordamerika	7
Marmorkrebs (<i>P. fallax</i> f. <i>virginalis</i>)	A	Neu	invasiv	O	Nordamerika	8

* siehe Seite 45 für die Einordnung als *Neue* NICS

Fragestellung

Die übergreifende Zielsetzung der vorliegenden Arbeit war unser Verständnis von biologischen Invasionen im Allgemeinen, und Flusskrebs-Invasionen im Speziellen, durch die Erforschung der Einfuhrvektoren, Lebenszyklus-Strategien und ökologischen Rollen und Auswirkungen von *Neuen NICS* voranzutreiben.

Neue NICS lagen aus den folgenden Gründen im Fokus meiner Dissertation: (1) die Einfuhr/Freisetzung von *Neuen NICS* ist ein noch andauernder Prozess (z.B. Marmorkrebs), (2) viele *Neue NICS* sind offensichtlich sehr erfolgreiche Invasoren (z.B. *Orconectes immunis*), (3) *Neue NICS* weisen ein großes ökologisches und ökonomisches Schadenspotenzial auf (z.B. *P. clarkii*), und (4) die Einfuhrvektoren und Ökologie von *Neuen NICS* sind kaum untersucht.

Im Speziellen habe ich die folgenden Forschungsthemen bearbeitet, die sich in drei Kapitel gliedern, in denen jeweils zwei Publikationen zusammengefasst sind:

Kapitel 1) *Invasoren zum Verkauf: Der Aquarienhandel als Einfuhr-/Freisetzungsvektor von Neuen NICS*

Kapitel 2) *Was macht Invasoren erfolgreich? Lebenszyklus-Strategien von Neuen NICS in Mitteleuropa*

Kapitel 3) *Ökologische Rolle und Auswirkungen von Neuen NICS*

Neben der Förderung unseres Verständnisses von biologischen Invasionen, beleuchten diese Forschungsthemen die mit *Neuen NICS* assoziierten Risiken und Probleme, sowie deren Prävention und Kontrolle, und sind somit auch für Interessengruppen, Behörden und im Umweltmanagement von Interesse.

Im Anhang befinden sich ferner drei Kurzmitteilungen, die, trotz ihres Fokus auf *Neuen NICS*, thematisch nicht in den engeren Rahmen meiner Doktorarbeit fielen. Die Themenstellungen dieser Kurzmitteilungen waren (1) die Klärung der Artidentität einer kürzlich in Ost-Frankreich nachgewiesenen *Neuen NICS* (Chucholl und Daudey 2008), (2) der Erstnachweis einer etablierten Marmorkrebs-Population in Deutschland (Chucholl und Pfeiffer 2010) und (3) die lokale Verbreitung und Invasionshistorie von *P. clarkii* in Süddeutschland (Chucholl 2011b).

Untersuchte Arten

In meiner Doktorarbeit habe ich mich schwerpunktmäßig mit den *Neuen* NICS Kalikokrebs, Roter Sumpfkrebs und Marmorkrebs beschäftigt, die alle Vertreter der nordamerikanischen Cambaridae (Crustacea, Decapoda, Astacida) sind.

Kalikokrebs (*Orconectes immunis* Hagen, 1870)



Orconectes immunis stammt aus dem nordöstlichen und zentralen Nordamerika, wo er in 26 US Bundesstaaten und drei kanadische Provinzen weit verbreitet ist (Hobbs 1989). Die Art wurde Mitte der 1990er erstmals in der Oberrheinebene nachgewiesen, wo sie hohe Populationsdichten erreicht und sich rasant ausbreitet (Gelmar *et al.* 2006; Chucholl und Dehus 2011). *Orconectes immunis* wurde vermutlich als lebender Angelköder von Angehörigen der Kanadischen Streitkräfte eingeführt, die in der Nähe der beiden Erstfundorte stationiert waren (Gelmar *et al.* 2006). Vor meiner Doktorarbeit war nur sehr wenig über die Ökologie von *O. immunis* bekannt, abgesehen von der Verdrängung seines invasiven Vorgängers, dem *Alten* NICS *O. limosus*, von bevorzugten Habitaten (Chucholl 2006).

Roter Sumpfkrebs [*Procambarus clarkii* (Girard, 1852)]



Procambarus clarkii kommt natürlicherweise im Süden der USA und dem nordöstlichen Mexiko vor. Die Art gehört zu den kommerziell wichtigsten Süßwasser-Dekapoden in der Aquakultur und ist auf allen Kontinenten mit Ausnahme von Australien und der Antarktis etabliert (Hobbs 1989; Gherardi 2006 und Zitate darin). Nach Europa gelangte *P. clarkii*

1973, als Spanien die Art zur Förderung der Binnenfischerei absichtlich einführte (Souty-Grosset *et al.* 2006). *Procambarus clarkii* ist daher eine *Alte* NICS in Europa. Die meisten, wenn nicht alle, Populationen in Mitteleuropa sind aber deutlich jüngeren Ursprungs und gehen auf andere Einfuhrvektoren zurück, wie ausgesetzte Aquarientiere oder Abwanderung aus Gartenteichen (Dehus *et al.* 1999; Soes und van Eekelen 2006; Dümpelmann *et al.* 2009). Im Rahmen dieser Arbeit habe ich *P. clarkii* daher als *Neue* NICS in Mitteleuropa betrachtet.

Marmorkrebs [*Procambarus fallax* f. *virginalis* (Hagen, 1870)]



Der Marmorkrebs ist aus drei Gründen eine interessante Art: (1) Marmorkrebse sind die einzigen bekannten dekapoden Krebse, die sich obligatorisch durch apomiktische Parthenogenese vermehren: Es existieren ausschließlich Weibchen, die genetisch uniforme Nachkommen produzieren (Martin *et al.* 2007). (2) Es ist keine autochthone Population bekannt; die Art tauchte Mitte der 1990er unvermittelt im deutschen Aquarienhhandel auf und alle Nachweise weltweit gehen höchstwahrscheinlich auf dieses Vorkommen in der Aquaristik zurück (zusammengefasst in CABI 2011). (3) Der Marmorkrebs war niemals im Speisehandel (mit Ausnahme von Madagaskar, Jones *et al.* 2009) oder der Aquakultur präsent. Alle Freiland-Nachweise in Europa gehen daher höchstwahrscheinlich auf ausgesetzte Aquarientiere zurück.

Über die phylogenetische Stellung und die Artidentität des Marmorkrebses wurde fast eine Dekade gerätselt (CABI 2011). Erst kürzlich zeigten Martin *et al.* (2010), dass Marmorkrebse eine parthenogenetische Linie von *Procambarus fallax* sind, und schlugen die vorläufige wissenschaftliche Bezeichnung *Procambarus fallax* f. *virginalis* vor. *Forma* (f.) wird jedoch von den Internationalen Regeln für Zoologische Nomenklatur nicht anerkannt (Martin *et al.* 2010). Ich habe in dieser Arbeit deshalb vorzugsweise den auch in Publikationen gebräuchlichen und eindeutigen Trivialnamen Marmorkrebs verwendet.

Zusammenfassung der Kapitel

Kapitel 1

Biologische Invasionen sind sequenzielle Prozesse, die Import/Transport, Freisetzung, Etablierung und Ausbreitung von gebietsfremden Organismen umfassen. Ein frühes und entscheidendes Invasionsstadium ist die Freisetzung, das heißt das beabsichtigte oder versehentliche Entlassen eines gebietsfremden Organismus von einer abgeschlossenen Umgebung in die Natur. Die Prävention von neuen Freisetzungen wird oft als primäre Zielsetzung zitiert (Hulme *et al.* 2009; Justo-Hanani *et al.* 2010; CBD 2011), allerdings sind die zugrundeliegenden Mechanismen und Einflussfaktoren oft kaum untersucht (D'Antonio *et al.* 2001; Duggan *et al.* 2006).

Ein neuartiger, und gegenwärtig sehr wahrscheinlich der bedeutendste, Vektor für die Einfuhr/Freisetzung von *Neuen* NICS in Mitteleuropa ist der Handel mit exotischen Flusskrebse für die Aquaristik (Tabelle 1; Holdich *et al.* 2009), der in den letzten zehn Jahren rapide zugenommen hat (Lukhaup und Pekny 2009). Im 1. Kapitel habe ich daher den Handel mit exotischen Flusskrebsarten näher beleuchtet und die bestimmenden Faktoren der Freisetzungswahrscheinlichkeit von Aquarienkrebse analysiert (**Chucholl im Druck**). Auf der Grundlage von vergleichbaren Studien über Aquarienfische, die einen klaren Zusammenhang zwischen ökologischen (z.B. Körperlänge) und ökonomischen (z.B. Popularität) Arteigenschaften und der Wahrscheinlichkeit, dass eine Art im Freiland nachgewiesen wurde, zeigten, habe ich postuliert, dass aus Aquarien freigesetzte Flusskrebsarten (1) häufiger und zu niedrigeren Preisen im Handel angeboten werden und (2) größer werden, als Arten die noch nie freigesetzt wurden.

Um diese Hypothese zu überprüfen, habe ich zwei Datensätze über im Zoohandel erhältliche Flusskrebse gesammelt und mit multiplen Regressionsmodellen analysiert inwieweit der Freisetzungstatus einer Art von Verfügbarkeit, Preis und maximaler Körperlänge abhängt (**Chucholl im Druck**). Der erste Datensatz umfasste Informationen darüber, welche Flusskrebsarten zwischen 2005 und 2009 nach Deutschland importiert wurden und über die generelle Verfügbarkeit der in diesem Zeitraum im Handel erhältlichen Arten. Der zweite Datensatz enthielt etwas objektivere Informationen über Verfügbarkeit und Preis von Flusskrebsarten, die 2009 von Onlineshops angeboten wurden. Außerdem habe ich die potenzielle Invasivität der online angebotenen NICS in einem Screening-Verfahren (FI-ISK) eingeschätzt (Cefas 2008; Tricarico *et al.* 2009). Den Fokus habe ich (1) auf den deutschen Zoohandel und (2) den Online-Handel gelegt, da der deutsche Zoohandel der europaweit größte Importeur von NICS ist (Pekny und Lukhaup 2005) und der Online-Handel

besser zugänglich ist als konventionelle Ladengeschäfte. Außerdem fördert der Online-Handel eine weiträumige Verbreitung der angebotenen Arten, auch über Ländergrenzen hinweg (Faulkes 2010; Peay *et al.* 2010; Soes und Koese 2010).

Insgesamt waren im betrachteten Zeitraum 120 NICS für die Aquarienhaltung verfügbar. Der Großteil dieser Arten (105) stammt aus Nord- oder Mittelamerika. Sie sind daher potenzielle Krebspest-Carrier. Trotz der hohen Zahl an importierten Arten wurden nur 20 NICS häufig oder sehr häufig vom Handel angeboten. Die Importrate seit 2005 betrug durchschnittlich 7 neue Arten pro Jahr, die hauptsächlich aus Nordamerika stammen. Die 16 analysierten Onlinehops boten 2009 mindestens 37 NICS an, von denen 6 hoch-risiko Arten darstellen (FI-ISK Bewertung >16). Fünf dieser hoch-risiko Arten wurden wahrscheinlich aus Aquarien in die freie Natur entlassen: *C. destructor*, *C. quadricarinatus*, Marmorkrebs, *P. alleni* und *P. clarkii*. Die Analyse der zwei Datensätze mit binären multiplen Regressionsmodellen zeigte übereinstimmend, dass die Freisetzungswahrscheinlichkeit einer Art statistisch hoch-signifikant von der maximalen Körperlänge und der Verfügbarkeit abhängt (**Chucholl im Druck**, Tabelle 3, Abbildung 5).

Meine Hypothese, wonach aus Aquarien ausgesetzte NICS ähnliche ökologische und ökonomische Arteigenschaften zeigen, wurde von allen Daten unterstützt, mit Ausnahme des Preises, der keinen signifikanten Effekt auf den Freisetzungsstatus hatte. Die wichtigsten Determinanten der Freisetzungswahrscheinlichkeit einer Art sind maximale Körperlänge und Verfügbarkeit, wobei große Arten (Körperlänge >13 cm), die häufig verfügbar sind, wahrscheinlich ausgesetzt werden. Die Körperlänge einer Art beeinflusst die Wahrscheinlichkeit der absichtlichen Freisetzung vermutlich in zweierlei Weise: (1) große Arten haben einen großen Platzbedarf, der die Kapazität eines Aquariums leicht überschreiten kann und (2) größere Arten können ein Aquarium rasch überfüllen, da sie wesentlich mehr Nachkommen produzieren als kleinere Arten. Hervorzuheben ist, dass die Körperlänge der analysierten NICS positiv mit der potenziellen Invasivität (FI-ISK Bewertung) korrelierte. Freisetzungseignisse sind daher wahrscheinlich mit hoch-risiko Arten assoziiert. *Neue* NICS, die aus Aquarien ausgesetzt werden, sind folglich ebenso gefährliche Invasoren wie viele *Alte* NICS, die bewusst auf eine große Körperlänge, hohe Fruchtbarkeit und Umwelttoleranz hin ausgewählt wurden (Holdich und Gherardi 1999; Lindqvist und Huner 1999; Holdich *et al.* 2009).

Ein Effekt der Verfügbarkeit auf den Freisetzungsstatus war von mir erwartet worden, da eine höhere Verfügbarkeit/Popularität potenziell zu einer höheren Anzahl von

Freisetzungseignissen führt und damit auch zu einem höheren Freisetzungs-Druck (engl.: propagule pressure) (Lockwood *et al.* 2005; Duggan *et al.* 2006; Lockwood *et al.* 2007).

Die Bedeutung der Popularität einer Art für die Freisetzungs- und Etablierungswahrscheinlichkeit ist besonders evident im Falle des Marmorkrebses. Für die zweite Studie in diesem Kapitel habe ich erstmals eine Zusammenstellung aller verifizierbaren Nachweise des Marmorkrebses in Europa erarbeitet. Die Ergebnisse belegen einen starken Anstieg der etablierten Populationen innerhalb der letzten drei Jahre (**Chucholl *et al.* im Druck**). Da zwischen Freisetzungseignis und dem Nachweis im Freiland wahrscheinlich eine längere Latenzphase liegt, gehen diese ansteigenden Populationszahlen vermutlich auf Freisetzungseignisse zurück, die mehrere Jahre zurückdatieren – wahrscheinlich um die Zeit als die Haltung von Marmorkrebsen im Aquarium populär wurde (**Chucholl *et al.* im Druck**, Abbildung 3). Die zunehmende Verbreitung von Marmorkrebsen in der Aquaristik führte wahrscheinlich zu einem höheren Freisetzungs-Druck und damit einer höheren Etablierungswahrscheinlichkeit in der Natur (Lockwood *et al.* 2005; Duggan *et al.* 2006; Lockwood *et al.* 2007). Durch eine hohe Anzahl unabhängiger Freisetzungseignisse werden negative Faktoren überwunden, die räumlich strukturiert sind, also etwa ein unpassendes Habitat am Ort der Freisetzung (Lockwood *et al.* 2005; Duggan *et al.* 2006). So konnten sich Marmorkrebse in kühlen, rasch-fließenden Bächen offensichtlich nicht etablieren, dafür gelang es der Art schließlich in Stillgewässern reproduzierende Bestände aufzubauen (**Chucholl *et al.* im Druck**, Tabelle 1). Als weitere Folge von vielen unabhängigen Freisetzungseignissen ist die aktuelle europäische Verbreitung des Marmorkrebses sehr zerstreut und disjunkt, wobei jede Population eine potenzielle Node für eine weitere aktive Ausbreitung darstellt (**Chucholl *et al.* im Druck**, Abbildung 2).

Zusammenfassend betrachtet habe ich gezeigt, dass der Handel mit lebenden exotischen Flusskrebsen ein tatsächliches Risiko darstellt. Die Propagierung von NICS in der Aquaristik hat einen erheblichen Freisetzungs-Druck zur Folge, wodurch die Zahl etablierter Populationen *Neuer* NICS wahrscheinlich weiter zunehmen wird. Die Entwicklung von Maßnahmen zur Prävention von neuen gefährlichen Freisetzungen erscheint dringend nötig. Dies ist insbesondere vor dem Hintergrund wichtig, dass bisher weder in Deutschland noch in der EU Regelungen für den Import, Handel und die Haltung von NICS bestehen (Genovesi 2007; Hulme *et al.* 2009; Shine *et al.* 2009).

Kapitel 2

Im Mittelpunkt des 2. Kapitels stand die Aufklärung des Lebens- und Reproduktionszyklus von *Neuen* NICS in Mitteleuropa, mit dem Ziel, die Invasivität dieser Arten besser zu verstehen. Speziell habe ich die Populationsökologie von *P. clarkii* und *O. immunis* untersucht (**Chucholl 2011a** und **Chucholl 2012**). *Procambarus clarkii* war von besonderem Interesse, da die Art in subtropischen Breiten heimisch ist, aber in letzter Zeit auch in kühleren Klimaten höherer Breiten invasiv wurde. *Orconectes immunis* ist dagegen in gemäßigten Zonen heimisch. Allerdings ist der Lebenszyklus der Art in Europa fast völlig unbekannt, obwohl sie hoch-invasiv ist. Erfolgreiche aquatische Invasoren besitzen häufig *r*-selektierte Lebenszyklen (Kley und Meier 2006; Füreder und Pöckl 2007; Grabowski *et al.* 2007), weshalb ich postuliert habe, dass beide NICS Eigenschaften eines *r*-Strategen aufweisen.

Die Populationsökologie von *P. clarkii* wurde von mir von 2007 bis 2009 in zwei mesotrophen Baggerseen im Donauried östlich von Ulm untersucht und die Populationsökologie von *O. immunis* 2007 in einem typischen Auengewässer entlang des Oberrheins. In beiden Studien habe ich standardisierte Reusenfänge zur Erfassung der Populationsdynamik verwendet. Die Lebenszyklusparameter der Populationen (Wachstumsrate, asymptotische Körperlänge, maximale Lebenserwartung, durchschnittliche Lebenserwartung und Mortalität) habe ich mithilfe der Von Bertalanffy Wachstumsfunktion (VBGF) aus den Längenverteilungen zu verschiedenen Zeitpunkten abgeleitet. Den Reproduktionszyklus habe ich anhand des gonadosomatischen Index (Verhältnis Gonadengewicht zu Körpergewicht), äußerlicher reproduktiver Merkmale, sowie der reproduktiven Form der gefangenen Krebse charakterisiert. Geschlechtreife Cambariden weisen einen zyklischen Dimorphismus zwischen einer sexual aktiven (I) und einer sexuell inaktiven Form (II) auf. Die reproduktive Form alterniert normalerweise mit jeder Häutung, das bedeutet, ein Individuum in Form I häutet sich in Form II und umgekehrt. In beiden Studien habe ich die zeitliche Variation des Anteils von Form I Individuen am Gesamtfang benutzt, um den Reproduktionszyklus aufzuklären. Im Falle von *O. immunis* habe ich mit dieser Methode ferner die Häutungsphasen der Adultpopulation beschrieben.

Die Wachstumsrate (*k*: Krümmungsparameter der VBGF, d.h. die Rate, mit der die asymptotische Körperlänge erreicht wird) von *P. clarkii* in Süddeutschland war geringer (0,45–0,49 Jahr⁻¹) als in niederen Breitengraden, während die maximale Lebenserwartung (6,1–6,6 Jahre), durchschnittliche Lebenserwartung (3,5–4,0 Jahre) und asymptotische Körperlänge (75–80 mm Carapaxlänge, *CL*) beträchtlich größer waren. Die Reproduktion war univoltin und konzentrierte sich von August bis Winteranfang, wobei ein kleiner Teil der

Weibchen auch während des Winters noch Eier trug. Dieses Reproduktionsmuster kontrastiert deutlich mit dem multivoltinen Reproduktionszyklus von *P. clarkii* in niederen Breiten, der von ganzjähriger Reproduktion mit mehreren Rekrutierungspeaks pro Jahr geprägt ist (**Chucholl 2011a**, Abbildung 9).

Die Wachstumsrate (k) von *O. immunis* war mit $1,15\text{--}1,22 \text{ Jahr}^{-1}$ vergleichsweise sehr hoch und fiel mit einer kurzen Lebenserwartung (maximal 2,5, durchschnittlich 2,1 Jahre) und einer geringen asymptotischen CL (50 mm) zusammen. Die Adultpopulation häutete sich während der Sommermonate bis zu viermal, wobei die sexuell inaktive Form (II) nur für eine bemerkenswert kurze Zeitdauer beibehalten wurde (**Chucholl 2012**, Abbildung 2). Die meisten Weibchen der untersuchten Population hatten wahrscheinlich bereits im Herbst Eier gelegt und trugen diese über den Winter aus. Diese Eiablage im Herbst ist ein distinkter Unterschied zu anderen *Orconectes*-Arten, wie dem Alten NICS *O. limosus*, die ihre Eier typischerweise erst im Frühjahr legen (Souty-Grosset *et al.* 2006). Jungtiere von *O. immunis* schlüpfen teilweise schon im März und können bereits zum Ende des ersten Lebenssommers geschlechtsreif werden. Dies stellt wahrscheinlich eine einzigartige Lebenszyklus-Eigenschaft dar, da von keiner anderen NICS oder ICS in Mitteleuropa ein Erreichen der Geschlechtsreife im ersten Lebenssommer bekannt ist.

Meine Ergebnisse legen nahe, dass *O. immunis* ein ausgeprägter r -Strategie ist. Dies kontrastiert mit den Lebenszyklen der ICS, die eher K -Strategen sind. ICS weisen eine deutlich höhere Lebenserwartung auf, wachsen langsamer und produzieren deutlich weniger Nachkommen (zusammengefasst in **Chucholl 2012**, Tabelle 4). *Procambarus clarkii* weist ebenfalls Eigenschaften eines r -Strategen auf, darunter eine sehr hohe Fekundität. Gleichzeitig zeigt der Lebenszyklus von *P. clarkii* in unseren gemäßigten Breiten aber im Vergleich zu seinem Lebenszyklus in niederen Breiten auch tendenziell K -selektierte Eigenschaften, wie eine geringere Wachstumsrate und eine deutlich höhere Lebenserwartung (**Chucholl 2011a**, Abbildung 8). Diese bemerkenswerte Lebenszyklus-Plastizität unterstreicht die Notwendigkeit, den effektiven Lebenszyklus einer IAS entlang verschiedener Umweltgradienten zu untersuchen (Bufford und Daehler 2011). Mit Blick auf ein Populationsmanagement implizieren die r -selektierten Eigenschaften von *P. clarkii* und *O. immunis* eine hohe Widerstandsfähigkeit gegenüber zeitlich limitierten Bekämpfungsmaßnahmen (z.B. Reusenfang), da die Populationen den Verlust von Individuen sehr rasch ausgleichen können (Gherardi *et al.* 2011).

Kapitel 3

Im 3. Kapitel habe ich mich schließlich den ökologischen Rollen und Auswirkungen von *Neuen* NICS zugewandt. Vergleichbaren Studien haben gezeigt, dass invasive Flusskrebse dazu tendieren autochthone Flusskrebsarten zu verdrängen (Capelli und Munjal 1982; Hill und Lodge 1994; Gherardi und Daniels 2004) und aquatische Gemeinschaften fundamental verändern können (Nyström 1999, 2002). Davon ausgehend habe ich postuliert, dass *Neue* NICS starke ökologische Auswirkungen haben, sowohl auf andere Flusskrebsarten, als auch auf litorale Biozönosen. Um diese Hypothese zu überprüfen, habe ich (1) die beobachtete Artverdrängung zwischen dem *Alten* NICS *O. limosus* und dem *Neuen* NICS *O. immunis* untersucht (**Chucholl et al. 2008**) und (2) die trophische Rolle und die ökologischen Auswirkungen von *P. clarkii* in Seen in Süddeutschland erforscht (**Chucholl eingereicht**).

Zeitgleich mit der Ausbreitung von *O. immunis* in der Oberrheinebene kam es zu einem Rückgang seines invasiven Vorgängers *O. limosus*. Direkte aggressive Interaktionen zwischen verschiedenen Flusskrebsarten werden als wichtiger Verdrängungsmechanismus angesehen. Ich habe deshalb in Laborexperimenten aggressive Interaktionen und Konkurrenz um Verstecke zwischen diesen beiden NICS untersucht. Direkte aggressive Interaktionen zwischen heterospezifischen 1:1 Paarungen wurden von mir beobachtet und mit einem etablierten Ethogram der Kampfdynamik von Flusskrebsen analysiert. Zusätzlich habe ich Experimente zur Versteckaffinität und Konkurrenz um Verstecke durchgeführt, um zu verifizieren, ob die Ergebnisse der Versuche zum direkten agonistischen Verhalten konsistent sind zu den Ergebnissen, wenn beide Arten um eine limitierte Ressource (Verstecke) konkurrieren. Der Großteil der Laborversuche wurde bereits im Rahmen meiner Diplomarbeit durchgeführt, einige Experimente wurden aber in meiner Doktorarbeit fortgeführt (**Chucholl et al. 2008**). Die ursprüngliche Hypothese, wonach *O. immunis* in direkten Interaktionen über *O. limosus* dominant ist, wurde von den Ergebnissen klar unterstützt. Selbst 4 mm kleinere *O. immunis* waren dominant über größere *O. limosus* und *O. immunis* war auch in der Konkurrenz um Verstecke klar überlegen (**Chucholl et al. 2008**).

Ergänzend zu der Laborstudie habe ich die Mechanismen der Artverdrängung in einer nachfolgenden Freilandstudie weiter erforscht, indem ich die Reproduktionsbiologie der beiden Arten in einem gemischten Bestand verglichen habe (beinhaltet in **Chucholl 2012**, Kapitel 2). Jungtiere von *O. immunis* schlüpfen saisonal früher als *O. limosus* Jungtiere (**Chucholl 2012**, Abbildung 5), und können bereits am Ende des ersten Lebenssommers, und damit deutlich früher als *O. limosus*, die Geschlechtsreife erreichen. Der frühere

Schlupftermin und das schnellere Erreichen der Geschlechtsreife verschaffen *O. immunis* wahrscheinlich einen zusätzlichen kompetitiven Vorteil gegenüber *O. limosus*.

Insgesamt wurde deutlich, dass *O. immunis* ein konkurrenzstarker Invasor ist, der *O. limosus* wahrscheinlich aufgrund von Überlegenheit in direkten Interaktionen und eines rascheren Lebenszyklus von bevorzugten Habitaten verdrängt. Ausgehend von diesen Erkenntnissen erwarte ich, dass *O. immunis* auch das Potenzial hat, ICS direkt auszukonkurrieren, zusätzlich zu einer möglichen Übertragung des Krebspestereggers (Maiwald *et al.* 2009). *Orconectes immunis* stellt daher eine ernsthafte Bedrohung für die Restbestände der gefährdeten ICS dar.

Zur Erforschung der trophischen Rolle und ökologischen Auswirkungen von *P. clarkii* habe ich zwei komplementäre Ansätze verwendet (**Chucholl eingereicht**): (1) Mageninhaltsanalysen zur Untersuchung der Ernährungsbiologie und (2) einen *in situ* Enclosure-Versuch zur Quantifizierung des ökologischen Einflusses von *P. clarkii* auf eine typische litorale Biozönose eines Sees.

Für die Mageninhaltsanalyse wurden von mir im Juni und September 2007 aktive Flusskrebse in der Uferzone eines kleinen, mesotrophen Baggersees gefangen und anschließend im Labor seziiert und der Mageninhalt untersucht. Um die systematischen Fehler, die entstehen, wenn nur eine Methode zur Quantifizierung des Mageninhalts verwendet wird, zu minimieren, habe ich zwei Quantifizierungsmethoden benutzt und daraus für jede Nahrungskategorie, respektive jeden Beuteorganismus, eine relative Gewichtung (engl.: relative importance index, *RI*) errechnet. Der *RI* der Beuteorganismen wurde anschließend mit der relativen Abundanz der Beuteorganismen in der Uferzone verglichen und aus beiden Werten ein Beuteselektions-Index berechnet.

Mit dieser Methode konnte ich klar zeigen, dass *P. clarkii* ein polytropher Omnivore ist, der sich von Makrophyten, Detritus und Makroinvertebraten ernährt (**Chucholl eingereicht**, Abbildung 1). Die Anzahl kleiner und agiler Beuteorganismen im Magen korrelierte negativ mit der Größe der Krebse, was auf eine ontogenetische Veränderung der Nahrungspräferenz hindeutet. Bemerkenswert war der Nachweis von terrestrischen Insekten, wie Hymenoptera und Scolytinae, im Mageninhalt von großen Krebsen. Wahrscheinlich wurden diese Insekten von den Krebsen von der Wasseroberfläche oder von Land abgesammelt. Die große Häufigkeit von Makroinvertebraten in der Nahrung von *P. clarkii* unterstreicht die Bedeutung von Flusskrebsen als funktionale Räuber (Momot 1995). Indem *P. clarkii* auf bevorzugte Beuteorganismen einen selektiven Räuberdruck ausübt, können für

Prädation anfällige Makroinvertebraten von den Krebsen rasch eliminieren werden (siehe unten). Insgesamt erhöht *P. clarkii* deutlich den Verknüpfungsgrad des Nahrungsnetzes und interagiert mit allen trophischen Stoffwegen und Energieflüssen. Die Art kann sich von fast jeglichem organischen Material ernähren, wodurch hohe Individuendichten erreicht und aufrechterhalten werden können.

Der fünfwöchige *in situ* Enclosure-Versuch wurde in einem mesotrophen See im Sommer 2008 durchgeführt. Das Versuchsdesign bestand aus drei Treatments, die sich in der eingesetzten Krebsdichte unterschieden (0, 2,5 und 5 Krebse m⁻²) und jeweils fünf Replikate umfassten. Jeder der 0,9 m × 0,9 m × 0,9 m Versuchskäfige wurde vor Versuchsbeginn mit den basalen Elementen eines typischen litoralen Nahrungsnetzes ausgestattet: Detritus (vorbehandeltes Laub), Primärproduzenten (drei Makrophytenarten), benthische Primärkonsumenten (drei Süßwasserschneckenarten) und benthische Planktivore (*Dreissena polymorpha*). Von den Krebsen entwurzelte, aufschwimmende Makrophyten-Fragmente wurden während des Versuchs regelmäßig von den Versuchskäfigen abgesammelt, gewogen und anschließend wieder verankert, um zwischen dem Makrophyten-Verlust durch Beweidung und dem Makrophyten-Verlust durch Entwurzelung unterscheiden zu können.

Wie von mir erwartet, hatte *P. clarkii* einen statistisch signifikanten Effekt auf die finale Makrophyten-Biomasse. Der Einflussmechanismus und das Resultat unterschieden sich allerdings sehr stark zwischen den Makrophytenarten (**Chucholl eingereicht**, Abbildungen 2-4): *Procambarus clarkii* führte zu einem starken Rückgang des indigenen Ährigen Tausendblatts (*Myriophyllum spicatum*), hauptsächlich aufgrund von Entwurzelung, und von Armluchteralgen (*Chara* sp.), hauptsächlich aufgrund von Beweidung, hatte aber keinen signifikanten Effekt auf die gebietsfremde Schmalblättrige Wasserpest (*Elodea nuttallii*). Die relative Abundanz dieser Makrophytenarten wurde dadurch zugunsten der Wasserpest verschoben. Die Krebse hatten ferner einen ausgeprägten, dichteabhängigen Effekt auf die finale Abundanz der Süßwasserschnecken und führten zu einem beschleunigtem Abbau von Detritus (**Chucholl eingereicht**, Abbildung 5 und 6).

Die Ergebnisse der Mageninhaltsanalyse und des *in situ* Enclosure-Versuchs lassen übereinstimmend darauf schließen, dass *P. clarkii* eine Schlüsselart ist, die Biozöosen *via* direkte trophische Interaktionen und nicht-trophische Effekte grundlegend verändern kann. Anfällige Makrophyten und Makroinvertebraten (z.B. *M. spicatum* und Süßwasserschnecken) wurden bei hohen Krebsdichten vollständig eliminiert. Überraschenderweise wurden andere IAS, wie *E. nuttallii* und *D. polymorpha*, von *P. clarkii* aber nicht beeinträchtigt, sondern möglicherweise sogar noch indirekt begünstigt. Speziell *E. nuttallii* schien von *P. clarkii* zu

profitieren. Umgekehrt sind *E. nuttallii*-Bestände vermutlich auch für *P. clarkii* von Vorteil, da die Wasserpest eine wichtige Nahrungsressource für *P. clarkii* darstellt und kleinen Krebsen wahrscheinlich abundante Versteckmöglichkeiten bietet. Beide IAS zeigen möglicherweise einen indirekten positiven Mutualismus. Positiven Mutualismen zwischen IAS sind ein regelmäßig beobachtetes Phänomen bei multiplen biologischen Invasionen und können die Dominanz von IAS fördern („invasional meltdown“; Simberloff und Von Holle 1999; Von Holle 2011).

Schlussfolgerungen

Während viele Freisetzungen aus Aquarien als vergleichsweise harmlos gelten (Duggan 2011), stellen aus Aquarien freigesetzte *Neue* NICS eine deutliche Ausnahme hiervon dar: (1) Freisetzungen von *Neuen* NICS sind wahrscheinlich mit hoch-risiko Arten assoziiert, die eine hohe potenzielle Invasivität aufweisen (**Chucholl im Druck**), (2) *Neue* NICS stellen eine ernsthafte Bedrohung für die gefährdeten ICS dar (**Chucholl 2012**) und (3) *Neue* NICS können gravierende ökologischen Auswirkungen haben (**Chucholl eingereicht**). Die Prävention von neuen Freisetzungen ist daher wichtig (CBD 2011), zumal eine Eradikation von NICS selten Erfolg hat und ausschließlich mit massivem Biozid-Einsatz gelingt (Holdich *et al.* 1999; Sandodden und Johnsen 2010; Gherardi *et al.* 2011). Die Freisetzungswahrscheinlichkeit einer Art war stark von deren Verfügbarkeit bestimmt (**Chucholl im Druck**, Tabelle 3, Abbildung 5). Alle Anstrengungen zur Verringerung des Risikos von gefährlichen Freisetzungen aus Aquarien sollten daher darauf abzielen, die Verfügbarkeit von hoch-risiko Arten drastisch zu senken. Eine Regulation des Imports, Handels und der Haltung von NICS würde die Freisetzungswahrscheinlichkeit von hoch-risiko Arten wahrscheinlich deutlich verringern (Souty-Grosset und Reynolds 2009; Justo-Hanani *et al.* 2010).

Der Invasionserfolg einer gebietsfremden Art wurde oft mit Arteigenschaften, wie Lebenszyklus-Strategie, Plastizität oder Nahrungsgeneralismus in Zusammenhang gebracht (zusammengefasst in Bufford und Daehler 2011 und Rejmánek 2011). Ein konsistentes Merkmal, das Invasivität generell erklärt, ist allerdings nur schwer fassbar, weshalb der Freisetzungs-Druck als Schlüssel-Element für den Etablierungserfolg vorgeschlagen wurde (Lockwood *et al.* 2005). Meine Ergebnisse legen nahe, dass alle diese Elemente eine Rolle spielen in der Invasivität von *Neuen* NICS.

Ein hoher Freisetzungs-Druck aus der Aquaristik ist höchstwahrscheinlich die Ursache für den Etablierungserfolg des Marmorkrebses in Mitteleuropa (**Chucholl *et al.* eingereicht**). Dasselbe gilt möglicherweise auch für *P. clarkii*, der ähnlich populär ist in der Aquaristik. Der

Invasionserfolg von *O. immunis* kann dagegen kaum über einen hohen Freisetzungs-Druck erklärt werden. Die Art wurde vermutlich nur einmal ausgesetzt und wegen ihrer geringen Größe und Verfügbarkeit wird sie wahrscheinlich nicht aus Aquarien freigesetzt. In Kapitel 2 konnte ich zeigen, dass *O. immunis* ein ausgeprägter *r*-Strategie ist, was ein stetiges, aber nicht exklusives oder konsistentes Merkmal von erfolgreichen IAS ist (Kley und Maier 2006; Füreder und Pöckl 2007; Bufford und Daehler 2011). Speziell weist *O. immunis* eine hohe Fekundität auf und besitzt den schnellsten bekannten Lebenszyklus unter den in Mitteleuropa vorkommenden Flusskrebsarten (zusammenfasst in **Chucholl 2012**, Tabelle 4). Diese Arteigenschaften tragen wahrscheinlich wesentlich zu seinem hohen Invasionserfolg bei. *r*-Strategen investieren einen Großteil ihrer Ressourcen in Wachstum und Reproduktion und der resultierende rasche Lebenszyklus und die hohe Reproduktionskapazität werden in allen Stadien des Invasionsprozesses als vorteilhaft angesehen (Lindqvist und Huner 1999; Bufford und Daehler 2011). *Procambarus clarkii* zeigt zusätzlich zu einer sehr hohen Fekundität eine bemerkenswerte Plastizität des Lebens- und Reproduktionszyklus (**Chucholl 2011a**), was vermutlich ein wichtiger Grund für die überraschend hohe Invasivität dieser Art in höheren Breitengraden ist (Lindqvist und Huner 1999; Capinha *et al.* 2011; Rejmánek 2011). Zuletzt fördert auch die omnivore Ernährung von Flusskrebsen, wie in meiner Doktorarbeit für *O. immunis* und *P. clarkii* gezeigt (**Chucholl 2012**, **Chucholl eingereicht**), die Invasivität von NICS, da hiermit eine hohe Flexibilität in der Ressourcennutzung verbunden ist (Bufford und Daehler 2011; Rejmánek 2011).

Omnivore NICS lassen sich nicht ohne weiteres in Nahrungsketten-Modelle einordnen, weshalb die Vorhersage oder Generalisierung ihrer Auswirkungen auf die aufnehmenden Biozönosen schwierig ist. Ein vorherrschendes Charakteristikum unter den ökologischen Auswirkungen ist jedoch ein starker top-down Einfluss auf Süßwasserschnecken und großwüchsige einsprossige Makrophyten, was durch meine Arbeit weiter bekräftigt wird (zusammengefasst in **Chucholl eingereicht**, Tabelle 3). Ein neuartiger Aspekt war dagegen, dass hohe Dichten von *P. clarkii* wahrscheinlich in *Elodea*-dominierten Habitaten resultieren. Dieses Szenario entspricht der Situation in dem untersuchten See, in dem *E. nuttallii* und *P. clarkii* seit Anfang der 2000er in hohen Dichten koexistieren. Diese Koexistenz zwischen einer abundanten Makrophytenart und *P. clarkii* kennzeichnet einen Unterschied zu den Auswirkungen von *P. clarkii* auf der Iberischen Halbinsel. Dort hat die Invasion von *P. clarkii* in Makrophyten-dominierte Habitate einen raschen Kollaps der Makrophyten-Bestände verursacht (Rodríguez *et al.*, 2005). Die überraschende Beobachtung, dass *E. nuttallii* und *P. clarkii* möglicherweise einem indirekten positiven Mutualismus

unterliegen, unterstreicht die Notwendigkeit, bei der Einschätzung der Auswirkungen von IAS auf Biozönosen auch Interaktionen zwischen IAS zu berücksichtigen (Simberloff und Von Holle 1999; Von Holle 2011).

Zusammenfassend betrachtet, legen meine Studien folgende Schlussfolgerungen nahe: (1) der Zoohandel ist ein wichtiger Einfuhr-/Freisetzungsvektor von *Neuen* NICS, indem ein großer Freisetzungs-Druck generiert wird, (2) *r*-selektierte Arteigenschaften und Plastizität fördern die Invasivität von *O. immunis* und *P. clarkii* und (3) *O. immunis* und *P. clarkii* sind ökologische Schlüsselarten, die Zusammensetzung und Struktur von Biozönosen gravierend verändern können. Neue Freisetzungen sind wahrscheinlich mit hoch-risiko Arten assoziiert, weshalb ein Management des Einfuhr-/Freisetzungsvektors dringend nötig erscheint. Bereits etablierte *Neue* NICS sollten kontrolliert und ihre weitere Ausbreitung verhindert werden, insbesondere dort, wo sie sensitive Ökosysteme oder ICS-Populationen gefährden.

Part 2
–
PUBLICATIONS
and
MANUSCRIPTS

Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish

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Abstract

The trade of live ornamental freshwater crayfish has grown rapidly in the last decade and has become the major pathway for new non-indigenous crayfish species (NICS) introductions into Europe. Here, I report on the German ornamental crayfish trade, the main importer of non-indigenous crayfish into Europe. In total, 120 NICS have been available as ornamental aquarium species. One hundred and five species originate from North or Central America and are therefore suspected to be crayfish plague vectors. The import rate since 2005 was estimated to be seven new species per year. Despite many species being imported, only eleven species were found to be very common in the trade. In 2009, 16 online shops offered at least 37 NICS. The availability, price, and size of the offered species were used to predict their introduction status. Multiple binary logistic regression analysis showed that species' availability and size were the principal predictors of the likelihood of being recorded as introduced from aquaria. NICS introduced from aquaria were found to be more available and larger than those present only in aquaria, and their potential invasiveness was also higher. The findings are consistent with the propagule pressure hypothesis in that a greater availability is likely related to more release events, and large species may be released more frequently as a result of overpopulating or outgrowing their aquaria. Efforts to mitigate the risk of further harmful crayfish introductions from aquaria should aim to drastically reduce the availability of high-risk species.

Key words: live animal trade, introduction pathway, alien crayfish, aquarium discards

Introduction

Non-indigenous species are recognized as an increasing major threat to global biodiversity and are considered to be the second most important cause of global biodiversity change after direct habitat destruction (CBD 2000). Non-indigenous species may perturb ecosystem functioning, wreak ecological havoc and compete with or even replace indigenous species (Lodge *et al.* 2000; Sala *et al.* 2000; McGeoch *et al.* 2010). Invasion pathways are mostly

human assisted mechanisms that move organisms outside of their natural range, either as a part of a commodity or by a transport vector (Hulme *et al.* 2008). The prevention of new introductions (either intentionally or by accident) is considered a major and primary objective (Hulme *et al.* 2009). This is especially true when the potential invader may act as keystone species or is a known disease carrier. One pathway of introduction of non-indigenous aquatic animals, such as freshwater fishes and snails, is through the import of live animals in aquarium and ornamental trades (Padilla and Williams 2004; Duggan 2010; Soes *et al.* 2011). There is no international regulatory framework for the trade of live animals, but regulations may be implemented on a national basis, *e.g.*, through a ban of potentially invasive species (Holdich and Pöckl 2005; Simons and De Poorter 2009; Justo-Hanani *et al.* 2010).

Freshwater crayfish (Crustacea, Decapoda, Astacida) are the largest mobile freshwater invertebrates. They are polytrophic omnivores and are frequently recognized as keystone members of littoral food webs (Nyström 2002). The three indigenous crayfish species (ICS) of central Europe face competition from at least twelve non-indigenous crayfish species (NICS; Souty-Grosset *et al.* 2006; Holdich *et al.* 2009; Table 1). NICS can have devastating effects on native ecosystems (Rodriguez *et al.* 2005) and displace the indigenous European crayfish (Souty-Grosset *et al.* 2006; Holdich *et al.* 2009). Eight of the NICS currently present in central Europe originate from North America and can be latent carriers of the crayfish plague, a disease caused by the parasitic Oomycete *Aphanomyces astaci* Schikora 1906, which is fatal for all European ICS. The crayfish plague was first reported in Europe in 1859 and inflicted pan-European mass mortalities in the susceptible European crayfish species within a few decades (Alderman 1997). The indigenous European crayfish stocks collapsed rapidly, and up to 95 % of the populations were lost because of the crayfish plague. Since the initial crayfish plague outbreak, at least four new strains of the crayfish plague have been introduced into European waters through the import of plague resistant and infected NICS from North America (Royo *et al.* 2004). The noble crayfish (*Astacus astacus*) has virtually disappeared from lowland rivers, which are its natural prime habitat, and most remaining populations have been artificially stocked and are spatially isolated (Souty-Grosset *et al.* 2006). Populations of ICS have continued vanishing at an alarming rate in most Central European countries during the last decades and are considered as 'critically endangered' (*A. astacus* and *Austropotamobius pallipes*) and 'endangered' (*Au. torrentium*) in Germany (Schulz *et al.* 2009; Chucholl and Dehus 2011). In France, *A. astacus* and *Au. torrentium* are now close to extinction (Holdich *et al.* 2009). All ICS are protected under the EU Habitats Directive and Special Areas of Conservation (SACs) have been set up to protect ICS and their habitats.

The presence and ongoing spread of NICS are among the greatest threats to the remaining European ICS stocks; in addition, they seriously jeopardize the success of reintroduction and conservation efforts (reviewed by Holdich *et al.* 2009; Souty-Grosset and Reynolds 2009). NICS have become widespread and abundant during the last 120 years, and virtually all countries now harbor at least one NICS (Souty-Grosset *et al.* 2006; Holdich *et al.* 2009). Holdich *et al.* (2009) coined the terms 'Old NICS' and 'New NICS' for naturalized NICS based on their introduction history and time. Early introductions of the 'Old NICS' *Orconectes limosus*, *Pacifastacus leniusculus*, and *Procambarus clarkii* were made

intentionally to compensate for the loss of the ICS stocks and to meet crayfish market demands. Similarly, *Astacus leptodactylus*, a Pontocaspian species that is susceptible to the crayfish plague, was introduced from Eastern Europe (Souty-Grosset *et al.* 2006). However, the crayfish harvest in Central Europe has never returned to pre-plague levels, and the food market demand has declined. Stocking of NICS is currently illegal in most European countries, and recent discoveries of 'New NICS' are the result of illegal stocking activities (Chucholl and Daudey 2008), one possible bait introduction (*Orconectes immunis*; Gelmar *et al.* 2006) and, more recently, garden pond escapes and aquarium releases (Dümpelmann *et al.* 2009; Marzano *et al.* 2009; Chucholl and Pfeiffer 2010).

Peay (2009) stated that the aquarium trade is the most likely source of new crayfish species introductions into Europe. There is striking evidence to suggest that keeping ornamental crayfish is responsible for the repeated release of an additional 'New NICS', the Marmorkrebs (*Procambarus fallax f. virginalis*), into European waters (Marten *et al.* 2004; Marzano *et al.* 2009; Chucholl and Pfeiffer 2010). Most *Procambarus clarkii* populations in Central Europe are also believed to originate from aquarium releases (Dehus *et al.* 1999b; Knuth 1999; Dümpelmann *et al.* 2009), as are single specimens of two *Cherax* species (*C. destructor* and *C. quadricarinatus*) that were discovered in Switzerland, Austria, the Netherlands, England and Germany (Stucki and Jean-Richard 1999; Pekny 2003; Holdich *et al.* 2009; Schulz *et al.* 2009). Furthermore, there are rumours of exotic 'blue crayfish' being found in or stocked in ponds in Germany.

The market for ornamental crayfish as pet species has grown rapidly in the last decade. New species have been imported at a very high rate, and keeping crayfish is now widespread and common among German aquarium enthusiasts (Pekny and Lukhaup 2005; Lukhaup and Pekny 2009). The 'exotic invertebrates boom' began in the late 1990s, when only a small number of species were available (chiefly *P. clarkii* and the Australian species *C. destructor* and *C. quadricarinatus* imported for commercial food sales; Schlüter 1989; Werner 1993; Pekny and Lukhaup 2005). The retail aquarium trade currently offers a remarkable variety of exotic invertebrate species, including more than 40 freshwater crayfish species from all around the world (Figure 1; Pekny and Lukhaup 2005; Lukhaup and Pekny 2009; Soes and Koese 2010). Pekny and Lukhaup (2005) reported that a total of 74 non-indigenous crayfish species had been imported to Central Europe by 2005 and that further imports of new species were likely. Some tropical *Cherax* species are farmed in south-eastern Asia for the ornamental crayfish trade (Figure 1A, B), including some species that have not yet been scientifically described (Lukhaup and Pekny 2005; Lukhaup and Pekny 2006; Lukhaup and Pekny 2008). Many cambarid species have been imported in low numbers by individuals from North and Central America (Figure 1C, D). Some of the imported species are of conservation concern within their indigenous range and Georgia has already banned the export of crayfish for the pet trade (Skelton 2010).

In the present study, I report on the trade of live ornamental freshwater crayfish in Germany. Freshwater crayfish are suitable model organisms and are associated with a high level of interest because new NICS could cause severe ecological damage and seriously threaten the remaining ICS stocks. For instance, North American crayfish species are a vector for the crayfish plague and could bring new, more aggressive or resistant crayfish plague

strains with them. Recent experience has shown that NICS eradication is rarely successful, even under optimal preconditions, and relies on brute-force chemical methods (Sandodden and Johnsen 2010). The prevention of new introductions is therefore imperative, and the trade of live crayfish should be followed carefully.

Based on related research that has suggested that relationships exist between a species' ecological (*e.g.*, size) and economic (*e.g.*, popularity) traits and the likelihood to be recorded as introduced or established (Duggan *et al.* 2006; Duggan 2011), I hypothesized that NICS introduced from aquaria share similar ecological and economic traits. Specifically, these species are expected to be widely available and inexpensive in the aquarium trade and to be typically larger than aquarium crayfish species that have not been introduced. To test this hypothesis, I analyzed the crayfish species in the German pet trade with regard to species' availability, pricing, and size, and examined the effect of these traits on the likelihood of being recorded as introduced from aquaria using multiple binary logistic regression analysis. In addition, I assessed the potential invasiveness of the offered species using an invasiveness screening tool (FI-ISK; Cefas 2008; Tricarico *et al.* 2009) and evaluated whether invasiveness is related to a species' introduction status. Understanding the underlying mechanisms of pet introductions is important to forecast which 'new' species are likely to occur in nature and to develop proactive strategies for risk mitigation. The latter aim is of particular interest because neither Germany nor the European Union has, to date, established a regulatory framework for the import or trade of NICS (Shine *et al.* 2009; Hulme *et al.* 2009). Possible management strategies are discussed.

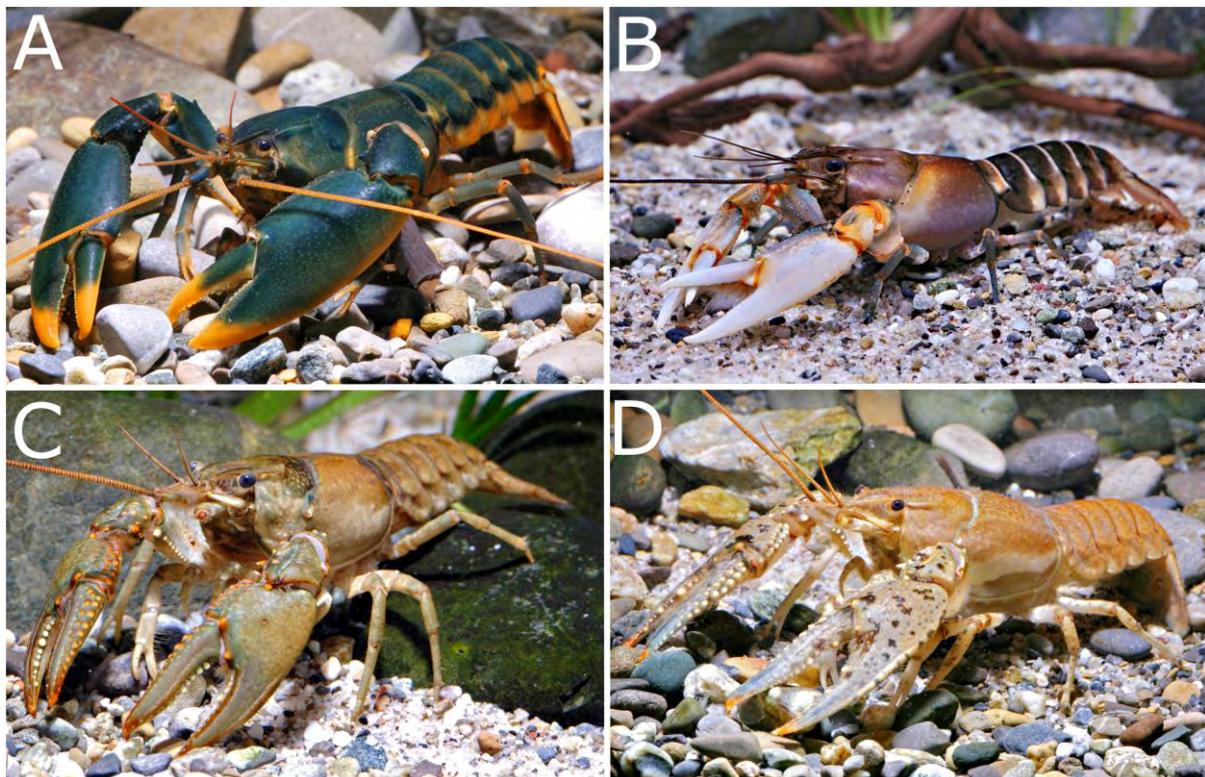


Figure 1. Low-risk (A, B) and high-risk (C, D) crayfish species available through the German pet trade (A: *Cherax holthuisi*, B: *Cherax peknyi*, C: *Orconectes virilis*, D: *Orconectes nais*). Photos courtesy of C. Lukhaup.

Table 1. Presumed introduction pathways, availability in the pet trade, NICS status, and origin of the NICS recorded from freshwater habitats in Central Europe. Introduction pathway abbreviations are as follows: *A* = aquarium trade, *C* = consumption trade, *FB* = fishing bait, and *S* = deliberate stocking. *Av_G* = general availability and *Av_{ON}* = online availability. *N.A.* means not available. The terms ‘Old NICS’ and ‘New NICS’ were coined by Holdich *et al.* (2009), based on initial introduction history and time.

Species	Introduction pathway(s)	Availability in the pet trade		NICS status	Origin
		<i>Av_G</i>	<i>Av_{ON}</i>		
<i>Astacus leptodactylus</i>	S, C	rare	<i>N.A.</i>	Old	Eastern Europe
<i>Cherax destructor</i>	<i>A</i>	common	0.31	New	Australia
<i>Cherax quadricarinatus</i>	<i>A</i>	very common	0.31	New	Australia
<i>Orconectes immunis</i>	<i>FB</i>	rare	<i>N.A.</i>	New	North America
<i>Orconectes juvenilis</i>	<i>S</i>	<i>N.A.</i>	<i>N.A.</i>	New	North America
<i>Orconectes limosus</i>	<i>S</i>	rare	0.06	Old	North America
<i>Orconectes virilis</i>	unknown	very rare	<i>N.A.</i>	New	North America
<i>Pacifastacus leniusculus</i>	<i>S</i>	very rare	<i>N.A.</i>	Old	North America
<i>Procambarus acutus/zonangulus</i>	unknown	very rare	<i>N.A.</i>	New	North America
<i>Procambarus alleni</i>	<i>A</i>	very common	0.63	New	North America
<i>Procambarus clarkii</i>	<i>A, C, S</i>	very common	0.56	Old	North America
Marmorcrebs (<i>P. fallax</i> f. <i>virginalis</i>)	<i>A</i>	very common	0.25	New	North America

Methods

Rationale

To identify the determinants of aquarium crayfish introductions, *i.e.*, deliberate releases of crayfish from aquaria into nature, I gathered two datasets on freshwater crayfish species in the German pet trade and attempted to predict species’ introduction status from its size, availability and price. Size and availability were selected as independent variables because previous research showed that these traits were determinants of the introduction of freshwater aquarium fish (Duggan *et al.* 2006; Duggan 2011). Price was also used as an independent variable because aquarium hobbyists may be more likely to offload excess stock of low-priced species than excess stock of expensive species. One dataset included data about which crayfish species have been imported into Germany and the general availability of the imported species in the pet trade, and the second dataset provided more objective data on the availability and price of crayfish species offered through online shops in 2009. Additionally, the potential invasiveness of the crayfish species offered online was assessed using the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK) and was tested for an effect on their introduction status.

Emphasis has been placed on the online pet trade because it is more easily accessible than conventional pet stores and because it facilitates the broad-scale spread of the offered species. Thus, the scope of this study was enlarged to neighboring countries, as pets are often sold across borders (Faulkes 2010) and because the German pet trade is most likely the main pathway of NICS imports into Europe (Pekny and Lukhaup 2005). For instance, the Marmorcrebs entered the European pet trade via Germany in the mid 1990s (Scholtz *et al.* 2002) and was subsequently imported from Germany to other European countries, including the Netherlands, Italy, Great Britain and Slovakia (Souty-Grosset *et al.* 2006; Marzano *et al.* 2009; Peay *et al.* 2010; Janský and Mutkovič 2010).

Imported species dataset

There are no official import statistics on NICS in Germany, and many species may have been introduced without proper declaration. An overview of the imported NICS was provided by Pekny and Lukhaup in 2005. A considerable number of species appear to have been imported since that time. To obtain an updated overview of the imported species, I gathered information from various sources. First, I interrogated two wholesalers who were known to be importers of crayfish species; however, little information could be obtained from these importers, and the validity of the reported species names was often problematic. Second, I consulted two experts on the German aquarium crayfish trade, both of whom are leading authors of aquarium crayfish reference books. Third, online pet forums and stock lists of aquarium marketplaces were searched for crayfish species at least once per month from January 2005 to June 2009, corresponding to an effort of approximately 20 man-hours per year. The obtained information was compiled, and the species names were verified by experts.

The general availability of each species (A_{VG}) was subsequently rated as either 'very rare' (only available for a short time and in low numbers), 'rare' (available occasionally in low numbers), 'common' (frequently available in low numbers) or 'very common' (always available) based on my own observations since 2005 and expert opinions. The annual importation rate of new species was estimated by dividing the number of newly imported species since 2005 by the elapsed time (four years).

Online shop dataset

The range of live ornamental crayfish products of 16 online pet shops was analyzed. Shops were selected by a Google search using the German terms for crayfish, crustacean, shrimp and invertebrate. From each shop, I recorded the offered species and the corresponding price. The price was noted on an ordinal scale, where $1 \leq 5$ €; $2 = 5-10$ €; $3 = 11-15$ €; $4 = 16-20$ €; $5 = 21-25$ € and $6 \geq 25$ €. The online availability (A_{VOn}) of each species was calculated as the proportion of shops that offered the species. The evaluated shops were also searched for information on crayfish plague or for advice given for preventing the release of NICS into nature. The shops were analyzed over a period of one month, beginning in May 2009.

Introduction status

Crayfish species were classified into two categories according to whether they have been presumably introduced into freshwater habitats from aquaria or not. The introduction status (S_{Intro}) of each species was thereby rated as either 'introduced from aquaria' (first category) or 'not introduced from aquaria' (second category).

The S_{Intro} of the NICS recorded from freshwater habitats in Central Europe were rated as follows (cf. Table 1): The Marmorkrebs and *P. alleni* were undoubtedly released from aquaria (Souty-Grosset *et al.* 2006, Schulz *et al.* 2009; Chucholl and Pfeiffer 2010) and circulated in the European pet trade for several years prior to the first records of free-living individuals (Marten *et al.* 2004; Souty-Grosset *et al.* 2006; Chucholl and Pfeiffer 2010). Both species were consequently classified as introduced from aquaria. Records of free-living *Cherax destructor* and *C. quadricarinatus* are also believed to originate from aquarium releases (Stucki and Jean-Richard 1999; Pekny 2003; Holdich *et al.* 2009; Schulz *et al.* 2009)

and both species were classified as introduced from aquaria. The introduction pathway of *Orconectes immunis* is unclear, and both an introduction from aquaria and an introduction as fishing bait were suggested (Dehus *et al.* 1999a; Gelmar *et al.* 2006). However, this species was not known in the pet trade prior to its introduction to the Upper Rhine plain (Gelmar *et al.* 2006), making an introduction as fishing bait more likely. The species was therefore classified as not introduced from aquaria. Substantial uncertainty also exists with regard to the introduction pathways of *O. virilis* and *P. acutus/zonangulus*, which are only present in the Netherlands. Although *O. virilis* is offered by a garden pond wholesaler, the initial introduction pathway is unknown (Soes and van Eekelen 2006; Soes and Kosese 2010). *Procambarus acutus/ zonangulus* may have been introduced via aquaculture, but this has never been verified (Soes and van Eekelen 2006; Soes and Kosese 2010). *Orconectes virilis* and *P. acutus/ zonangulus* were therefore classified as not introduced from aquaria. The 'Old NICS' *A. leptodactylus*, *O. limosus*, and *Pa. leniusculus* were also classified as not introduced from aquaria because they were clearly introduced by deliberate stocking (Souty-Grosset *et al.* 2006; Holdich *et al.* 2009). Although *Procambarus clarkii* was initially also introduced by stocking (Souty-Grosset *et al.* 2006), most populations in Germany are believed to be the result of aquarium introductions (Dehus *et al.* 1999b; Dümpelmann *et al.* 2009; Groß 2011). The species was therefore classified as introduced from aquaria. *Orconectes juvenilis* was introduced by stocking into a small pond in eastern France (Chucholl and Daudey 2008) and was therefore classified as not introduced from aquaria.

Risk assessment

To coherently assess and compare the potential invasiveness and risk of NICS offered online, species were rated using the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK, v.1.19; Cefas 2008). The FI-ISK system assesses the biogeography and history of the species, the presence of 'undesirable traits' and species biology and ecology based on 49 questions (see Tricarico *et al.* 2009 for a comprehensive description). Climate data for the source area (indigenous range) and the introduction area (Germany) were compared using the CLIMATCH tool (v.1.0; Invasive Animals CRC, Bureau of Rural Sciences 2008). Because the indigenous range of the as yet undescribed *Cherax* spp. species from New Guinea (*Cherax* sp. 'Blue moon', *C.* sp. 'Ajamaru' and *C.* sp. 'Hoa Creek') is only poorly known, it was assumed to consist of the entirety of Irian Jaya (*i.e.*, the western part of New Guinea). Based on preliminary aquarium observations by the author and Lukhaup and Pekny (2005), the fecundity for this species group (along with *C. peknyi*, *C. holthuisi* and *C. lorentzi*) was rated as 'normal', and the minimum age at maturity was assumed to be one year. The natural range of the Marmorkrebs was assumed to be within the natural range of its closest relative *P. fallax* (Souty-Grosset *et al.* 2006; Martin *et al.* 2010). Biogeographic and ecological data were compiled from Hobbs (1974; 1989), Holthuis (1986), Hobbs *et al.* (1989), Huner and Barr (1991), Jimenez and Faulkes (2010), Lukhaup (2004), Taylor and Schuster (2004), Beatty *et al.* (2005), Seitz *et al.* (2005), Lukhaup and Pekny (2005; 2006; 2008), Rabalais and Magoulick (2006), Souty-Grosset *et al.* (2006), Adams (2008), Lukhaup and Herbert (2008) and Holdich *et al.* (2009).

Statistical analyses

All statistical analyses were performed using SigmaPlot 10 (with SigmaStat 3.5 Integration) except for the binary logistic regression models, which were run in the software package R 2.14 (R Development Core Team, 2011). The proportion of NICS introduced from aquaria was compared between the Av_G categories by Fisher's exact tests, followed by a Bonferroni's adjustment of the P level. To assess whether the two assessments of availability, Av_G and Av_{On} , are consistent, the relation between Av_G and Av_{On} was described using a Spearman's rank order correlation. The FI-ISK score, mean price class (Pr), Av_{On} , and maximum total body length (TL_{max}) were compared for each genus using Kruskal-Wallis one way ANOVA on rank with Dunn's post-hoc test (excluding genera with a sample size smaller than five species) and based on S_{Intro} using Mann-Whitney rank sum tests.

Multiple binary logistic regression models were used to assess whether species' availability (Av_{On} or Av_G), mean price class (Pr), and maximum total body length (TL_{max}) had a significant effect on its introduction status (S_{Intro}). The regression models predicted S_{Intro} as a binary response variable from three initial sets of independent variables, using the imported species dataset or the online shop dataset. The first model (*RM1*) used the imported species dataset and Av_G and TL_{max} as independent variables, and the second model (*RM2*) used the online shop dataset and Av_{On} , Pr , and TL_{max} as independent variables. To test whether the FI-ISK score had also an effect on S_{Intro} , a third binary logistic regression model (*RM3*) was run that used only the FI-ISK score as an independent variable. The variable types and the corresponding datasets are summarized in Table 2. Analysis of deviance, based on likelihood-ratio chi-squared test statistics, was used to assess whether an independent variable had a significant effect on S_{Intro} (Chambers and Hastie 1992; Venables and Ripley 2002). Independent variables that showed no significant effect were removed from each model by stepwise backward selection until the final regression models contained only significant predictor variables. Akaike's information criterion (*AIC*), Bayesian information criterion (*BIC*), and the residual deviance (*RD*) were reported for each final regression model to provide information on model fit (Chambers and Hastie 1992), and the partial eta-square statistic (η^2) was given for all significant effects as a measure of effect size (Muller and Peterson, 1984). In addition, the adjusted count R^2 was calculated for each model, indicating the proportion of correct classifications, corrected for the most frequent value of the response variable (UCLA/ATS 2011). Receiver operating characteristics (ROC) curve analysis was used to determine optimal cutoff values for numeric predictor variables. Species that exceed the cutoff values of the predictor variables are likely to occur in nature as a result of aquarium introductions.

Table 2. The response variable and the independent variables used for the binary logistic regression analysis. Variable abbreviations are as follows: S_{Intro} = introduction status, AV_G = general availability, TL_{max} = maximum total body length, AV_{ON} = online availability, and Pr = mean price class. The FI-ISK score (invasiveness) was calculated using the Freshwater Invertebrate Invasiveness Scoring Kit.

Response variable			Independent variables		
Name	Type	State	Name	Type	Dataset
S_{Intro}	binary	introduced from aquaria	AV_G	ordered factor	imported species
		not introduced from aquaria	TL_{max}	numeric	imported sp. /online shops
		AV_{ON}	numeric	online shops	
		Pr	numeric	online shops	
		FI-ISK score	numeric	online shops	

Results

Imported species

A total of 123 crayfish species were found to be kept as aquarium species (excluding probable synonyms and misidentified specimens; see Online Resource 1 for a full list of all species). Only three of these species are indigenous, *i.e.*, 120 NICS were available as aquarium species. Three species belong to the Astacidae, 16 to the Parastacidae and 104 to the Cambaridae. One hundred five species originate from North or Central America and are therefore suspected to be crayfish plague vectors (Souty-Grosset *et al.* 2006). The vast majority of the imported species (74) were very rare in the aquarium trade, while 26 were rare, nine were common and eleven were very common. Among the very rare and rare species, there were no NICS introduced from aquaria, but six NICS that had been introduced via other pathways (*cf.* Online Resource 1 and Table 1). Common aquarium species included one species introduced from aquaria (*C. destructor*), while the very common aquarium species included four species introduced from aquaria: *C. quadricarinatus*, Marmorcrebs, *P. alleni*, and *P. clarkii*. The proportion of NICS introduced from aquaria was significantly higher among the very common aquarium species than among the very rare or rare aquarium species (Fisher's exact test, followed by a Bonferroni's adjustment of the P level: $P < 0.001$ and $P = 0.005$, respectively; Figure 2). The proportion did not differ significantly between the very common and common aquarium species, between the very rare and rare, between the common and very rare, or between the common and rare aquarium species (Fisher's exact tests, followed by a Bonferroni's adjustment of the P level: $P > 0.017$; Figure 2). The importation rate was estimated to be seven new species per year.

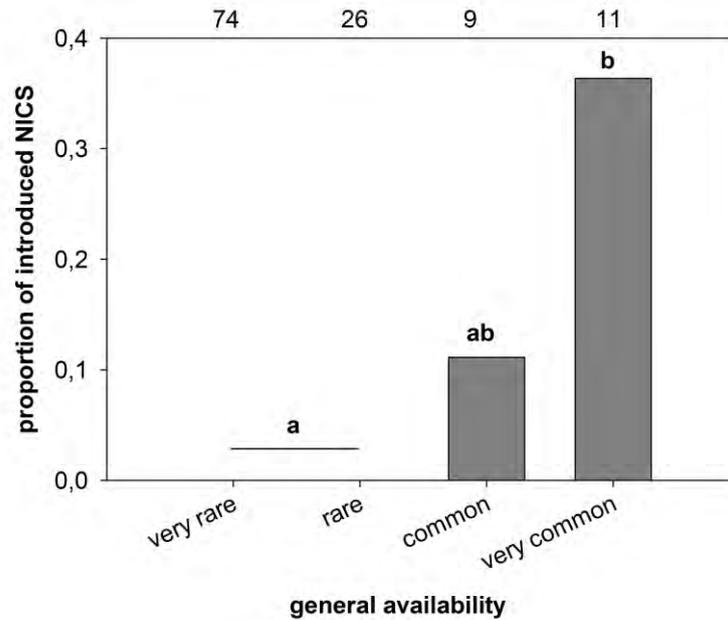


Figure 2. Proportion of non-indigenous crayfish species introduced from aquaria among very rare, rare, common and very common aquarium species. The number of species in each category is shown on top of each bar. Different letters denote significant differences in the proportion of species introduced from aquaria shown by Fisher's exact tests, followed by a Bonferroni's adjustment of the P level ($P < 0.016$).

Online trade

The 16 analyzed online shops offered 37 NICS (see Online Resource 2 for a list of all species). The offered species were primarily from the genus *Procambarus* (13 species), along with ten each from the genera *Cambarellus* and *Cherax*, three *Orconectes* and one *Cambarus* species. Ten species were offered from only one shop, while the most common species, *Cambarellus patzcuarensis* var. 'Orange', was available from all shops. The two assessments of availability, Av_G and Av_{On} , were significantly correlated (Spearman's rank order correlation: $N = 37$, $P < 0.001$; cf. Online Resource 1 and 2). Five of the analyzed shops mentioned the crayfish plague, and four provided a reference to the risks associated with the introduction of NICS into nature.

Some species were offered at prices above 25 € per individual (e.g., *Cherax preissii*, *C. sp.* 'Hoa Creek', and *Orconectes durelli*), while others were sold at prices below 10 € per individual (e.g., *Procambarus clarkii*, *P. alleni*, *C. quadricarinatus*, and most *Cambarellus* sp.). The cheapest species was the Marmorcrebs, which was advertised at approximately 5 € per individual. There was no significant relationship between Pr and Av_{On} (Pearson product moment correlation: $N = 36$, $P = 0.16$) or between Pr and TL_{max} (Pearson product moment correlation: $N = 36$, $P = 0.20$).

The FI-ISK scores ranged between 2 (*Cambarellus chapalanus*) and 33 (*Procambarus clarkii*); six species had scores higher than 16, where the FI-ISK outcome would advise 'reject' (i.e., high-risk species; Tricarico *et al.* 2009). Five of those high-risk species have been introduced into Central European freshwater habitats, four of them probably as a result

of aquarium introductions (*C. destructor*, Marmorkrebs, *P. alleni*, and *P. clarkii*; cf. Online resource 2). There was no significant relationship between the FI-ISK score and Pr or Av_{On} (Pearson product moment correlations; $P > 0.05$), whereas TL_{max} showed a significant correlation with the FI-ISK score (Pearson product moment correlation: $N = 37$, $P < 0.001$).

Among the species offered online, there were six introduced NICS, of which five were introduced from aquaria: *C. destructor*, *C. quadricarinatus*, Marmorkrebs, *P. alleni*, and *P. clarkii*. The five NICS introduced from aquaria had higher FI-ISK scores (Mann-Whitney rank sum test: $U = 5.50$, $P < 0.001$; Figure 3.A) and a greater Av_{On} (Mann-Whitney rank sum test: $U = 28.00$, $P = 0.018$; Figure 3.B) and were larger (t-test: $t = -4.86$, $P < 0.001$; Figure 3.D) than the offered species not introduced from aquaria. The species introduced from aquaria also tended to be less expensive than species not introduced from aquaria; however, this difference was not significant (Mann-Whitney rank sum test: $U = 100.00$, $P = 0.301$; Figure 3.C).

The *Cherax* species presented lower FI-ISK scores than the *Procambarus* species (Kruskal-Wallis one way ANOVA on rank with Dunn's post-hoc test: $H_2 = 6.44$, $P = 0.04$; Figure 4.A), were more expensive than the *Procambarus* and *Cambarellus* species (Kruskal-Wallis one way ANOVA on rank with Dunn's post hoc-test: $H_2 = 14.34$, $P < 0.001$; Figure 4.C), and were larger than the *Cambarellus* species (Kruskal-Wallis one way ANOVA on rank with Dunn's post hoc-test: $H_2 = 20.98$, $P < 0.001$; Figure 4.D). The *Cambarellus* species were also smaller than the *Procambarus* species (Kruskal-Wallis one way ANOVA on rank with Dunn's post hoc test: $H_2 = 20.98$, $P < 0.001$). Av_{On} did not differ significantly between the offered crayfish genera (Kruskal-Wallis one way ANOVA on rank with Dunn's post-hoc test: $H_2 = 1.66$, $P = 0.437$; Figure 4.B).

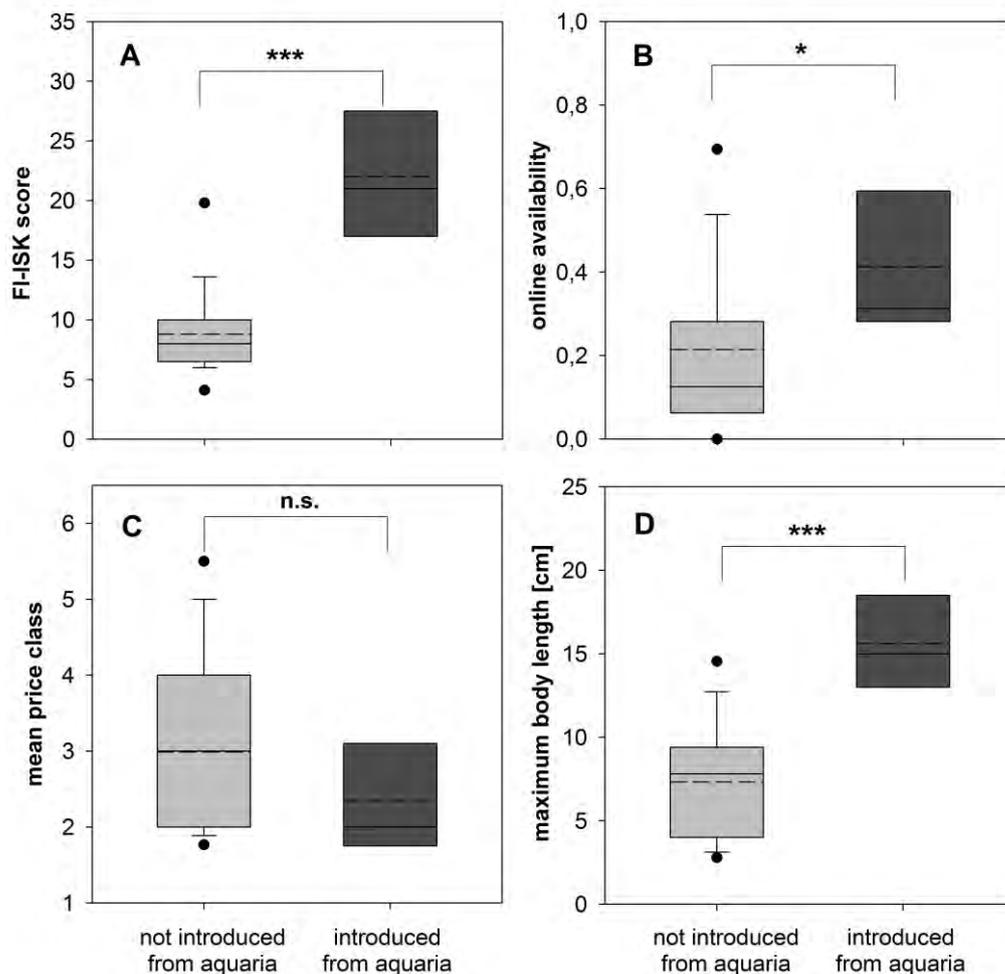


Figure 3. Potential invasiveness (A; FI-ISK score), online availability (B), mean price class (C), and maximum total body length (D) of non-indigenous crayfish species introduced from aquaria (dark grey; $N = 5$) and not introduced from aquaria (grey; $N = 32$) that were offered by 16 online shops in 2009. Solid lines indicate the median value, broken lines the mean value, whiskers the 5th/ 95th percentile, and dots outliers. Significant differences shown by Mann-Whitney rank sum tests. * means $P < 0.05$, *** means $P < 0.001$ and n.s. means not significant.

Binary logistic regression analysis

The obtained significant predictor variables for S_{Intro} and the information on the final regression model fit (AIC , BIC , RD , and adjusted count R^2) are summarized for each model in Table 3. Species availability (Av_{On} or Av_{G}) and size (TL_{max}) had a significant effect on S_{Intro} in both multiple regression models (analysis of deviance: $P < 0.01$; Table 3) using either the imported species dataset ($RM1$; Figure 5.A) or the online shops dataset ($RM2$; Figure 5.B). The effect size (η^2) of species' availability and TL_{max} showed no consistent pattern; in $RM1$, availability had a bigger effect on S_{Intro} than TL_{max} , while in $RM2$, the reverse situation occurred (Table 3). Pr was found to have no significant effect on S_{Intro} (analysis of deviance: $LR \chi^2_{1,35} = 1.61, P = 0.205$; $RM2$), while the FI-ISK score had a significant effect on S_{Intro} (analysis of deviance: $P < 0.001$; Table 3, $RM3$). The overall model fit of the two multiple

regression models was good ($RD < 0.001$; Table 3, $RM1$ and $RM2$), and both models had an adjusted count R^2 of 1.0, indicating that all species were correctly classified. The regression model that used only the FI-ISK score as predictor variable ($RM3$) produced a significantly higher RD than the multiple regression model that used AV_{On} and TL_{max} as predictor variables ($RM2$; analysis of deviance: $P < 0.001$; cf. Table 3), indicating that the model fit was inferior to the multiple regression model.

The optimal cutoff values obtained by ROC curve analyses were 12.7 cm for TL_{max} (sensitivity = 1.00, specificity = 0.94, ROC curve area = 0.98, and $P < 0.001$; and sensitivity = 0.80, specificity = 0.85, ROC curve area = 0.89, and $P = 0.003$ for the online shops dataset and imported species dataset, respectively), 0.22 for AV_{On} (sensitivity = 1.00, specificity = 0.58, ROC curve area = 0.83, and $P = 0.02$), and 12.5 for the FI-ISK score (sensitivity = 1.00, specificity = 0.88, ROC curve area = 0.93, and $P = 0.002$).

Table 3. Binary logistic regression models ($RM1-3$) and predictor variables for the introduction status (S_{intro}) of freshwater crayfish in the German pet trade. The models differ in the dataset used and the initial set of independent variables (see footnotes and methods section for details). Likelihood-ratio (LR) χ^2 and P values were calculated using analysis of deviance, and asterisks denote significant effects (**: $P < 0.01$, ***: $P < 0.001$). AIC indicates the Akaike's information criterion, BIC indicates the Bayesian information criterion and RD gives the residual deviance for each model. The adjusted count R^2 gives the proportion of correct classifications, corrected for the most frequent value of the response variable. Variables are defined in Table 2.

Regression model	Dataset	Response variable	AIC	BIC	RD	Adjusted count R^2	Predictor variables	df	η^2	$LR\chi^2$	P
$RM1$ ¹⁾	imported species	S_{intro}	10.00	23.36	<0.001	1.00	AV_G :	3	0.64	37.27	<0.001***
							TL_{max} :	1	0.36	20.70	<0.001***
							Residuals:	102			
$RM2$ ²⁾	online shops	S_{intro}	6.00	10.91	<0.001	1.00	AV_{ON} :	1	0.24	8.49	0.004**
							TL_{max} :	1	0.76	26.51	<0.001***
							Residuals:	35			
$RM3$ ³⁾	online shops	S_{intro}	22.23	25.51	18.23	0.63	FI-ISK score:	1	0.94	11.36	<0.001***
							Residuals:	36			

¹⁾: initial set of independent variables: AV_G and TL_{max} ;

²⁾: initial set of independent variables: AV_G , TL_{max} , and Pr ;

³⁾: initial independent variable: FI-ISK score.

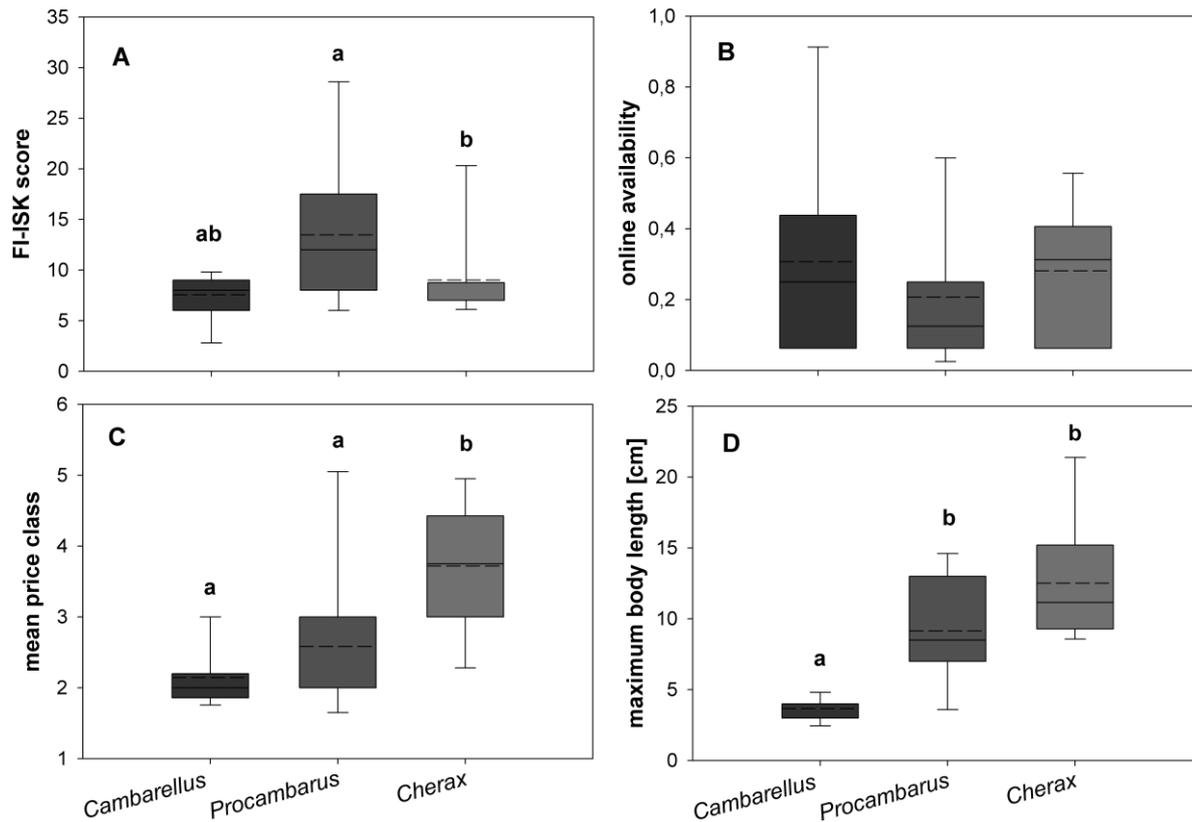


Figure 4. Potential invasiveness (A; FI-ISK score), online availability (B), mean price class (C), and maximum total body length (D) of the three main crayfish genera offered by 16 online shops in 2009 (number of species in each genus: *Cambarellus*: $N = 10$; *Procambarus*: $N = 13$, and *Cherax*: $N = 10$). Solid lines indicate the median value, broken lines the mean value, and whiskers the 5th/ 95th percentile. Letters indicate significant differences shown by Kruskal-Wallis one way ANOVA on rank with Dunn's post hoc test ($P < 0.05$).

Discussion

The prevention of new introductions of non-indigenous species is often cited as a major and primary objective (Hulme *et al.* 2009; Justo-Hanani *et al.* 2010; CBD 2011), yet the underlying mechanisms and determinants of introductions remain under-studied topics (Duggan *et al.* 2006). Here, I report on introductions from aquaria, a newly emerging NICS introduction pathway in Central Europe that has the potential to add yet another chapter to the notorious history of NICS introductions in Europe (Henttonen and Huner 1999; Holdich *et al.* 2009; Schulz *et al.* 2009) and to become a threat on other continents as the popularity of crayfish as pets increases (*cf.* Faulkes 2010; Jones *et al.* 2009). Consistent with related research on aquarium fish introductions, the present analysis provides evidence that the likelihood of being recorded as introduced from aquaria depends largely on a species' availability in the pet trade and the species' size.

An effect of species' availability in the pet trade on the likelihood of being introduced from aquaria was anticipated because greater availability is likely related to a higher number of release events and, thus, higher propagule pressure (*i.e.*, introduction effort; Lockwood *et al.* 2007). A similar phenomenon has been seen in freshwater aquarium fishes in North America, where clear relationships exist between the popularity of a fish and the likelihood of

being recorded as introduced or established (Duggan *et al.* 2006; Duggan 2011). However, it is important to acknowledge that by relating species' availability in the pet trade to propagule pressure, species' availability is taken as a surrogate measure for the popularity of the species in home aquaria, as aquarium species are most likely introduced from home aquaria (Courtenay 1999; Duggan *et al.* 2006; Duggan 2011). The popularity of fish and crayfish species in home aquaria seems to be an important determinant of propagule pressure, and this relationship presumably also applies to other common aquarium taxa, such as snails, freshwater shrimps, and plants. For instance, *Neocaridina heteroptera*, a popular aquarium shrimp species, was recently observed in a small brook in Germany (Bauer 2011). The two assessments of aquarium crayfish availability, Av_G and Av_{On} , were significantly correlated and showed the same trend where only a few species were commonly offered in the trade and where the majority of species were seldom available. *Procambarus alleni*, *P. clarkii*, *Ca. patzcuarensis* var. 'Orange', *Ca. puer*, *Ca. montezumae*, *C. peknyi*, and *C. holthuisi* were available from more than 40% of the analyzed online shops in 2009, and these species were also rated as very common in the imported species dataset, in addition to Marmorkrebs, *C. quadricarinatus*, *Ca. shufeldtii*, and *Ca. chapalanus*. Interestingly, Soes and Koese (2010) recently reported that the three most popular aquarium crayfish genera in the Netherlands were *Procambarus*, *Cambarellus*, and *Cherax* and that the species most available in the Dutch aquarium trade were *P. clarkii*, Marmorkrebs, *P. alleni*, and dwarf crayfish (*Cambarellus* sp.). These findings are remarkably consistent with the results obtained here and suggest that species' popularity in the German pet trade may indeed be indicative of the situation in neighboring countries. The most popular aquarium crayfish species, namely *P. clarkii*, *P. alleni*, Marmorkrebs and *Cambarellus* sp., can be reared easily in aquaria (Lukhaup and Pekny 2005), and both retail sellers and aquarium hobbyists may be able to maintain their own breeding stocks instead of purchasing animals, which may explain the high availability of these species. *Procambarus* species, such as *P. clarkii* and Marmorkrebs, have a particularly high reproductive potential and produce many offspring (Huner and Barr 1991; Seitz *et al.* 2005; Souty-Grosset *et al.* 2006). It is also likely that this characteristic accounts for the finding that the *Procambarus* species were found to be sold at lower prices than the *Cherax* species, which have a lower reproductive potential (with the exceptions of *C. quadricarinatus* and *C. destructor*).

Crayfish size was also found to have a significant effect on the likelihood of being recorded as introduced from aquaria. Species' size may influence the likelihood of deliberate release in two ways: first, large species may outgrow their aquaria; and second, large species may quickly overpopulate their aquaria because the fecundity of crayfish is size-dependant, with larger species being usually more fecund than smaller species. For instance, dwarf crayfish (*Cambarellus* sp.) carry, on average, between 25 and 50 eggs per female (Lukhaup and Pekny 2005), while the fecundity of larger species such as *P. clarkii* and *C. quadricarinatus* can easily exceed 500 eggs per female (Lukhaup and Pekny 2005; Chucholl 2011b). Aquarium hobbyists may therefore be more likely to offload excess stocks of large species than excess stocks of small species (*cf.* Chucholl 2011a). Both of these effects, *i.e.*, animals becoming too large and prolific for their aquaria, have been previously cited as reasons for deliberate aquarium fish releases (Courtenay 1999; Crossman and Cudmore

1999). However, an alternative reason confounding the effect of species' size (and the correlated potential invasiveness) on the introduction status is that species' size/invasiveness may also affect the probability of detection, such that introductions of small low-risk species are less likely to be detected than introductions of large high-risk crayfish species (*cf.* Duggan *et al.* 2006). Larger species may simply be more likely to be found (Thompson 2004), and high-risk species have a greater chance of successful establishment, thus making their detection more likely. These potentially confounding effects are difficult to assess and must be acknowledged; however, these effects presumably had little to no influence on the present analysis for several reasons. First, introductions from aquaria were recorded for different sized crayfish species, namely, Marmorkrebs, *P. clarkii*, *P. alleni*, *C. destructor*, and *C. quadricarinatus*; at least two of these records are based on the collection of small individuals that were similar or smaller in size than the dwarf crayfish species (*Cambarellus* sp.) available in the pet trade (Marten *et al.* 2004; Marzano *et al.* 2009). For instance, the first record of a free-living Marmorkrebs from Germany is based on the collection of a single juvenile individual, measuring only approximately 1 cm in total body length (Marten *et al.* 2004), suggesting that crayfish size may not be the major or exclusive determinant of detection probability. Many aquarium crayfish species have a vivid, conspicuous coloring (*cf.* Figure 1.A, B), which may increase the probability of their detection (Thompson 2004). Second, several failed crayfish introductions from aquaria have been recorded (Knuth 1999; Stucki and Jean-Richard 1999; Marten *et al.* 2004; Marzano *et al.* 2009; Martin *et al.* 2010). For instance, single Marmorkrebs individuals have been repeatedly found in Germany and most likely represent failed introductions (*e.g.*, Marten *et al.* 2004; Martin *et al.* 2010). Assuming that small low-risk species that are as equally abundant in the pet trade as Marmorkrebs, such as *Ca. patzcuarensis* var. 'Orange', were also introduced from aquaria, one could argue that at least one record of an introduced small low-risk species should exist. However, the lack of any record of a small low-risk species from freshwater habitats in Central Europe presumably indicates that these species have not, or at least very rarely, been introduced from aquaria, as suggested here.

A significant effect of species' availability and size on the likelihood of being recorded as introduced from aquaria was demonstrated in two multiple binary logistic regression models, using either the imported species dataset or the more objective online shop dataset. The effect size (η^2) of these traits on the introduction status varied between the two multiple binary logistic regression models, and this difference is most likely due to the different nature of the two datasets; the imported species dataset was largely composed of very rare or rare species, highlighting the effect of species' availability on the introduction status, as none of these rare or very rare species have been recorded as introduced from aquaria. In contrast, the online shop dataset was composed of slightly more available species, *i.e.*, only three of the very rare species of the imported species dataset were included, thus giving more predictive power to species' size. An effect of species' availability and size on the introduction status is also supported by the finding that crayfish introduced from aquaria were more available and larger than those not introduced from aquaria.

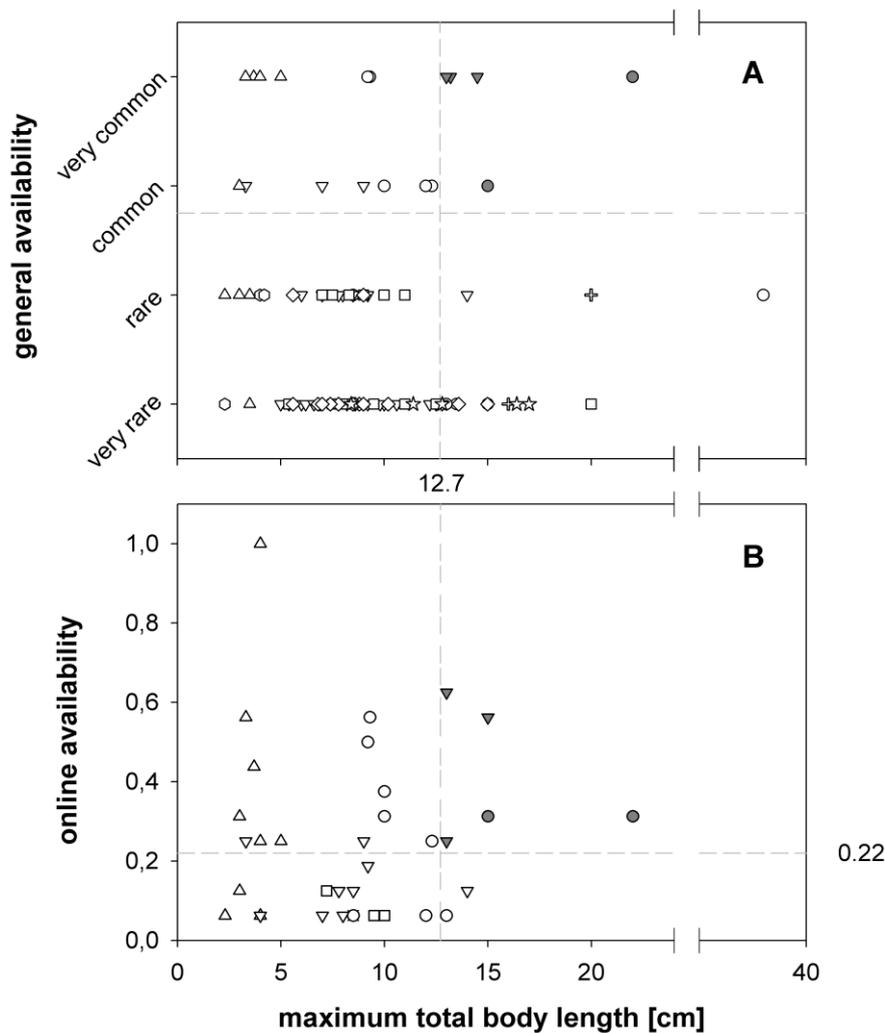


Figure 5. Availability and size as principal predictors of the introduced status of crayfish species in the German pet trade (white: not introduced from aquaria, dark grey: introduced from aquaria); A: imported species dataset ($N = 120$), B: online shops dataset ($N = 37$). Dashed lines and numbers provide optimal cutoff values obtained by ROC curve analysis (except for general availability), above which species are likely to occur in nature. Symbol shape indicates genus: circle = *Cherax*, triangle up = *Cambarellus*, triangle down = *Procambarus*, diamond = *Cambarus*, square = *Orconectes*, hexagon = *Hobbseus* and *Bouchardina*, cross = *Astacus* and *Pacifastacus*, and star = *Astacoides*, *Parastacus*, and *Samastacus*.

Species' potential invasiveness, as calculated by the FI-ISK tool, was also found to have an effect on the introduction status. However, this effect needs to be carefully interpreted, as the FI-ISK score correlated with species' size, and the observed effect on the introduction status may therefore merely represent the effect of species' size. Nonetheless, the analysis of the FI-ISK scores clearly demonstrated that several high-risk crayfish species are available through the pet trade and that NICS introduced from aquaria presented a higher potential invasiveness than those species that were not introduced from aquaria. An unexpected finding was that price had no significant influence on the introduction status. This finding is likely a result of the many *Cambarellus* species that were offered at low prices but have never been recorded as introduced.

Overall, the initial hypothesis that NICS introduced from aquaria show similar ecological and economic traits was supported, with the exception of pricing, which had no significant influence on the introduction status. Large species ($TL_{\max} > 13$ cm) that are widely available through the aquarium trade ($Av_{On} > 0.22$) are likely to be introduced from aquaria. Because species' size was found to correlate with potential invasiveness, as calculated by the FI-ISK tool, release events are likely to be associated with high-risk species. Thus, NICS originating from aquarium introductions are presumably equally 'dangerous' invaders as many 'Old NICS' are, which were introduced by aquaculture or stocking and have been intentionally pre-selected for large size, high fecundity and environmental tolerance (Holdich and Gherardi 1999; Lindqvist and Huner 1999; Holdich *et al.* 2009). Interestingly, the invasion success of NICS introduced from aquaria is quite similar to the invasion success reported for freshwater fish species potentially introduced via the aquarium trade in North America (Duggan 2011); in both taxa, approximately 40% of the introduced species became established (two out of five introduced crayfish species and 34 out of 94 introduced fish species). One factor not affected by species' availability and size that is likely to determine the invasion success upon introduction is the climate suitability of the receiving habitat (Lockwood *et al.* 2007). This is well-illustrated by the case of *C. quadricarinatus*, a tropical Australian species, which failed to establish a self-sustaining population in a temperate lake in Germany (Schulz *et al.* 2009) but became established in an oxbow lake fed by thermal hot water springs in eastern Slovenia (Jaklič and Vrezec 2011). However, the effect of climate suitability on the invasion success of NICS should be interpreted with care because *P. clarkii*, often referred to as a 'warm water' species (Henttonen and Huner 1999), has recently been found to thrive in colder climates by modulating its life history (Chucholl 2011b).

Inappropriate trade names may further facilitate pet introductions into nature: one shop advertised *P. clarkii* as 'Teichkrebs', which is German for 'pond crayfish'. This misleading name is widely used among aquarium enthusiasts and may provoke introduction into ponds (*cf.* Chucholl 2011a). In fact, there are various reports of *P. clarkii* being held in garden ponds. Because crayfish can migrate considerable distances over land and readily leave unsuitable habitats, it is likely that some of these 'pond crayfish' eventually invaded natural waterways (Dehus *et al.* 1999b). Although the stocking of NICS in ponds is illegal in many federal states of Germany, there are virtually no controls to enforce these regulations. In fact, *Pa. leniusculus* was long advertised as a pond species, and in 2008, a pet shop in northwestern Germany sold *Orconectes* cf. *virilis* from the Netherlands for garden pond stocking. In 2006, large numbers of berried *O. limosus* females captured from the River Elbe were sold at an aquarium trade show in southwestern Germany for garden pond stocking. Even dedicated aquarium species, such as *Ca. patzcuarensis* var. 'Orange' (CPO), have been advertised as pond species in aquarium magazines (*e.g.*, Blankenhaus 2010). The false labeling of crayfish is of equal concern: both *Pa. leniusculus* and *A. leptodactylus* have frequently been offered as indigenous 'noble crayfish' (Chucholl and Dehus 2011).

Since 2005, 28 new NICS have been imported, which is equivalent to an average import rate of seven new species per year. It is worrying that 26 of these species originate from North or Central America and are therefore suspected to be crayfish plague vectors (Souty-Grosset *et al.* 2006). Furthermore, two of these species represent high-risk species (*O.*

nais and *O. neglectus*; Figure 1.D), which are likely to become invasive once set free (Larson and Olden 2010). Pekny (2003) provided a preliminary list of high-risk species: *Orconectes rusticus*, *O. virilis* (Figure 1.C), *O. neglectus*, and *P. clarkii*. By 2009, all of these species were either present in nature or available through the pet trade. Similarly, Holdich (1999) stated approximately a decade ago that “we can be thankful that Europe has not been invaded by *Orconectes rusticus* - yet”. His concern has become a bitter reality, as *O. rusticus* has been imported for the German pet trade. Wholesalers and importers often lack a profound understanding of imported species and are rarely aware of the associated risks. Even high-risk species, such as *O. rusticus*, *O. nais*, and *O. neglectus*, have been imported without hesitation. However, it is interesting to note that none of the species imported since 2005 have yet been recorded as introduced. This finding is most likely because these species were either (yet) too rarely available (22 species, cf. Online Resource 1) and/or too small (e.g., five *Cambarellus* species) to be introduced from aquaria. With these species becoming more available in the pet trade, the likelihood of introduction may increase, though.

The availability of several high-risk species through the pet trade constitutes a major obstacle for the prevention of further harmful crayfish introductions, particularly because public awareness of the risks associated with NICS is typically low. As Peay *et al.* (2010) stressed, a risk assessment should have been carried out prior to the importation of these species, and high-risk species should have been banned initially. However, there have been no effective import regulations in Germany, leaving customer education, voluntary self-regulation of the aquarium trade, and public risk awareness as the sole means to prevent ornamental crayfish releases. However, public awareness related to the impacts of non-indigenous species in Europe is alarming low and barely exceeds 2% (Holdich *et al.* 2009; Hulme *et al.* 2009). Many wholesalers and vendors are not aware of the risks associated with NICS, and they often exhibit no or poor knowledge about the crayfish plague, despite numerous public and trade education efforts (e.g., Groß 2011). In the present study, only five shops mentioned the crayfish plague, although most of the 16 shops specialized in crayfish and all offered potential crayfish plague carriers. Only four shops provided a reference to the risks associated with the introduction of NICS into nature. Voluntary self-regulation did occur to some extent in the case of Marmorkrebs, as this species has a bad reputation because of its tendency to quickly degrade typical aquarium communities. However, such an initiative is not the rule, and Marmorkrebs are still widespread and readily available through wholesalers, the retail trade and online marketplaces (Lukhaup and Pekny 2009).

Recent records of Marmorkrebs and *P. clarkii* introductions from aquaria (e.g., Chucholl and Pfeiffer 2010; Janský and Mutkovič 2010; Chucholl 2011a; Groß 2011; Wendt 2011) clearly demonstrate that the aforementioned efforts are insufficient to halt NICS introductions, and, for this reason, it remains doubtful whether such mechanisms will ever be effective. In 2010, the UN International Year of Biodiversity, NICS were still an increasing major threat to the endangered European ICS stocks (Holdich *et al.* 2009). Aquarium discards are likely to further worsen the situation, both in terms of creating new bridgeheads for already established NICS and introducing new (presumably high-risk) species and their associated pathogens and parasites (e.g., crayfish plague). With the aquarium hobby growing and the propagation of freshwater crayfish as pets continuing, it is likely that the propagule

pressure from the aquarium trade will further increase with time, leading to an increase of established NICS introduced from aquaria (*cf.* Duggan *et al.* 2006). This trend is already illustrated by the case of the Marmorcrebs, for which only one established population was known in Central Europe in 2009 (Soes and van Eekelen 2006; Schulz *et al.* 2009), but at least five established populations were known by mid-2011 (Chucholl and Pfeiffer 2010; Janský and Mutkovič 2010; Wendt 2011; Chucholl unpublished data).

The development of proactive management strategies is clearly needed (*cf.* Shine *et al.* 2009; Hulme *et al.* 2009). For instance, a regulatory framework for the import, trade and keeping of NICS could greatly mitigate the risk of harmful crayfish releases (Souty-Grosset and Reynolds 2009; Justo-Hanani *et al.* 2010). Given the wide range of the obtained FI-ISK scores and the considerable market demand for ornamental crayfish, import and trade regulations based on a 'white list' approach appear more feasible than a total ban of all NICS. For example, most tropical *Cherax* spp. from Irian Jaya pose only a minor threat, whereas many of the larger, *r*-selected cambarid species are especially risk-entailing. A 'white list' approach, *i.e.*, a ban of all but appropriately screened low-risk species, probably offers better risk mitigation potential than a 'black list' (ban of high-risk species; Hulme *et al.* 2009; Duggan 2011) because little is known about the ecology of most of the imported species, and new imports occur at a high rate. Arriving at a manageable number of 'legal' species may also facilitate effective controls because control authorities will only have to be able to identify the legal species. However, the existing regulatory framework of the European Union is presently not applicable to the keeping of ornamental aquatic animals in pet shops, garden centers, contained garden ponds or aquaria (Shine *et al.* 2009; Hulme *et al.* 2009). According to the World Organisation of Animal Health (Office International des Epizootics - OIE) Aquatic Health Code (OIE 2009), a risk assessment of potential crayfish plague vectors (*i.e.*, all crayfish species indigenous to North or Central America) should be accomplished prior to their import. Because Germany and all neighboring countries are members of the OIE, the ongoing import of North American crayfish should be strictly regulated according to the existing OIE recommendations (*cf.* Riley 2005). Implementation of quarantine-based trade restrictions will probably discourage wholesalers from importing new high-risk species (*i.e.*, potential crayfish plague vectors). As outlined by Peay (2009), a wholesaler who wants to import NICS should bear the expenses for crayfish plague risk assessment.

The ornamental freshwater crayfish trade constitutes an actual risk, and the present situation is worrying. Several high-risk species are widely available through the aquarium trade and are likely to be introduced into nature. As Jimenez and Faulkes (2010) put it aptly, the pet trade has an "unimpressive record of keeping species captive", and any efforts focused on the mitigation of the risk of harmful aquarium releases should therefore aim to drastically reduce the availability of high-risk species.

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References

- Adams, S.B., 2008. *Cambarellus shufeldtii*. Version 1.0. USDA Forest Service, Crayfishes of Mississippi website, Oxford, MS. <http://maps.fs.fed.us/crayfish/factsheets/FS0056.pdf>. Accessed 23 August 2009
- Alderman, D.J., 1997. Crustaceans : Bacterial and Fungal Diseases. *OIE Sci Tech Rev* 15
- Bauer, U., 2011. *Neocaridina* in Deutschland aufgetaucht. <http://www.crustahunter.com/de/node/1248>. Accessed 22 November 2011
- Beatty, S., Morgan, D., Gill, H., 2005. Role of life history strategy in the colonisation of Western Australian aquatic systems by the introduced crayfish *Cherax destructor* Clark, 1936. *Hydrobiologia* 549: 219–237
- Blankenhaus, R., 2010. CPO - *Cambarellus patzcuarensis* “Orange”. *Amazonas* 6: 28–35
- CBD, 2000. Global strategy on invasive alien species. Convention on Biological Diversity, UNEP/CBD/SBT/TA/6/INF/9: 1-52. UNEP, Montreal, Canada Publ Internet. <http://www.cbd.int/programmes/cross-cutting/alien/documents.aspx> Accessed 22 December 2008
- CBD, 2011. Aichi Biodiversity Targets, Convention on Biological Diversity. <http://www.cbd.int/sp/targets/>. Accessed 19 December 2011
- Chambers, J.M., Hastie, T.J. 1992. Statistical Models in S. Wadsworth, Pacific Grove
- Chucholl, C., 2011a. Der Handel mit exotischen Flusskrebse. *Forum Flusskrebse* 15: 33–39
- Chucholl, C., 2011b. Population ecology of an alien “warm water” crayfish (*Procambarus clarkii*) in a new cold habitat. *KMAE* 401, 29. doi: 10.1051/kmae/2011053
- Chucholl, C., Daudey, T., 2008. First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). *Aquat Inv* 3: 105–107

- Chucholl, C., Pfeiffer, M., 2010. First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquat Inv* 5: 405–412
- Chucholl, C., Dehus, P., 2011. Flusskrebse in Baden-Württemberg. Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen
- Courtenay, Jr. W.R., 1999. Aquariums and water gardens as vectors of introduction. In: Claudi R and Leach JH (eds) *Nonindigenous Freshwater Organisms: Vectors, Biology, and impacts*, Lewis Publishers, Boca Raton, pp 127–128
- Crossman, E.J., Cudmore, B.C., 1999. Summary of North American fish introductions through the aquarium/horticulture trade. In: Claudi R and Leach JH (eds) *Nonindigenous Freshwater Organisms: Vectors, Biology, and impacts*, Lewis Publishers, Boca Raton, pp 129–134
- Dehus, P., Dussling, U., Hoffmann, C., 1999a. Notes on the occurrence of the calico crayfish (*Orconectes immunis*) in Germany. *Freshw Crayfish* 12: 786–790
- Dehus, P., Phillipson, S., Bohl, E., Oidtmann, B., Keller, M., Lechleiter, S., 1999b. German conservation strategies for native crayfish species with regard to alien species. *Crustac Issues* 11: 149–159
- Duggan, I., 2010. The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biol Inv.* doi: 10.1007/s10530-010-9768-x
- Duggan, I., 2011. Aquaria. In: Simberloff D, Rejmánek M (eds.) *Encyclopedia of Biological Invasions*, University of California Press, Berkley, Los Angeles, London, pp 32–35
- Duggan, I., Rixon, C.A.M., MacIsaac, H.J., 2006. Popularity and Propagule Pressure: Determinants of Introduction and Establishment of Aquarium Fish. *Biol Inv* 8: 377–382
- Dümpelmann, C., Bonacker, F., Häckl, M., 2009. Erstnachweis des Rotem Amerikanischen Sumpfkrebsses *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia* 67: 39–47
- Faulkes, Z., 2010. The spread of the parthenogenetic marbled crayfish, Marmorkrebs (*Procambarus* sp.), in the North American pet trade. *Aquat Inv* 5: 447–450
- Gelmar, C.F., Pätzold, F., Grabow, K., Martens, A., 2006. Der Kalikokrebs *Orconectes immunis* am nördlichen Oberrhein: ein neuer amerikanischer Flusskrebs breitet sich schnell in Mitteleuropa aus (Crustacea: Cambaridae). *Lauterbornia* 56: 15–25

- Groß, H., 2011. Edelkrebsprojekt NRW. http://www.edelkrebsnrw.de/krebse_frame.htm. Accessed 12 January 2011
- Henttonen, P., Huner, J.V., 1999. The Introduction of alien species of crayfish in Europe: A historical introduction. *Crustac Issues* 11: 13–22
- Hobbs, H.H., 1974. A checklist of the North and Middle American crayfishes (Decapoda: Astacidae and Cambaridae). *Smithson Contrib Zool* 166. 161 pp
- Hobbs, H.H., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithson Contrib Zool* 480. 236 pp
- Hobbs, H.H., Jass, J.P., Huner, J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56: 299–316
- Holdich, D.M., 1999. The negative effects of established crayfish introductions. *Crustac Issues* 11: 31–48
- Holdich, D.M., Gherardi, F., 1999. Native and alien crayfish in Europe: An introduction. *Crustac Issues* 11: 3–12
- Holdich, D.M., Pöckl, M., 2005. Does legislation work in protecting vulnerable species? *Bull Fr Peche Piscicult* 376-377: 809–827
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *KMAE* 11, 394-395. doi: 10.1051/kmae/2009025
- Holthuis, L.B., 1986. The freshwater Crayfish of New Guinea. *Freshw Crayfish* 6: 48–58
- Hulme, P.E., Bacher, S., Kenis, M., Klotz, S., Kühn, I., Minchin, D., Nentwig, W., Olenin, S., Panov, V., Pergl, J., Py, P., Roques, A., Sol, D., Solarz, W., Vilà, M., 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. *J Appl Ecol*. doi: 10.1111/j.1365-2664.2007.01442.x
- Hulme, P.E., Pysek, P., Nentwig, W., Vilà, M., 2009. Will Threat of Biological Invasions Unite the European Union? *Science* 324: 40–41
- Huner, J.V., Barr, L.E., 1991. Red Swamp Crawfish: Biology, Culture, and Exploitation. Louisiana State University Sea Grant College System, Louisiana State University, Baton Rouge, Louisiana

- Jaklič, M., Vrezec, A., 2011. The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae). *Crustaceana* 84: 651–665
- Janský, V., Mutkovič, A., 2010. Rak *Procambarus* sp. (Crustacea: Decapoda: Cambaridae) – Prvý Nález na Slovensku. *Acta Rer Natur Mus Nat Slov* 56: 64–67
- Jimenez, S.A., Faulkes, Z., 2010. Can the parthenogenetic marbled crayfish Marmorokrebs compete with other crayfish species in fights? *J Ethol*. doi: 10.1007/s10164-010-0232-2
- Jones, J.P.G., Rasamy, J.R., Harvey, A., Toon, A., Oidtmann, B., Randrianarison, M.H., Raminosoa, N., Ravoahangimalala, O.R., 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biol Inv* 11: 1475–1482
- Justo-Hanani, R., Dayan, T., Tal, A., 2010. The role of regulatory decision-making on non-indigenous species introductions. *Biol Inv* 12: 2815-2824. doi: 10.1007/s10530-010-9687-x
- Knuth, D., 1999. Erstnachweis des Roten Amerikanischen Sumpfkrebse *Procambarus (Scapulicambarus) clarkii* (GIRARD, 1852) für Brandenburg in der Havel bei Werder. *Beitr Tierwelt Mark* 14: 117–118
- Larson, E.R., Olden, J.D., 2010. Latent Extinction and Invasion Risk of Crayfishes in the Southeastern United States. *Conserv Biol* 24: 1099–1110. doi: 10.1111/j.1523-1739.2010.01462.x
- Lindqvist, O.V., Huner, J.V., 1999. Life history characteristics of crayfish: what makes them good colonizers? *Crustac Issues* 11: 23–30
- Lodge, D.M., Taylor, C.A., Holdich, D.M., Skurdal, J., 2000. Nonindigenous Crayfishes threaten North American Freshwater Biodiversity. *Fisheries* 25: 7–19
- Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2007. *Invasion Ecology*. Blackwell publishing, Oxford
- Lukhaup, C., 2004. Süßwasserkrebse aus aller Welt. Dähne Verlag, Ettlingen
- Lukhaup, C., Herbert, B., 2008. A new species of freshwater crayfish (Crustacea: Decapoda: Parastacidae) from the Fly River drainage, Western Province, Papua New Guinea. *Mem Queensl Mus* 52: 213–219

- Lukhaup, C., Pekny, R., 2005. *Krebse im Aquarium*. Dähne Verlag, Ettlingen
- Lukhaup, C., Pekny, R., 2006. *Cherax (Cherax) holthuisi*, a new species of crayfish (Crustacea: Decapoda: Parastacidae) from the centre of the Vogelkop Peninsula in Irian Jaya (West New Guinea), Indonesia. *Zool Med Leiden* 80-1: 101–107
- Lukhaup, C., Pekny, R., 2008. *Cherax (Astaconephrops) boesemani*, a new species of crayfish (Crustacea: Decapoda: Parastacidae) from the centre of the Vogelkop Peninsula in Irian Jaya (West New Guinea), Indonesia. *Zool Med Leiden* 82: 1–10
- Lukhaup, C., Pekny, R., 2009. Flusskrebse in der Aquaristik. In: Füreder L. (ed.) *Flusskrebse: Biologie, Ökologie, Gefährdung*. Veröffentlichungen des Naturmuseums Südtirol, 6, Folio Verlag, Bozen, Wien, pp 129–132
- Marten, M., Werth, C., Marten, D., 2004. Der Marmorkrebs (Cambaridae, Decapoda) in Deutschland - ein weiteres Neozoon im Einzugsgebiet des Rheins. *Lauterbornia* 50: 17–23
- Martin, P., Shen, H., Füllner, G., Scholtz, G., 2010. The first record of the parthenogenetic Marmorkrebs (Decapoda, Astacida, Cambaridae) in the wild in Saxony (Germany) raises the question of its actual threat to European freshwater ecosystems. *Aquat Inv* 5: 397–403
- Marzano, F.N., Scalici, M., Chiesa, S., Gherardi, F., Piccinini, A., Gibertini, G., 2009. The first record of the marbled crayfish adds further threats to fresh waters in Italy. *Aquat Inv* 4. doi: 10.3391/ai.2009.4.2
- McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J., Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Divers Distrib* 16: 95–108
- Muller, K.E., Peterson, B.L., 1984. Practical methods for computing power in testing the Multivariate General Linear Hypothesis. *Comput Stat Data An* 2: 143–158.
- Nyström, P., 2002. Ecology. In: Holdich, D.M. (ed.) *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, pp 192–224
- OIE, 2009. Aquatic Animal Health Code. Publ Internet. http://www.oie.int/eng/normes/fcode/en_sommaire.htm. Accessed 1 July 2010
- Padilla, D.K., Williams, S.L., 2004. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Front Ecol Environ* 2:131–138

- Peay, S., 2009. Invasive non-indigenous crayfish species in Europe: Recommendations on managing them. *KMAE* 394-395, 03. doi: 10.1051/kmae/2010009
- Peay, S., Holdich, D.M., Brickland, J., 2010. Risk assessments of non-indigenous crayfish in Great Britain. *Freshw Crayfish* 17. (In press)
- Pekny, R., 2003. Flusskrebse aus aller Welt - Mögliche Invasoren in Europa. Internationales Flusskrebsforum Augsburg 2003, Tagungsband: 27–34
- Pekny, R., Lukhaup, C., 2005. Aquarienkrebse in Europa - eine rasante Entwicklung! 2. Internationale Flusskrebstagung, Baden 2005, Tagungsband: 78–94
- Rabalais, M.R., Magoulick, D.D., 2006. Is competition with the invasive crayfish *Orconectes neglectus chaenodactylus* responsible for the displacement of the native crayfish *Orconectes eupunctus*? *Biol Inv* 8: 1039–1048
- Riley, S., 2005. Invasive alien species and the protection of biodiversity: The role of quarantine laws in resolving inadequacies in the international legal regime. *J Environ Law* 17: 323–359
- Rodríguez, C.F., Bécares, E., Fernández-Aláez, M., Fernández-Aláez, C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biol Inv* 7: 75–85
- Royo, F., Andersson, G., Bangyeekun, E., Múzquiz, J.L., Söderhäll, K., Cerenius, L., 2004. Physiological and genetic characterisation of some new *Aphanomyces* strains isolated from freshwater crayfish. *Vet Microbiol* 104: 103–112
- Sala, O.E., Chapin, III F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, B.H., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity scenario for the year 2100. *Science* 287: 1770–1774
- Schlüter, M., 1989. Flußkrebse aus Australien. Ritter in farbenfroher Rüstung. *DATZ* 42: 526–528
- Scholtz, G., Bradand, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F., Vogt, G., 2002. Parthenogenesis in an outsider crayfish. *Nature* 421: 806
- Sandodden, R., Johnsen, S.I., 2010. Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquat Inv*: 5. doi: 10.3391/ai.2010.5.1

- Schulz, H., Groß, H., Dümpelmann, C., Schulz, R., 2009. Flusskrebse Deutschlands. In: Füreder L. (ed.) Flusskrebse: Biologie, Ökologie, Gefährdung. Veröffentlichungen des Naturmuseums Südtirol, 6, Folio-Verlag, Bozen, Wien, pp 71–81
- Seitz, R., Vilpoux, K., Hopp, U., Harzsch, S., Maier, G., 2005. Ontogeny of the Marmorokrebs (Marbled Crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. *J Exp Zool* 303: 393–405
- Shine, C., Kettunen, M., ten Brink, P., Genovesi, P., Gollasch, S., 2009. Technical support to EU strategy on invasive species (IAS) – Recommendations on policy options to control the negative impacts of IAS on biodiversity in Europe and the EU. Final report for the European Commission. Institute for European Environmental Policy (IEEP), Brussels, Belgium
- Simons, S.A., De Poorter, M., (eds.) 2009. Best Practices in Pre-Import Risk Screening for Species of Live Animals in International Trade. Proceedings of an Expert Workshop on Preventing Biological Invasions, University of Notre Dame, Indiana, USA, 9-11 April 2008. Global Invasive Species Programme, Nairobi, Kenya
- Skelton, C.E., 2010. History, Status, and Conservation of Georgia Crayfishes. *Southeast Nat* 9 (Special Issue 3):127–138
- Soes, M., van Eekelen, R., 2006. Rivierkrefeten, een oprukkend probleem? *De Levede Natuur* 107: 56–59
- Soes, M., Koese, B., 2010. Invasive crayfish in the Netherlands: a preliminary risk analysis. Interim report, Bureau Waardenburg bv, Stichting EIS-Nederland, Invasive Alien Species Team, Waardenburg
- Soes, M., Majoor, G.D., Keulen, S.M.A., 2011. *Bellamyia chinensis* (Gray, 1834) (Gastropoda: Viviparidae), a new alien snail species for the European fauna. *Aquat Inv* 6: 97–102
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P., (eds.) 2006. Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris (Patrimoines naturels, 64)
- Souty-Grosset, C., Reynolds, J.D., 2009. Current ideas on methodological approaches in European crayfish conservation and restocking procedures. *KMAE* 394-395, 01. doi: 10.1051/kmae/2009021
- Stucki, T., Jean-Richard, P., 1999. Verbreitung der Flusskrebse in der Schweiz. Mitteilungen zur Fischerei 65. BUWAL, Bern

- Taylor, C.A., Schuster, G.A., 2004. Crayfishes of Kentucky. Illinois Natural History Survey, Special Publication 28
- Tricarico, E., Vilizzi, L., Gherardi, F., Copp, G.H., 2009. Calibration of FI-ISK, an Invasiveness Screening Tool for Nonnative Freshwater Invertebrates. *Risk Anal.* doi: 10.1111/j.1539-6924.2009.01255.x
- Thompson, W.L., (ed) 2004. Sampling rare or elusive species: concepts, designs, and techniques for estimating population parameters. Island Press, Washington
- UCLA/ATS, 2011. Pseudo *R*-squareds. University of California: Academic Technology Services, Statistical Consulting Group. http://www.ats.ucla.edu/stat/mult_pkg/faq/general/pseudo_rsquareds.htm. Accessed 12 November 2011
- Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S. Springer, New York
- Wendt, W., 2011. Erstnachweis des invasiven Marmorkrebse, *Procambarus fallax* (HAGEN, 1870) f. *virginalis*, für Sachsen. *Forum Flusskrebse* 15: 39–42
- Werner, U., 1993. Ausgefallene Aquarienfleglinge. Landbuch-Verlag, Hannover

Online Resource 1. Year of import, general availability, and presumed introduction pathways of freshwater crayfish in the German pet trade. Introduction pathway abbreviations are as follows: *C* = consumption trade, *FB* = fishing bait, and *S* = deliberate stocking. Please note that the list includes probable synonyms and misidentified specimens: */# = presumably the same species; \$ = status unsure, probably *Ca. shufeldtii* and *Ca. schmitti*; § = probably misidentified. The main references are as follows: 1= Pekny and Lukhaup (2005), 2 = present study, 3 = Lukhaup and Pekny (2005), and 4 = Holdich *et al.* (2009).

Species	Taxon	Year of import		General availability	Introduced from aquaria	Introduced via other pathways	Established in Central Europe	Main ref.
		before 2005	after 2005					
<i>Astacoides betsileonensis</i>	Parastacidae		x	very rare				2
<i>Astacoides granulimanus</i>	Parastacidae	x		very rare				2
<i>Astacoides cf. madagascariensis</i>	Parastacidae		x	very rare				2
<i>Astacus astacus</i>	Astacidae	indigenous		rare	indigenous	indigenous	indigenous	1, 3
<i>Astacus leptodactylus</i>	Astacidae	x		rare		yes (C, S)	yes	1, 3, 4
<i>Austropotamobius pallipes</i>	Astacidae	indigenous		very rare	indigenous	indigenous	indigenous	1, 3
<i>Austropotamobius torrentium</i>	Astacidae	indigenous		very rare	indigenous	indigenous	indigenous	1, 3
<i>Bouchardina robisoni</i>	Cambaridae		x	very rare				2
<i>Cambarellus chapalanus</i>	Cambaridae		x	very common				2
<i>Cambarellus diminutus</i>	Cambaridae	x		common				1
<i>Cambarellus montezumae</i>	Cambaridae	x		very common				1, 3
<i>Cambarellus ninae</i>	Cambaridae		x	rare				2
<i>Cambarellus patzcuarensis</i>	Cambaridae	x		very common				1, 3
<i>Cambarellus puer</i>	Cambaridae		x	very common				2
<i>Cambarellus schmitti</i>	Cambaridae	x		rare				1
<i>Cambarellus shufeldtii</i>	Cambaridae	x		very common				1, 3
<i>Cambarellus sp.</i> 'ALA' [§]	Cambaridae		x	rare				2
<i>Cambarellus sp.</i> 'LOU' ^{*/#}	Cambaridae		x	rare				2
<i>Cambarellus sp.</i> 'HUB' ^{*/#}	Cambaridae		x	rare				2
<i>Cambarellus texanus</i>	Cambaridae		x	rare				2
<i>Cambarellus zempoalensis</i> [§]	Cambaridae	x		very rare				2
<i>Cambarus asperimanus</i>	Cambaridae	x		very rare				1
<i>Cambarus bartonii</i>	Cambaridae	x		very rare				1
<i>Cambarus cf. chaugaensis</i>	Cambaridae	x		very rare				2
<i>Cambarus conasaugaensis</i>	Cambaridae	x		very rare				1
<i>Cambarus coosae</i>	Cambaridae	x		very rare				1
<i>Cambarus coosawattae</i>	Cambaridae	x		very rare				1
<i>Cambarus crinipes</i>	Cambaridae	x		very rare				1
<i>Cambarus diogenes</i>	Cambaridae	x		very rare				3
<i>Cambarus extraneus</i>	Cambaridae	x		very rare				1
<i>Cambarus fasciatus</i>	Cambaridae	x		very rare				1
<i>Cambarus friaufi</i>	Cambaridae	x		very rare				1
<i>Cambarus girardianus</i>	Cambaridae		x	very rare				2

Species	Taxon	Year of import		General availability	Introduced from aquaria	Introduced via other pathways	Established in Central Europe	Main ref.
		before 2005	after 2005					
<i>Cambarus graysoni</i>	Cambaridae	x		very rare				1
<i>Cambarus halli</i>	Cambaridae	x		very rare				1
<i>Cambarus howardi</i>	Cambaridae	x		very rare				1
<i>Cambarus ludovicianus</i>	Cambaridae		x	very rare				2
<i>Cambarus maculatus</i>	Cambaridae	x		very rare				2
<i>Cambarus manningi</i>	Cambaridae	x		rare				1
<i>Cambarus reduncus</i>	Cambaridae	x		very rare				1
<i>Cambarus rusticiformis</i>	Cambaridae	x		rare				1
<i>Cambarus scotti</i>	Cambaridae		x	very rare				2
<i>Cambarus</i> sp. 1	Cambaridae	x		very rare				2
<i>Cambarus</i> sp. 2	Cambaridae	x		very rare				2
<i>Cambarus speciosus</i>	Cambaridae	x		rare				1
<i>Cambarus williami</i>	Cambaridae		x	very rare				2
<i>Cherax albertisii</i>	Parastacidae	x		very rare				1, 3
<i>Cherax boesemani</i> *	Parastacidae	x		common				2, 3
<i>Cherax cainii</i>	Parastacidae	x		rare				1, 3
<i>Cherax destructor</i>	Parastacidae	x		common	yes		no	1, 3, 4
<i>Cherax holthuisi</i>	Parastacidae	x		very common				1, 3
<i>Cherax lorentzi</i>	Parastacidae	x		common				1, 3
<i>Cherax monticola</i>	Parastacidae	x		very rare				1
<i>Cherax peknyi</i>	Parastacidae	x		very common				1, 3
<i>Cherax preissii</i>	Parastacidae	x		rare				1, 3
<i>Cherax quadricarinatus</i>	Parastacidae	x		very common	yes		no	1, 3, 4
<i>Cherax</i> sp. 'Ajamaru' *	Parastacidae	x		very rare				2
<i>Cherax</i> sp. 'blue moon'	Parastacidae	x		common				1, 3
<i>Cherax</i> sp. 'Hoa Creek'	Parastacidae	x		common				1, 3
<i>Cherax tenuimanus</i> §	Parastacidae	x		very rare				2
<i>Hobbseus</i> sp.	Cambaridae	x		rare				1
<i>Hobbseus yalobushensis</i>	Cambaridae	x		rare				1
<i>Orconectes chickasawae</i>	Cambaridae	x		very rare				1
<i>Orconectes compressus</i>	Cambaridae	x		very rare				1
<i>Orconectes durrelli</i>	Cambaridae	x		rare				1
<i>Orconectes erichsonianus</i>	Cambaridae	x		very rare				1
<i>Orconectes eupunctus</i>	Cambaridae		x	rare				2
<i>Orconectes immunis</i>	Cambaridae	x		rare		yes (FB)	yes	1, 3, 4
<i>Orconectes limosus</i>	Cambaridae	x		rare		yes (S)	yes	1, 3, 4
<i>Orconectes longidictus</i>	Cambaridae		x	very rare				2
<i>Orconectes luteus</i>	Cambaridae	x		very rare				2
<i>Orconectes marchandi</i>	Cambaridae		x	very rare				2
<i>Orconectes menae</i>	Cambaridae		x	very rare				2
<i>Orconectes mississippiensis</i>	Cambaridae		x	very rare				2
<i>Orconectes nais</i>	Cambaridae		x	rare				2
<i>Orconectes nana</i>	Cambaridae	x		very rare				1

Species	Taxon	Year of import		General availability	Introduced from aquaria	Introduced via other pathways	Established in Central Europe	Main ref.
		before 2005	after 2005					
<i>Orconectes neglectus</i>	Cambaridae		x	very rare				2
<i>Orconectes ozarkae</i>	Cambaridae	x		very rare				1
<i>Orconectes palmeri palmeri</i>	Cambaridae	?		rare				2
<i>Orconectes placidus</i>	Cambaridae	x		very rare				2
<i>Orconectes propinque</i>	Cambaridae	x		rare				1
<i>Orconectes punctimanus</i>	Cambaridae	x		very rare				1
<i>Orconectes rusticus</i>	Cambaridae	x		unknown				2
<i>Orconectes</i> sp.	Cambaridae	x		very rare				2
<i>Orconectes</i> sp. 'Leopard'	Cambaridae		x	very rare				2
<i>Orconectes</i> sp. 3	Cambaridae		x	very rare				2
<i>Orconectes spinosus</i>	Cambaridae	x		very rare				2
<i>Orconectes virilis</i> (cf. <i>causeyi</i>)	Cambaridae	x		very rare		yes (unknown)	yes	1, 4
<i>Pacifastacus leniusculus</i>	Astacidae	x		very rare		yes (S)	yes	1, 3, 4
<i>Parastacus nicoletti</i>	Parastacidae	x		very rare				2
<i>Procambarus acantophorus</i>	Cambaridae	x		very rare				1
<i>Procambarus acutus/zonangulus</i>	Cambaridae	x		very rare		yes (unknown)	yes	1
<i>Procambarus alleni</i>	Cambaridae	x		very common	yes		unknown	1, 3
<i>Procambarus apalachicola</i>	Cambaridae	x		very rare				1
<i>Procambarus blandingii</i>	Cambaridae	x		very rare				1
<i>Procambarus bouvieri</i>	Cambaridae	x		very rare				2
<i>Procambarus</i> cf. <i>chacei</i>	Cambaridae	x		very rare				1
<i>Procambarus</i> cf. <i>echinatus</i>	Cambaridae	x		very rare				1
<i>Procambarus clarkii</i>	Cambaridae	x		very common	yes	yes (C, S)	yes	1, 3, 4
<i>Procambarus clemmeri</i>	Cambaridae		x	rare				2
<i>Procambarus cubensis</i>	Cambaridae	x		common				1, 3, 4
<i>Procambarus dupratzi</i>	Cambaridae		x	very rare				2
<i>Procambarus enoplosternum</i>	Cambaridae	x		rare				1
<i>Procambarus epicyrtus</i>	Cambaridae	x		very rare				1
<i>Procambarus fallax</i>	Cambaridae	x		common				2
Marmorcrebs (<i>P. fallax</i> f. <i>virginalis</i>)	Cambaridae	x		very common	yes		yes	1, 3, 4
<i>Procambarus geodytes</i>	Cambaridae	x		very rare				2
<i>Procambarus hirsutus</i>	Cambaridae		x	very rare				2
<i>Procambarus hofmani</i>	Cambaridae	x		very rare				1
<i>Procambarus leonensis</i>	Cambaridae	x		very rare				1
<i>Procambarus llamsi</i>	Cambaridae		x	rare				2
<i>Procambarus lophotus</i>	Cambaridae	x		very rare				1
<i>Procambarus mancus</i>	Cambaridae	x		very rare				1
<i>Procambarus milleri</i>	Cambaridae	x		rare				1, 3
<i>Procambarus paeninsulanus</i>	Cambaridae	x		very rare				1
<i>Procambarus petersi</i>	Cambaridae	x		very rare				1
<i>Procambarus pictus</i>	Cambaridae	x		very rare				1
<i>Procambarus pubescens</i>	Cambaridae	x		very rare				1
<i>Procambarus pygmaeus</i>	Cambaridae	x		very rare				2

Species	Taxon	Year of import		General availability	Introduced from aquaria	Introduced via other pathways	Established in Central Europe	Main ref.
		before 2005	after 2005					
<i>Procambarus rodriguezii</i>	Cambaridae	x		very rare				2
<i>Procambarus seminola</i>	Cambaridae	x		very rare				1
<i>Procambarus</i> sp.	Cambaridae		x	very rare				2
<i>Procambarus spiculifer</i>	Cambaridae	x		rare				1, 3
<i>Procambarus strenthi</i>	Cambaridae	x		very rare				1
<i>Procambarus tolteace</i>	Cambaridae	x		rare				1
<i>Procambarus vasquezae</i>	Cambaridae	x		common				1
<i>Procambarus versutus</i>	Cambaridae		x	rare				2
<i>Procambarus vioscai</i>	Cambaridae		x	very rare				2
<i>Procambarus youngi</i>	Cambaridae		x	rare				2
<i>Samastacus spinifrons</i>	Parastacidae	x		very rare				2

Online Resource 2. Mean price class, online availability, potential invasiveness (FI-ISK score), natural habitat, and native and introduced range of non-indigenous crayfish species offered by 16 online shops in 2009. *N.A.* means that the price was not depicted or no available information on the natural habitat.

Species	Mean price class	Online avail.	FI-ISK score	FI-ISK outcome	Habitat	Native range	Introduced range
<i>Cambarellus chapalonus</i>	2.0	0.25	2	evaluate	lentic situations	Mexico	
<i>Cambarellus diminutus</i>	2.2	0.32	8	evaluate	pools, sluggish portions of streams and ditches	southern USA	
<i>Cambarellus montezumae</i>	1.9	0.44	8	evaluate	sluggish streams and lentic situations	Mexico	
<i>Cambarellus patzcuarensis</i>	2.0	0.25	9	evaluate	lake	Mexico	
<i>Cambarellus patzcuarensis</i> var. 'Orange'	2.0	1.00	9	evaluate	lake	Mexico	
<i>Cambarellus puer</i>	1.8	0.56	9	evaluate	swamps, ditches, sloughs, ponds, and sluggish streams	southern USA	
<i>Cambarellus schmitti</i>	2.0	0.06	8	evaluate	clear streams and springs	southeastern USA	
<i>Cambarellus shufeldtii</i>	1.8	0.25	8	evaluate	swamps, ditches, sloughs, lakes, ponds, and sluggish streams	southern USA	USA (Louisiana and Harris County, Georgia)
<i>Cambarellus</i> sp. 'ALA'	3.0	0.06	6	evaluate	<i>N.A.</i>	Alabama	
<i>Cambarellus</i> sp. 'LOU'	3.0	0.06	6	evaluate	<i>N.A.</i>	Louisiana	
<i>Cambarellus texanus</i>	2	0.13	10	evaluate	roadside ditches	southern USA	
<i>Cambarus maculatus</i>	<i>N.A.</i>	0.06	5	evaluate	streams	central N-America	
<i>Cherax boesemani</i>	4.3	0.25	7	evaluate	lake	New Guinea	
<i>Cherax destructor</i>	4.0	0.31	21	reject	lentic and lotic habitats	southeastern Australia	Spain, Italy, Switzerland, western Australia
<i>Cherax holthuisi</i>	3.3	0.56	7	evaluate	lentic and lotic	New Guinea	
<i>Cherax lorentzi</i>	3.0	0.06	7	evaluate	streams	New Guinea	
<i>Cherax pekneyi</i>	3.5	0.50	7	evaluate	sreeks, streams	New Guinea	
<i>Cherax preissii</i>	5.0	0.06	6	evaluate	streams	southwestern Australia	
<i>Cherax quadricarinatus</i>	2.2	0.31	14	evaluate	lentic and lotic situations	northeastern Australasia	Germany, Slovenia, Central America, south-east Asia
<i>Cherax</i> sp. 'Ajamaru'	3.0	0.06	7	evaluate	<i>N.A.</i>	New Guinea	
<i>Cherax</i> sp. 'Blue Moon'	4.4	0.31	7	evaluate	<i>N.A.</i>	New Guinea	
<i>Cherax</i> sp. 'Hoa Creek'	4.5	0.38	7	evaluate	<i>N.A.</i>	New Guinea	
<i>Orconectes durelli</i>	5.5	0.13	6	evaluate	creeks, streams	eastern N-America	
<i>Orconectes limosus</i>	2.0	0.06	24	reject	streams	northeastern N-America	
<i>Orconectes neglectus</i>	5.0	0.06	18	reject	streams	central N-America	USA (Oregon, southeastern New York, Missouri, Arkansas)
<i>Procambarus alleni</i>	2.0	0.63	21	reject	lentic and lotic situations and burrows	Florida	France
<i>Procambarus clarkii</i>	2.0	0.56	33	reject	lentic situations, burrows as water disappears from habitat	southern N-America	America, Europe, Africa, Asia

Species	Mean price class	Online avail.	FI-ISK score	FI-ISK outcome	Habitat	Native range	Introduced range
<i>Procambarus clemmeri</i>	4.0	0.06	8	evaluate	streams	southern USA	
<i>Procambarus cubensis</i>	2.0	0.06	8	evaluate	lentic and lotic situations	Cuba	
<i>Procambarus enoplosternum</i>	3.0	0.19	10	evaluate	streams and lotic situations	southeastern USA	
<i>Procambarus fallax</i>	2.0	0.25	12	evaluate	lentic and lotic situations and burrows	southeastern USA	
Marmorkrebs (<i>P. fallax</i> f. <i>virginalis</i>)	1.5	0.25	22	reject	<i>N.A.</i>	southeastern USA	Germany, Netherlands, Italy, Slovakia, Madagascar, Japan
<i>Procambarus llamasii</i>	5.5	0.13	13	evaluate	small streams and drainage ditches	Mexico/ Guatemala	
<i>Procambarus spiculifer</i>	3.0	0.13	14	evaluate	streams	southeastern USA	
<i>Procambarus toltecaae</i>	<i>N.A.</i>	0.06	6	evaluate	epigean and hypogean streams	Mexico	
<i>Procambarus vazquezae</i>	2.0	0.25	6	evaluate	lentic situations	Mexico	
<i>Procambarus versutus</i>	2.0	0.13	12	evaluate	streams	southeastern USA	
<i>Procambarus youngi</i>	2.0	0.06	10	evaluate	streams	Florida	

The clones are coming – strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe

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Abstract

We describe a new occurrence of parthenogenetic Marmorkrebs in southwestern Germany and give a synopsis of recent records of this species in Europe. Including the most recent records, 15 Marmorkrebs records are currently known, most of which are from Germany. At least six records represent established populations, which is an alarming increase beyond the one Marmorkrebs population known prior to 2010. Most established populations occur in lentic habitats near conurbations, typically in highly frequented secondary habitats, such as gravel pit lakes. In three instances, Marmorkrebs migrated over land, demonstrating their potential for active spread, and two invasive populations endanger indigenous crayfish populations. Most Marmorkrebs populations are large and are most likely several years old, suggesting a considerable lag between introduction and detection. Marmorkrebs populations in Europe are most likely the result of deliberate releases from aquaria, although secondary introductions may have occurred in one instance. Because Marmorkrebs are still widespread in the European pet trade, which most likely generates substantial propagule pressure, it is likely that the number of established populations will further increase over time. To mitigate the risk of further harmful crayfish releases, we suggest the prohibition of trading live high-risk crayfish species, including Marmorkrebs.

Key words: aquarium introductions, marbled crayfish, invasiveness

Introduction

The Marmorkrebs is an enigmatic crayfish species of North American origin. It was first discovered in the German pet trade in the mid 1990s, when aquarium enthusiasts reported an all-female crayfish species that reproduces without males (Lukhaup 2001). Due to their characteristic and conspicuous color pattern, these crayfish quickly became known as ‘Marmorkrebs’ (German, which translates into English as ‘marbled crayfish’). Scientists have puzzled for almost a decade about their phylogenetic position and status. Using genetic and morphological comparisons, Martin *et al.* (2010a) recently showed that Marmorkrebs are the parthenogenetic form of *Procambarus fallax* (Hagen, 1870) and proposed the tentative scientific name *Procambarus fallax* f. *virginalis*. The Marmorkrebs is unique because it is the only known decapod crustacean that obligatorily reproduces by apomictic parthenogenesis: only females exist, which lay unfertilized eggs that develop into genetically identical offspring (Scholtz *et al.* 2003; Martin *et al.* 2007; Vogt *et al.* 2008). No males have been found in the laboratory or introduced, wild populations (Seitz *et al.* 2005; Jones *et al.* 2009; Janský and Mutkovič 2010).

Marmorkrebs had circulated in the European pet trade for several years before the first free-living individuals were captured in Europe and Madagascar in 2003 (Soes and van Eekelen 2006; Jones *et al.* 2009). The major pathway for Marmorkrebs introduction is the deliberate release of aquarium specimens (Soes and van Eekelen 2006; Souty-Grosset *et al.* 2006; Chucholl 2011). Their appealing coloration, undemanding nature and exceptional mode of reproduction make Marmorkrebs attractive to aquarium hobbyists. However, parthenogenesis permits a high reproductive potential, and Marmorkrebs can overpopulate an aquarium quickly. Aquarium hobbyists will likely offload their excess stock to either other aquarium hobbyists or natural habitats (Souty-Grosset *et al.* 2006; Chucholl 2011). Because Marmorkrebs reproduce by parthenogenesis, the risk that released Marmorkrebs may seed viable populations in the wild is considerably greater than that for sexually reproducing crayfish species – a single Marmorkrebs is sufficient to create a new population.

Marmorkrebs quickly became established in Madagascar and are considered to be ‘perfect invaders’ there (Jones *et al.* 2009). On the contrary, in Europe, almost all published Marmorkrebs records between 2004 and 2009 are accounts of single individuals, suggesting that Marmorkrebs might fail to establish self-sustaining populations in temperate zones (Marten *et al.* 2004; Souty-Grosset *et al.* 2006; Nonnis Marzano *et al.* 2009; Martin *et al.* 2010b). In 2010, the situation dramatically changed when the first informal evidence for an established Marmorkrebs population in Germany was published by newspapers (Privenau 2010): the local media repeatedly reported on Marmorkrebs emerging from a small pond in a village near Halle (Saxony-Anhalt; Wendt 2011). Shortly afterward, a research paper provided evidence that Marmorkrebs had established another stable, reproducing population in a small lake near the city of Freiburg (Baden-Württemberg; Chucholl and Pfeiffer 2010). Since then, numerous additional Marmorkrebs records from Europe were reported, including more reproducing populations.

The purpose of the present article was twofold: (1) to describe a new occurrence of Marmorkrebs in southwestern Germany that was discovered in 2011, and (2) to give a

synopsis of the recent records of this species in Europe, including newly discovered populations in other parts of Germany and Slovakia. We then use the available information to discuss the introduction history, population trend, and invasiveness of Marmorkrebs in Europe. Because Marmorkrebs are primarily introduced from aquaria (Souty-Grosset *et al.* 2006; Chucholl 2011), we focus on the risks associated with aquarium introductions, a newly emerging introduction pathway of non-indigenous crayfish species (NICS) that may add yet another chapter to the notorious history of NICS introductions in Europe (*cf.* Holdich *et al.* 2009; Chucholl 2010). Our findings may therefore help with invasive species risk assessment and the prioritization of introduction pathway management.

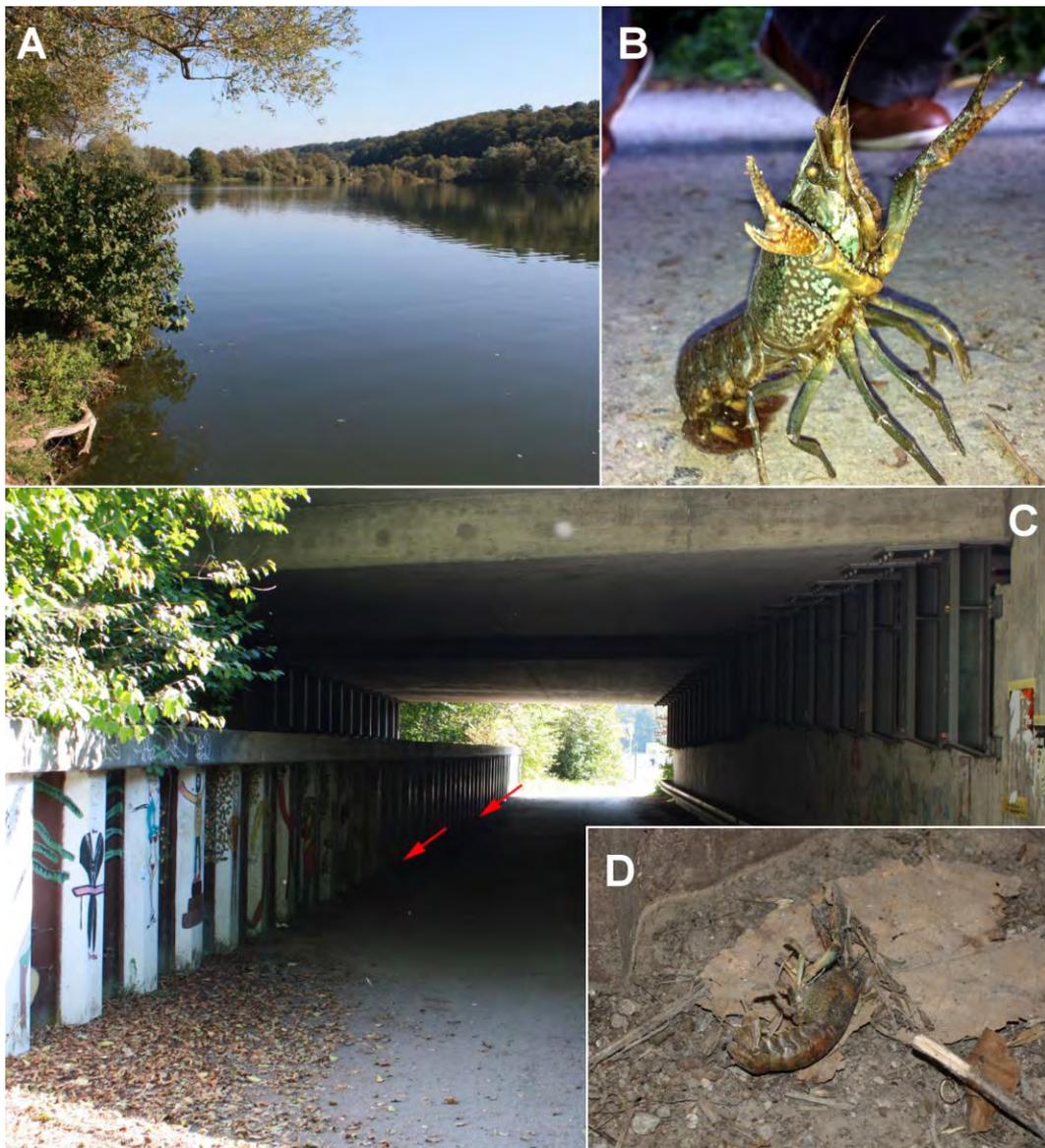


Figure 1. Lake Epplesee (A) and the pedestrian underpass (C), where Marmorkrebs were first found (B: live individual on September 14, and D: dead individual on September 30, 2011; red arrows mark the locations where the two dead Marmorkrebs were found). The pedestrian underpass is located 130 m northeast of Lake Epplesee, and it is separated from the lake by a 100 m stretch of sunbathing area (visible in the background of C). Behind the metal boarding on the left side of the walkway is the brook Schlierbach, which is inhabited by the endangered stone crayfish (*Austropotamobius torrentium*). Photographs A, C, and D by CC, photograph B courtesy of KM.

New occurrence of established Marmorkrebs in southwestern Germany

On September 14, 2011, one of the authors (KM) discovered a live Marmorkrebs at a pedestrian underpass, 130 m from a gravel pit lake (Lake Epplesee; Figure 1A, B; Table 1, Nr. 12). Lake Epplesee (*LE*) is a shallow, eutrophic lake with a surface area of 26.6 ha and a maximum depth of 5 m. It lies 302 m above sea level, in close proximity to the River Neckar. The lake is popular for several recreational activities, including swimming, sunbathing, and angling, and is highly frequented because of its easy accessibility from a nearby highway. *LE* is situated in the vicinity of Tübingen but also attracts people from Reutlingen and Stuttgart.

To verify the sighting, the northern lake margin was sampled using a hand-held net on September 30, 2011. Within five minutes, two Marmorkrebs were captured within an area of approximately 2 m², and a third individual was spotted but escaped. One of the captured females carried hatchlings, while the other female exhibited active glair glands on the ventral side of the pleon, indicating breeding condition. In addition, two dead individuals were found at the pedestrian underpass, which is separated from the northern lake margin by a 100 m stretch of sunbathing area (Figure 1C, D). The Marmorkrebs captured from the lake measured 29.6 and 50.2 mm in carapace length (*CL*: measured from the tip of the rostrum to the dorsal posterior margin of the cephalothorax with a digital slide caliper), and the two dead individuals measured 47.1 and 44.5 mm *CL*.

The occurrence of a 'new crayfish' in *LE* has been known to recreational fishermen since at least 2010, when the species was reportedly already very abundant. Close to *LE* is the brook Schlierbach, which is inhabited by the endangered stone crayfish [*Austropotamobius torrentium* (Schrank, 1803); cf. Figure 1C; Chucholl and Dehus 2011]. The environmental and fisheries agencies currently attempt to control and to contain the Marmorkrebs population, e.g., through stocking and managing of predatory fish and discouraging the public from transplanting and fostering Marmorkrebs (M. Konrad, pers. com. 2011).

Synopsis of Marmorkrebs records from Europe

The year of the initial recording, country, location, habitat, population status, collection method and additional comments are summarized for each of the known Marmorkrebs records from Europe in Table 1, and the present European distribution of Marmorkrebs is shown in Figure 2.

In total, 15 Marmorkrebs records are currently known in Europe, excluding putative records that could not be verified by voucher specimens or photos. Most of the records were initially discovered after 2005, and 13 of the 15 records were only discovered within the past four years (Figure 3). The vast majority of the records were reported in Germany (12), while one record each was reported from the Netherlands, Italy and Slovakia (Figure 2). Marmorkrebs were found in both lotic and lentic freshwater habitats, including brooks, rivers, canals, natural and artificial lakes, and ponds; however, established populations have only been found in lentic habitats (cf. Table 1). Out of the 15 records, six clearly represent established populations, and on the basis of the collection of several individuals, five additional records might also represent established populations. Single individuals that most

likely failed to propagate, *i.e.*, failed introductions, were reported in three cases, of which two involved captures from small brooks (Table 1, Nr. 3 and 7). The methods used to detect Marmorkrebs varied considerably and included accidental by-catch, observations/captures of individuals crawling on land, electrofishing, trapping, manual search, and observations made by snorkeling/diving. In at least four cases, Marmorkrebs occurred in sympatry with *Orconectes limosus* (Rafinesque, 1817) or *Procambarus clarkii* (Girard, 1852) (Table 1, Nr. 1, 4, 6, and 15).

Apart from the records summarized above, at least four additional putative records of Marmorkrebs in Germany have been reported and cover three federal states; however, these records have not been verified yet (Chucholl and Dehus 2011; H. Groß, unpublished data). In Slovakia, Marmorkrebs apparently also formed breeding populations in garden ponds (Stloukal 2009). Dead Marmorkrebs found on the land were repeatedly reported from North Rhine-Westphalia (Germany; H. Groß unpublished data), suggesting that the estimated number of unreported Marmorkrebs occurrences is still high (*cf.* Chucholl and Pfeiffer 2010).

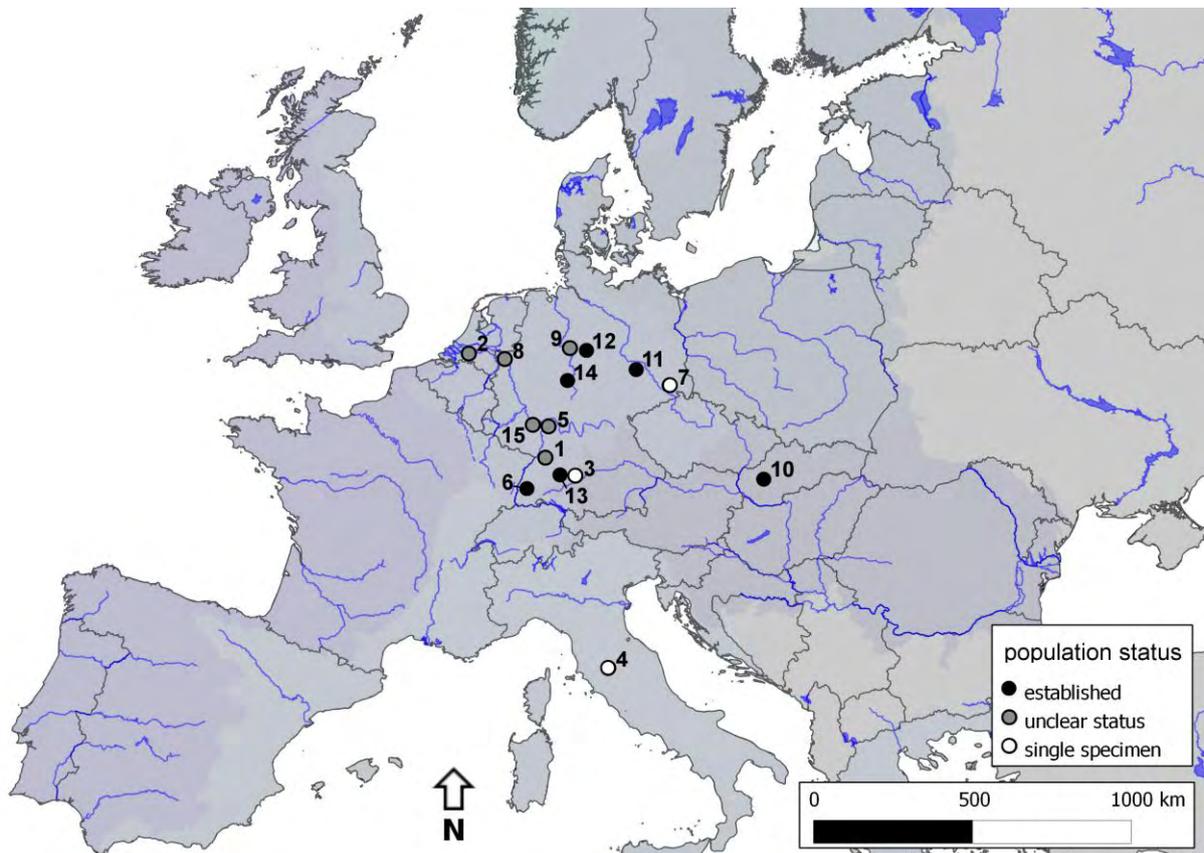


Figure 2. Current distribution of Marmorkrebs in Europe, shown as presence in CGRS grid squares. Numbers refer to the records summarized in Table 1.

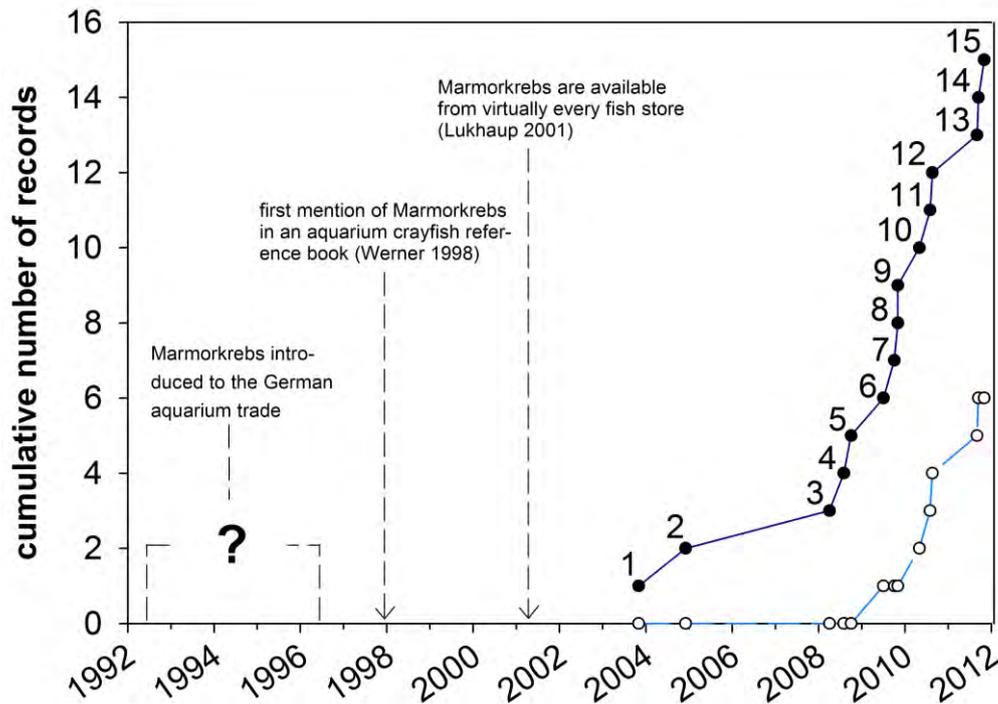


Figure 3. Important events and cumulative number of Marmorkrebs records in Europe in relation to time. The total number of records is depicted as a solid line with solid symbols, and the number of established populations is depicted as a broken line with open symbols. Numbers refer to the records summarized in Table 1.

Discussion

The Marmorkrebs is an extraordinarily successful crayfish species; it was first discovered in the German pet trade in the mid-1990s (*cf.* Werner 1998; Figure 3) and was first mentioned in a scientific publication in 2002 (Scholtz *et al.* 2002). By this time, it was already very popular as an aquarium pet (Lukhaup 2001; Figure 3). Only ten years later, it has become a highly invasive species in Madagascar (Jones *et al.* 2009), was introduced into freshwater habitats in Japan (Kawai and Takahata 2010), and is well established in various parts of Europe (summarized in Table 1; Figures 2 and 3). Including the new occurrence described here, at least six established Marmorkrebs populations are currently known in Europe, representing an alarming increase beyond the one population known prior to 2010 (*cf.* Figure 3). Most populations have accumulated great numbers and are most likely several years old, suggesting a considerable lag between introduction and detection. Given this probable lag between introduction and detection, it is likely that the currently observed trend of increasing population numbers is a result of introductions that occurred several years ago, presumably around the time that Marmorkrebs became popular in home aquaria (*cf.* Figure 3). This interpretation is in accordance with the propagule pressure hypothesis, which states that a major determinant of the invasion success of a non-indigenous species is the number of independent release events (propagule number) plus the number of individuals released in any one event (propagule size), that is, introduction effort or propagule pressure (Lockwood *et al.* 2005; Lockwood *et al.* 2007).

The proliferation of Marmorkrebs as aquarium pets likely increased propagule pressure, and thereby the likelihood of establishment in nature (Lockwood *et al.* 2005; Duggan *et al.* 2006; Lockwood *et al.* 2007). Specifically, the great popularity of Marmorkrebs as aquarium pets probably results in many independent release events, *i.e.*, a high propagule number, which serves to overcome negative forces that are spatially structured, such as an unsuitable habitat at the site of introduction (Lockwood *et al.* 2005; Duggan *et al.* 2006). For instance, Marmorkrebs apparently failed to establish in cool, rapid-flowing brooks but eventually invaded lentic habitats. Marmorkrebs propagule pressure and, thus, the likelihood of establishment in nature, is probably highest in countries where (1) Marmorkrebs are popular aquarium pets and (2) human population density is high (*cf.* Lockwood *et al.* 2005; Perdikaris *et al.* 2012). Both of the criteria apply to Germany (Lukhaup 2001; Perdikaris *et al.* 2012), where five out of the six currently known Marmorkrebs populations in Europe are located (Figure 2).

Because Marmorkrebs are still abundant in the European pet trade (Stloukal 2009; Chucholl 2010; Soes and Koese 2010) and there is potentially a lag between introduction and detection, it is likely that the number of records of established populations will further increase with time. Marmorkrebs are acquired through retail pet shops, various online sources, and personal contacts between aquarium hobbyists (Stloukal 2009; Faulkes 2010; Soes and Koese 2010). Retail stores offer Marmorkrebs at approximately 5 € per animal, whereas aquarium hobbyists often give their excess stock away for free (*cf.* Chucholl 2011). For instance, a search for ‘Marmorkrebs’ at a German online marketplace (www.kleinanzeigen.ebay.de) on January 12, 2012 resulted in 36 hits, and most of these vendors offered the animals for free or at prices below 5 € per crayfish. In recent years, Marmorkrebs have also become popular as live food for predatory aquarium fish and ornamental turtles owing to their undemanding nature and high reproductive potential. Because ornamental turtles are frequently kept in outdoor ponds, using Marmorkrebs as a food source may facilitate accidental introductions. It is even more concerning that Marmorkrebs are deliberately released into garden ponds (Stloukal 2009), from which they can easily escape to nearby freshwater habitats.

Introduced Marmorkrebs have been found in both lentic and lotic freshwater habitats (*e.g.*, Marten *et al.* 2004; Martin *et al.* 2010b). However, established populations in Europe have been found in lentic habitats only (*cf.* Table 1), which agrees with Chucholl’s and Pfeiffer’s (2010) suggestion that Marmorkrebs are most likely able to colonize summer-warm, lentic habitats in most parts of Europe. In Madagascar, Marmorkrebs were reported from a great variety of habitats, including rice paddies, rivers, lakes and swamps (Heimer 2010), as well as brick pits, drainage ditches and fish ponds (Jones *et al.* 2009). Most established populations in Europe occur in habitats near conurbations, typically in highly frequented secondary habitats, such as gravel pit lakes. Introductions from aquaria are more likely to occur in highly frequented habitats than in remote habitats because highly frequented habitats are usually more easily accessible and may already be known to the potential pet releaser. However, the probability of detection is presumably also higher in highly frequented habitats, which may confound this effect.

In at least three instances, Marmorkrebs were observed to migrate over land (Table 1, Nr. 2, 11, and 13; Figure 1), and single dead Marmorkrebs found on the land were repeatedly reported from North Rhine-Westphalia (H. Groß, unpublished data). The frequent observation of Marmorkrebs migrating over land suggests that this behavior is most likely an inherent dispersal mechanism for this species, rather than an escape mechanism in response to adverse environmental conditions. Established Marmorkrebs populations may therefore act as latent 'bridgeheads' for a further active range expansion both *via* waterways and land. Any attempts to contain local populations should take both pathways into account. A further potential pathway for spreading is translocation by humans, *i.e.*, secondary introductions. This mechanism of spreading most likely occurred in Saxony-Anhalt (Table 1, Nr. 11), where people collected Marmorkrebs migrating over land and most likely transplanted them to garden ponds (Wendt 2011).

During the last decade, Marmorkrebs were primarily known as aquarium pets. Most research on these organisms was carried out in the laboratory and was concerned with their exceptional mode of reproduction or use as a model organism for development, epigenetics and toxicology (*e.g.*, Seitz *et al.* 2005; Martin *et al.* 2007; Vogt 2008). Marmorkrebs being an invasive species is a rather new phenomenon, and their life history and trophic ecology in nature are understudied topics. For instance, nothing is known about the trophic position and ecological impact of Marmorkrebs. Given the major impacts of the related species *P. clarkii* on recipient ecosystems (Souty-Grosset *et al.* 2006, and citations therein), Marmorkrebs may have a profound impact on the species richness, functioning and integrity of ecosystems.

Marmorkrebs most likely pose a serious threat to the indigenous European crayfish species because of competition for food and space and crayfish plague transmission (Jones *et al.* 2009; Chucholl and Pfeiffer 2010). Jimenez and Faulkes (2011) studied direct aggressive interactions between Marmorkrebs and *P. clarkii* and concluded that Marmorkrebs have the potential to compete with other crayfish species. Furthermore, Marmorkrebs differ ecologically from the more *K*-selected indigenous European crayfish because Marmorkrebs have a fast growth rate, very high fecundity and an extended breeding period (Seitz *et al.* 2005; Jones *et al.* 2009; Chucholl and Pfeiffer 2010), all of which might give an additional competitive advantage to Marmorkrebs. The risk of devastating consequences for indigenous crayfish would dramatically increase if Marmorkrebs were infected with the causative agent of crayfish plague, *Aphanomyces astaci* Schikora, 1903: any contact between Marmorkrebs and the susceptible European crayfish would almost certainly result in mass mortalities among the susceptible species. This potential threat to indigenous crayfish is alarming, especially because at least two of the six established Marmorkrebs populations already endanger indigenous crayfish populations (Table 1, Nr. 6 and 13).

Conclusion

Without much doubt, all Marmorkrebs introductions trace back to this species' first occurrence in the German pet trade in the mid-1990s. It circulated in the European pet trade for several years before the first free-living individuals were captured in Germany and the Netherlands (*cf.* Table 1 and Figure 3). The currently observed and alarming increase in established populations is likely to continue over time, unless Marmorkrebs propagule

pressure from the pet trade decreases. The introductions occurred despite public education efforts, explicitly advising to not release Marmorkrebs into nature (*e.g.*, Lukhaup 2001; Lukhaup and Pekny 2005; Lukhaup and Pekny 2007; Edelkrebsprojekt NRW 2009). In fact, one of the first articles on Marmorkrebs in a popular German aquarium magazine (Lukhaup 2001) and the first scientific mention of Marmorkrebs, published in *Nature* (Scholtz *et al.* 2002), both implied warning that Marmorkrebs may become a pest if released from captivity. Unfortunately, there is no ‘magic silver bullet’ to eradicate established NICS populations (Gherardi *et al.* 2011) and Marmorkrebs do not need to maintain a minimum viable population size. Their resilience against small population size renders eradication of established Marmorkrebs populations even more difficult or even impossible. The prevention of new introductions is therefore imperative. Given the apparent ineffectiveness of public education efforts to halt exotic crayfish releases, we suggest the prohibition of trading live high-risk crayfish species, including Marmorkrebs.

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References

- Chucholl, C., 2010. Invaders for sale: Does the ornamental freshwater crayfish trade constitute an actual and overlooked risk? In: Souty-Grosset C, Grandjean F, Mirebeau C (eds) (2010) Proceedings of the European crayfish, food, flagship and ecosystem services conference, Poitiers, France, October 26-29, 2010. Imprimerie Copy-Media, p108
- Chucholl, C., 2011. Der Handel mit exotischen Flusskrebse. *Forum Flusskrebse* 15: 33–39
- Chucholl, C., Pfeiffer, M., 2010. First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquatic Invasions* 5: 405–412
- Chucholl, C., Dehus, P., 2011. Flusskrebse in Baden-Württemberg. Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen, Germany, 92 pp
- Duggan, I., Rixon, C.A.M., MacIsaac, H.J., 2006. Popularity and Propagule Pressure: Determinants of Introduction and Establishment of Aquarium Fish. *Biological Invasions* 8: 377–382

- Dümpelmann, C., Bonacker, F., (in press). Erstnachweis des Marmorkrebse *Procambarus fallax* f. *virginalis* (Decapoda: Cambaridae) in Hessen. *Forum Flusskrebse*
- Edelkrebisprojekt NRW, 2009. Flusskrebse im Aquarium und Gartenteich. Edelkrebisprojekt NRW, Bad Münstereifel-Schönau, Germany, 6 pp
- Faulkes, Z., 2010. The spread of the parthenogenetic marbled crayfish, Marmorkrebs (*Procambarus* sp.), in the North American pet trade. *Aquatic Invasions* 5: 447–450
- Gherardi, F., Aquiloni, L., Diéguez-Uribeondo, J., Tricarico, E., 2011. Managing invasive crayfish: is there a hope? *Aquatic Sciences*, doi 10.1007/s00027-011-0181-z.
- Heimer, K., 2010. Invasion of self-cloning crayfish alarms Madagascar. Deutsche Presse-Agentur wire story. <http://www.earthtimes.org/articles/news/339974,alarms-madagascar-feature.html> (Accessed 12 January 2011)
- Hessenfischer, 2011. Vorkommen des invasiven Marmorkrebse (*Procambarus fallax* f. *virginalis*) im Singliser See. http://www.hessenfischer.net/natur/natur_18.htm (Accessed 9 January 2012)
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 11: 394–395. doi: 10.1051/kmae/2009025
- Janský, V., Mutkovič, A., 2010. Rak *Procambarus* sp. (Crustacea: Decapoda: Cambaridae) – Prvý Nález na Slovensku. *Zborník Slovenského Národného Múzea (Acta rerum naturalium Musei Nationalis Slovaci Bratislava)* 56: 64–67
- Jimenez, S.A., Faulkes, Z., 2011. Can the parthenogenetic marbled crayfish Marmorkrebs compete with other crayfish species in fights? *Journal of Ethology* 29: 115–120
- Jones, J.P.G., Rasamy, J.R., Harvey, A., Toon, A., Oidtmann, B., Randrianarison, M.H., Raminosoa, N., Ravoahangimalala, O.R., 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions* 11: 1475–1482
- Kawai, T., Takahata, M., (eds) 2010. Biology of Crayfish. Sapporo, Japan: Hokkaido University Press, Japan, 556 pp
- Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20: 223–228

- Lockwood, J.L., Hoopes, M.F., Marchetti, M.P., 2007. *Invasion Ecology*. Blackwell publishing, Oxford, UK, 304 pp
- Lukhaup, C., 2001. *Procambarus* sp. - Der Marmorkrebs. *Aquaristik Aktuell* 7–8: 48–51
- Lukhaup, C., Pekny, R., 2005. *Krebse im Aquarium*. Dähne Verlag, Ettlingen, Germany, 160 pp
- Lukhaup, C., Pekny, R., 2007. Marmorkrebs bedroht madagassische Flusskrebarten. www.crusta10.de (Accessed 30 May 2007)
- Marten, M., Werth, C., Marten, D., 2004. Der Marmorkrebs (Cambaridae, Decapoda) in Deutschland – ein weiteres Neozoon im Einzugsgebiet des Rheins. *Lauterbornia* 50: 17–23
- Martin, P., Kohlmann, K., Scholtz, G., 2007. The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. *Naturwissenschaften* 94: 843–846
- Martin, P., Dorn, N., Kawai, T., van der Heiden, C., Scholtz, G., 2010a. The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contributions to Zoology* 79: 107–118
- Martin, P., Shen, H., Füllner, G., Scholtz, G., 2010b. The first record of the parthenogenetic Marmorkrebs (Decapoda, Astacida, Cambaridae) in the wild in Saxony (Germany) raises the question of its actual threat to European freshwater ecosystems. *Aquatic Invasions* 5: 397–403
- Nonnis Marzano, F.N., Scalici, M., Chiesa, S., Gherardi, F., Piccinini, A., Gibertini, G., 2009. The first record of the marbled crayfish adds further threats to fresh waters in Italy. *Aquatic Invasions* 4: 401–404
- Perdikaris, C., Kozák, P., Kouba, A., Konstantinidis, E., Paschos, I., 2012. Socio-economic drivers and non-indigenous freshwater crayfish species in Europe. *Knowledge and Management of Aquatic Ecosystems* 402, doi: 10.1051/kmae/2011077
- Privenau, K., 2010. Marmorkrebs bringt Pest. Mitteldeutsche Zeitung news story. <http://www.mz-web.de/servlet/ContentServer?pagename=ksta/page&atype=ksArtikel&aid=1286541132341&calledPageId=987490165154> (Accessed 2 December 2010)
- Pyka, H., 2010. Marmorkrebs im Giftener Teich. <http://www.fvhannover.de/joomla/index.php/neuigkeiten/469-marmorkrebs-im-giftener-teich.html> (Accessed 6 March 2012)

- Scholtz, G., Braband, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F., Vogt, G., 2002. Parthenogenesis in an outsider crayfish. *Nature* 421: 806–806
- Seitz, R., Vilpoux, K., Hopp, U., Harzsch, S., Maier, G., 2005. Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. *Journal of Experimental Zoology* 303: 393–405
- Soes, M., van Eekelen, R., 2006. Rivierkreeften, een oprukkend probleem? *De Levende Natuur* 107: 56–59
- Soes, M., Koese, B., 2010. Invasive crayfish in the Netherlands: a preliminary risk analysis. Interim report, Bureau Waardenburg bv, Stichting EIS-Nederland, Invasive Alien Species Team, Waardenburg, The Netherlands, 69 pp
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P., (eds) 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France, 187 pp
- Stloukal, E., 2009. Recent distribution of non-indigenous (sic) crayfish species in Slovakia. *Folia faunistica Slovaca* 14: 119–122
- Vogt, G., 2008. The marbled crayfish: a new model organism for research on development, epigenetics and evolutionary biology. *Journal of Zoology* 276: 1–13
- Vogt, G., Huber, M., Thiemann, M., van den Boogaart, G., Schmitz, O.J., Schubart, C.D., 2008. Production of different phenotypes from the same genotype in the same environment by developmental variation. *The Journal of Experimental Biology* 211: 510–523
- Wendt, W., 2011. Erstnachweis des invasiven Marmorkrebses, *Procambarus fallax* (HAGEN, 1870) f. *virginalis*, für Sachsen. *Forum Flusskrebse* 15: 39–42
- Werner, U., 1998. Garnelen, Krebse und Krabben im Süßwasseraquarium, Aqualog Spezial, A.C.S., Mörfelden-Walldorf, Germany, 48 pp

Table 1. Current records of Marmorkrebs in Europe. Only records verified by voucher specimens or photos are included. NA means not available.

Nr	year of record	country, region	location	habitat	population status	collection method	comments	references
1	2003	Germany, Baden-Württemberg	49°5'19"N, 8°22'17"E	gravel pit lake	unclear; single specimen	by-catch during <i>Lymnomyxis</i> sampling	the specimen was first taken to be <i>Orconectes limosus</i> , but was later identified as Marmorkrebs; current status unclear	Marten <i>et al.</i> 2004
2	2004	Holland, Dordrecht, Vlij	51°48'49"N, 04°41'36"E	canal	unclear; established from 2004-2008	capture of crayfish crawling on the land	the current status is unclear (Soes and Koese, 2010)	Soes and van Eekelen 2006
3	2008	Germany, Bavaria	48°26'46"N, 10°09'28"E	brook	single specimen	electrofishing	nocturnal manual search yielded no further specimens	C. Chucholl, unpublished data
4	2008	Italy, Tuscany	43°16'51"N, 11°50'12"E	canal	single specimen	electrofishing, trapping	single Marmorkrebs in sympatry with a large <i>Procambarus clarkii</i> population	Nonnis Marzano <i>et al.</i> 2009
5	2008	Germany, Hesse	49°58'43"N, 08°36'59"E	shallow ditch	unclear; three indiv. were captured	trapping	the ditch was almost dry, and the Marmorkrebs were captured from a pool	R. Hennings, pers. com. 2012
6	2009	Germany, Bden-Württemberg	48°01'53"N, 07°48'18"E	lake	established population	observation (snorkeling), manual search	in sympatry with <i>O. limosus</i> ; a few Marmorkrebs were later observed in the lake outflow	Chucholl and Pfeiffer 2010
7	2009	Germany, Saxony	51°20'17"N, 13°33'33"E	brook	single specimen	electrofishing	subsequent trapping yielded no further specimens	Martin <i>et al.</i> 2010
8	2009	Germany, North Rhine-Westphalia	51°27'36"N, 06°43'24"E	Rhine harbor	unclear; several specimens were collected	NA	the harbor was later subject to construction works, possibly destroying the potential population	H. Groß, unpublished data
9	2009	Germany, Lower Saxony	52°10'14"N, 09°05'48"E	river	unclear; several specimens were collected	NA	no further information available	H. Groß, unpublished data
10	2010	Slovakia, Western Slovakia	48°28' N, 17°49' E	gravel pit lake	established population	electrofishing	more than 150 Marmorkrebs were captured	Jansky and Mutkovic 2010
11	2010	Germany, Saxony-Anhalt	51°28'58"N, 12°07'13"E	pond	established population	observation of crayfish crawling on the land	people collected Marmorkrebs migrating over land and probably transplanted them to garden ponds	Privenau 2010; Wendt 2011
12	2010	Germany, Lower Saxony	52°12'55"N, 09°49'33"E	pond	established population	observation made by divers, netting	Divers took a picture of a Marmorkrebs in 2010; an unconfirmed record dates back to 2007	Pyka 2010
13	2011	Germany, Baden-Württemberg	48°32'36"N, 09°09'14"E	gravel pit lake	established population	observation of crayfish crawling on the land, manual search	Marmorkrebs were observed to migrate over land; probably a large population	present study
14	2011	Germany, Hesse	51°03'33"N, 09°18'22"E	pit mine lake	established population	observation, dip netting	Marmorkrebs were first observed by a recreational angler; probably a large population	Dümpelmann and Bonacker (in press); Hessenfischer 2011
15	2011	Germany, Rhineland-Palatinate/Hesse	49°58' N, 07°53' E	Rhine River	unclear; one reproducing indiv. was captured	NA	one Marmorkrebs with attached hatchlings and six <i>O. limosus</i> were collected from the Rhine River	H. Groß, unpublished data

Population ecology of an alien “warm water” crayfish (*Procambarus clarkii*) in a new cold habitat

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Abstract

Procambarus clarkii is one of the worst invasive and best-studied crayfish species worldwide, but its life history at higher latitudes is poorly understood. In the present study, the population ecology of *P. clarkii* was studied for the first time within its northeastern range limit in Europe (southern Germany) for a two-year period, and the findings are used to discuss several life-history parameters across different latitudes of its current distribution range. The reproductive cycle was tracked using the gonadosomatic index and reproductive traits in females and the reproductive form in males. Life-history parameters were estimated using Von Bertalanffy's growth function. Reproduction was univoltine and occurred from late summer to autumn. A small proportion of females carried eggs throughout the winter. This contrasts with the species' multi-voltine life cycle at lower latitudes, with year-round breeding and several reproduction peaks per year. Growth was estimated to be slower than at lower latitudes, whereas longevity, mean lifetime and size increased. These changes in life history probably reflect a general phenomenon at higher latitudes and, thus, latitudinal clines. The presented findings provide evidence that *P. clarkii* is able to cope well with new cold habitats by modulating its life history.

Key words: life-history plasticity, non-indigenous crayfish, latitudinal clines

Introduction

Invasive alien species (IAS) are an increasing, major threat to global biodiversity. This is especially true when IAS act as keystone species (Lodge *et al.* 2000; Sala *et al.* 2000; McGeoch *et al.* 2010). Crayfish are the largest mobile freshwater invertebrates and have been frequently recognised as key members of littoral food webs (Nyström 1999, 2002). The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is the world's most invasive crayfish species and is listed among the “100 of the worst” IAS in Europe (DAISIE 2010). *Procambarus clarkii* shows considerable ecological plasticity and is one of the most important freshwater decapods farmed for food consumption (Gutiérrez-Yurrita *et al.* 1999;

Huner 2002; Gherardi 2006). The natural range of *P. clarkii* comprises the central south of the USA and northeastern Mexico. However, it is now established on every continent except Australia and Antarctica and has invaded even remote islands like the Azores, Canaries and Hawaii as a result of human-mediated translocation (Hobbs *et al.* 1989; Huner 2002; Souty-Grosset *et al.* 2006). The red swamp crayfish was first imported into Europe in 1973 when Spain intentionally introduced the species for commercial crayfish production. *Procambarus clarkii* rapidly established self-sustaining populations and is now the most abundant crayfish species on the Iberian Peninsula. The first illegal introductions into France and Italy occurred soon after its introduction to Spain (Gutiérrez-Yurrita *et al.* 1999; Huner 2002; Gherardi 2006). Today, *P. clarkii* is widespread and abundant throughout southwestern Europe and northern and central Italy, whereas northeastern populations in the Netherlands, Belgium, Great Britain, Germany, Switzerland and Austria are mainly scattered and isolated from each other (*cf.*, Figure 9). *Procambarus clarkii* has also become popular as an ornamental aquarium species, and many populations in Central Europe are the result of pet releases (Dehus *et al.* 1999; Soes and van Eekelen 2006; Dümpelmann *et al.* 2009).

The red swamp crayfish is a polytrophic omnivore, and its ecological impact on native ecosystems can be devastating (Rodríguez *et al.* 2005; Gherardi 2006; Souty-Grosset *et al.* 2006). *Procambarus clarkii* also poses a serious threat to the imperilled indigenous European crayfish, as it is a carrier of *Aphanomyces astaci* Schikora 1906, the causative agent of the crayfish plague. *Aphanomyces astaci* is a parasitic oomycete native to North America and fatal for all European crayfish. Further adverse impacts include damage to dams and dykes resulting from burrowing. *Procambarus clarkii* is especially considered a pest in agricultural areas, such as rice paddies, where it damages young rice plants as well as irrigation systems and dams (Gherardi 2006; Souty-Grosset *et al.* 2006). Hobbs *et al.* (1989) reported that the majority of *P. clarkii* introductions have had negative consequences.

Although *P. clarkii* is often considered as a 'warm water' species (Henttonn and Huner 1999), it has also been found to thrive in colder climates at higher latitudes and altitudes in North America, Japan and Europe (Suko 1958; Frutiger *et al.* 1999; Lindquist and Huner 1999; Mueller 2007; Dümpelmann *et al.* 2009; Chucholl 2011). The life history of *P. clarkii* at warmer temperature regimes is well understood, and it is characterised by fast growth rates, early maturation, a short life-span and year-round breeding with reproduction peaks in the spring and the autumn in permanent habitats (Gherardi *et al.* 1999; Gutiérrez-Yurrita *et al.* 1999; Scalici and Gherardi 2007). Individual egg-bearing females are present at any time during the year, which represents a unique life history trait and enhances this species' potential to become invasive (Lindquist and Huner 1999). However, there is an apparent lack of information on the life history of *P. clarkii* in colder environments, where low winter temperatures probably shorten the breeding season and restrict growth (*cf.*, Frutiger *et al.* 1999).

The major motivation for the present study was to fill this gap in knowledge and to present life-history parameters and population dynamics of naturalised *P. clarkii* populations in southern Germany, which is within its northeastern range limit in Europe. Von Bertalanffy's growth function was used to estimate growth characteristics, longevity and mortality. The reproductive cycle was followed using the gonadosomatic index and

reproductive traits in females, and the reproductive form in males. The findings are used to discuss several life-history parameters of *P. clarkii* across different latitudes of its current distribution range, thus providing a better understanding of its remarkable invasive success.

Methods

Study sites and sampling period

To assess population dynamics and life-history parameters of *P. clarkii*, a population in Lake Riedheim (LR), a mesotrophic gravel pit lake (9.3 ha; 450 m above sea level; 48°27'32.4''N, 10°10'32.3''E), was sampled at least once per month from May 1 to October 2, 2007 and from April 16 to August 29, 2008. Furthermore, female reproductive traits were followed closely in Lake Burlafingen (LB; also a mesotrophic gravel pit lake. 5.1 ha; 456 m above sea level; 48°25'28.7''N, 10°04'27.4''E) from September 12, 2008 through March 19, 2009. Both lakes are part of an artificial lake system along the River Danube in southern Germany. *Procambarus clarkii* is known from at least ten localities in this lake system and was introduced into LR and LB in the late 1990s or early 2000s (a detailed description of the study area is available in Chucholl 2011).

Trapping and measurements

Crayfish were captured with 60 cm long, cylindrical minnow traps with 4 mm mesh and 2 entrances, that were baited with dry cat food. In LR, the traps were set approximately every 10 m along the shoreline at a distance from shore of between 1 and 4 m and a depth of between 0.2 and 3 m. The number of traps used was 13, except in April, July and October 2007, when only 7, 8 and 5 traps were used, respectively. Traps were exposed for 16 hours beginning at 8 pm and one catch per unit effort (CPUE) corresponds to the catch of one trap set for 16 hours. The same trapping scheme was used in LB, however, traps were exposed between one day and two weeks as a result of low capture rates and ice cover in the winter. Surface water temperature, conductivity and pH were measured at both study lakes at a 1 m distance from shore on each sampling occasion (Konduktometer LF 191 WTW, pH 197-S WTW).

Upon capture, the carapace length (*CL*: measured from the tip of the rostrum to the dorsal posterior margin of the cephalothorax with a digital slide calliper) and sex were noted for each specimen. Mature male Cambarid crayfish show a cyclic dimorphism between a sexually active and a sexually inactive form. Sexually active males are referred to as being first form (I) and can be distinguished from sexually inactive males (form II) by having greatly inflated chelae, prominent copulatory hooks and cornified gonopodia (Huner and Barr, 1991; Huner, 2002). The reproductive form was noted for each captured male. Active glair glands, eggs, juveniles and egg-remains on female pleopods were also noted. The presence of those reproductive traits was interpreted as sexual maturity in females, whereas the form I condition was used to determine sexual maturity in males. To assess the relationship between *CL* and body weight (*W*), 261 females and 52 form I males were weighed (Kern balance, type 822-67). After measurements, all males were returned to the lake and all females were transported to the laboratory.

Growth

The obtained size frequency data were analysed in FiSAT 2 (v.1.2.2.; Gayanilo and Pauly 1997; Ligas 2008, Scalici *et al.* 2009). Growth was described using Von Bertalanffy's growth function (VBGF). Because of the pronounced seasonal changes in temperature, the "seasonalized" equation derived by Pauly and Morgan (1987) was used:

$$L(t) = L_{\infty} \{1 - e^{[-k(t-t_0) - (Ck/2\pi)(\sin 2\pi(t-t_s) - \sin 2\pi(t_0-t_s))]} \},$$

where $L(t)$ is the *CL* at age t , L_{∞} the asymptotic length (*i.e.*, the theoretical possible *CL*), k the curvature parameter (*i.e.*, the rate at which L_{∞} is approached) and t_0 the initial condition parameter (*i.e.*, the hypothesised age at which *CL* is zero). T_s refers to the onset of the first oscillation relative to $t = 0$, but for practical purposes it was replaced by the winter point ($WP = t_s + 0.5$), *i.e.*, the moment in which the growth rate is the slowest in the annual cycle. C is a constant parameter expressing the amplitude of the seasonal growth oscillation.

L_{∞} and k were estimated using the ELEFAN I procedures included in FiSAT 2 (non-parametric scoring; Pauly and David 1981; Gayanilo and Pauly 1997) and t_0 by non-linear regression analysis. Longevity (t_{\max}) was estimated by $t_{\max} = (3/k) + t_0$ and mean life-time by $t_{1/2} = \{\Sigma[n(t)t]\}/N$, where n is the number of individuals at time t and N the total number of individuals (Gayanilo and Pauly 1997; Scalici and Gherardi 2007). The growth performance index (ϕ') was derived from k and L_{∞} by applying the equation $\phi' = \log(k) + 2\log(L_{\infty})$ (Pauly and Munro 1984). ϕ' allows to compare different stocks' growth performance in terms of length. Populations of the same species present similar values of ϕ' and ϕ' can therefore be used to identify biased growth parameter estimates (Pauly and Monroe 1984). The total mortality index (Z) was obtained in FiSAT2 from the Powell–Wetherall plot equation, which computed L_{∞} and the ratio Z/k using length-frequency data. Z is the sum of natural mortality (M) and the mortality resulting from fishing (F). M was obtained by solving the equation $\log(M) = -0.0066 - 0.279\log(L_{\infty}) + 0.6543\log(k) - 0.463\log(T)$, where T is the mean environmental temperature during the study period (Pauly 1980). F was calculated by subtracting M from Z (Pauly 1980).

Female reproductive pattern

The females captured from LR were transported to the laboratory, frozen to -18°C and later dissected to calculate the gonadosomatic index ($\text{GSI} = \text{gonad weight} / W \cdot 100$). Additional female specimens were collected by hand from the shallow lake margin during the night in June and October 2007 and again in September 2008. Only sane females were used for the GSI calculation to avoid bias from the lower W of specimens with regenerated or missing chelae. Prior to weight measurement, female specimens were put on blotting paper and were gently shaken 10 times to remove adherent water drops. Dissected gonads were also gently shaken 10 times prior to weighing (Kern balance, type 822-67). In addition to the GSI, the colour of the dissected gonads was used to assess the ovary development stage by adopting the scheme provided by Dörr *et al.* (2006): white = ovary at rest, orange to brown = maturing ovary, dark brown to black = mature ovary.

Female reproductive traits were followed closely in LB during the fall and winter months of 2008 and during the spring of 2009. The relationship between the proportion of

females with active glair glands and water temperature was assessed by a Pearson product moment correlation.

Fecundity

Fecundity data were collected from ten egg-bearing females captured from LB on September 25, 2008. Upon capture, females were carefully transported to the laboratory and frozen to -18 °C. Pleopodal eggs and those ones attached on the pleon were later stripped off with a forceps and counted.

Size at maturity

The CL at which 50 % (CL_{50}) and 95 % (CL_{95}) of individuals matured was assessed by a logistic regression analysis of the proportion of mature specimens in each size class (Beatty *et al.* 2005). The probability of maturity (p) at each size class (CL) was given by $p(CL) = 1/\{1+\exp[-\ln(19)(CL-CL_{50})/(CL_{95}-CL_{50})]\}$. Estimates for the parameters CL_{50} and CL_{95} were derived by fitting the model to the data in SigmaStat 3.5.

Trap bias

Trap data are often biased towards more competitive individuals, *e.g.*, form I males and larger females (France *et al.* 1991; Dorn *et al.* 2005; Price and Welch 2009). To assess this bias, trap data were compared with hand captures obtained by SCUBA divers, which are likely to be less biased (Somers and Stetchey 1986; France *et al.* 1991). France *et al.* (1991) suggest a minimum SCUBA sample size of 200 specimens to gain a reasonable picture of a crayfish population.

Five traps were set along a transect across the western part of LR (every 30 m, with a distance of 150 m between opposite lake shores) on October 1, 2007. The maximum depth was 4 m, and the vegetation was composed of dense stands of *Elodea nuttallii* (Planchon) St. John and scattered patches of *Chara* spp. Traps were checked in the morning of the next day, and captured crayfish were not returned to the lake. Beginning at 7 pm, three pairs of SCUBA divers entered the lake and captured crayfish along three parallel transects (one transect was the same as that used for trapping, and the other two were laid out 20 m to the left and 20 m to the right of the original). Crayfish were collected by hand or with small hand nets. Additionally, two people searched the opposite sides of the shallow lake margin enclosed by the two outermost transects for crayfish. The SCUBA captures lasted for one hour.

Population size estimate

The trappable population size was estimated for both study lakes in 2008. Crayfish that were to be returned to the lakes were marked by clipping the distal margin of one uropod. Only males were marked at LR, and the size of the trappable male population was estimated by the weighted mean model (Begon 1979). At LB, the uropod clipped was alternated with every marking occasion, resulting in three distinct batch markings. Crayfish caught more than once were newly marked. The size of the trappable population was estimated by Bailey's triple-catch method (Henderson 2003).

The influence of latitude on life-history parameters

The obtained life-history parameters of the study population in LR were used with data reported in the literature to test for significant interactions between latitude and k , t_{\max} and L_{∞} , using Pearson product moment correlations. The latitude of all the studied populations was directly obtained from the literature or by GIS software (Quantum GIS v.1.6.0).

Statistical analyses

Apart from the above-cited analyses, Pearson product moment correlations were used to assess the relationships between total, male and female CPUE and water temperature in LR. The relationship between CL and W was described using the power equation $y = a x^b$, with $y = W$ and $x = CL$. Parameters a and b were estimated by employing a linear regression after log transformation of the data. The size-frequencies of the crayfish captured from LR were pooled per sex and month and compared to the values of the previous month by non-parametric Mann-Whitney rank sum tests. The GSI values of the captured females from LR were also pooled per month and compared to the values of the previous month by non-parametric Mann-Whitney rank sum tests. The interaction between CL and GSI was assessed for each sampling occasion by a Pearson product moment correlation. The relation between CL and fecundity was assessed by a Pearson product moment correlation. The sex ratio of the SCUBA catch and the sex ratio of the trap catch were compared by a chi-square-test. The same test was used to compare the proportion of form I males and to test whether the sex ratio obtained by either method differs significantly from 1:1. The size-frequencies obtained from the SCUBA catch and the trap catch were compared per sex by parametric t-tests. All statistical analyses were performed with SigmaPlot 10.0 (with SigmaStat 3.5 Integration). The data were tested for normality prior to statistical testing for differences.

Results

Trap bias

The SCUBA divers captured 192 *P. clarkii* specimens. The sex ratio did not differ significantly from 1:1 (chi-square-test: $\chi^2 = 0.375$, $P = 0.54$). In comparison to the SCUBA catch, the traps largely overestimated the proportion of males within the total catch (trap bias = 17 %; chi-square-test: $\chi^2 = 5.016$, $P = 0.025$) and the proportion of form I males among the total male population (trap bias = 24 %; chi-square-test: $\chi^2 = 7.746$, $P = 0.005$). The size frequency derived from the SCUBA catch was composed of significantly smaller male individuals than the size frequency of males derived from the trap catch (t-test: $t_{142} = 3.104$, $P = 0.002$), but this was not the case for females (t-test: $t_{96} = 1.278$, $P = 0.204$). The sex ratio of the trap catch was significantly unbalanced in favour of males (chi-square-test: $\chi^2 = 5.362$, $P = 0.02$).

Population size estimate

Out of the 524 marked males in LR, 26 were recaptured at least once, whereas 156 crayfish were marked in LB, of which 21 were recaptured at least once. The estimated trappable male population size in LR (6708, SE = 1343; weighed mean model of 7 trapping occasions) was larger than the trappable population size in LB, which was estimated at 1922 (SE = 610; triple-catch method).

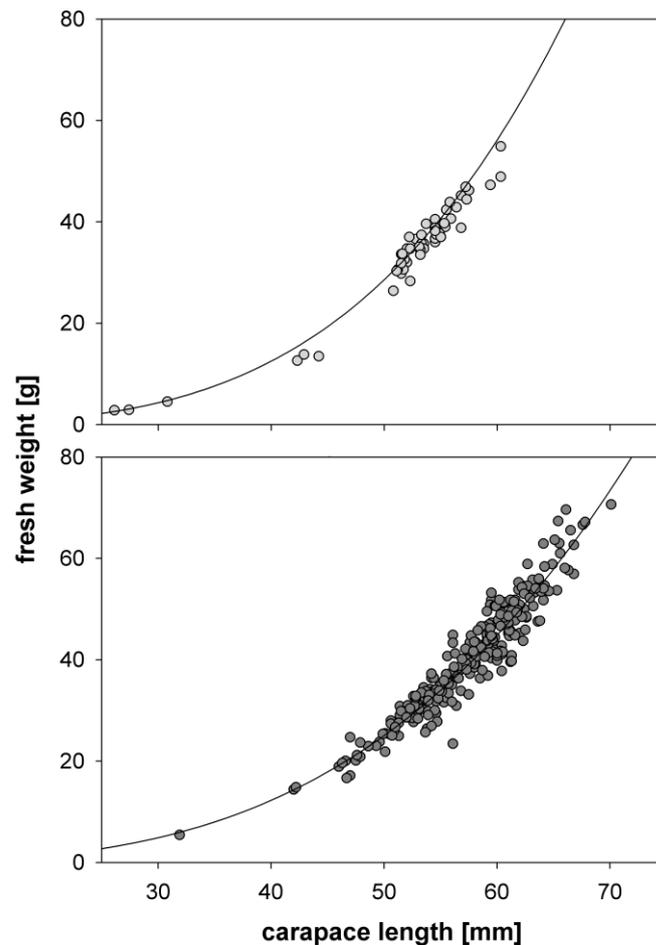


Figure 1. The length-weight relationship in male (light grey; form I in adults) and female (dark grey) *P. clarkii* from LR. Power equations were as follows: $W [g] = 10^{-4.853} CL [mm]^{3.703}$ in form I males and $W [g] = 10^{-4.033} CL [mm]^{3.197}$ in females.

Population dynamics

A total of 1585 crayfish from LR and 436 crayfish from LB were trapped and measured during the study period. The largest captured female specimens measured 76.1 and 76.0 mm in *CL* and the largest captured males measured 66.6 and 71.4 mm in *CL*, for LB and LR respectively. There was a significant positive relationship between *CL* and *W* in both sexes (form I in males; linear regression: $N = 52$, $R^2 = 0.985$, $P < 0.001$ and $N = 261$, $R^2 = 0.929$, $P < 0.001$, respectively; Figure 1).

The CPUE in LR was highest in August and October 2007 and in July 2008 (Table 1). Male CPUE was consistently higher than female CPUE and reached its maximum in both years in August. Female CPUE increased in the summer but decreased in August. However, female CPUE increased again in October 2007 (no data for October 2008 because several traps were stolen). There was no significant relationship between total, male or female CPUE and surface water temperature (Pearson product moment correlation, $P = 0.58$, 0.29 and 0.28 , respectively; Table 1). The mean *CL* of males increased in both years until July and declined distinctly in August (Table 1, Figure 2). The mean *CL* of females followed the same pattern with the exception of the period from April to July, 2007, when there was no increase in mean *CL*. Differences in the size-frequencies between months were assessed by Mann-Whitney rank sum tests and the results are summarized per sex in Table 1. The proportion of form II males was generally low and was highest in the spring and autumn; form II males were virtually absent from the trap catches in the midsummer months (Table 1).

Table 1. *Procambarus clarkii* population dynamics and surface water temperature (SWT) in LR. *f* - females, *m* - males (form I and II pooled), *m* I - form I males. Asterisks indicate significant differences in size-frequency from the previous month, given by a Mann-Whitney rank sum test (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

	Mean <i>CL</i> <i>m</i>	Mean <i>CL</i> <i>f</i>	<i>N</i>	CPUE	CPUE <i>m</i>	CPUE <i>f</i>	% <i>m</i> I / <i>m</i>	SWT [°C]
Apr 07	56.1	59.1	12	1.71	1.14	0.6	87.5	18.3
May 07	56.3	55.9	87	3.1	2.2	0.9	87.1	19.0
Jun 07	57.2	59.1	128	4.0	3.2	0.8	94.3	22.1
Jul 07	57.2	59.0	85	11.33	8.0	3.3	100.0	19.3
Aug 07	53.4***	54.8**	146	12.2	10.8	1.5	100.0	23.9
Oct 07	55.1*	56.8	62	12.4	9.0	3.4	86.7	17.3
Apr 08	54.7	55.7	83	6.6	3.8	2.9	76.6	11.5
May 08	56.6	54.1	29	3.0	1.9	1.1	72.2	19.8
Jun 08	56.6	57.8	249	6.5	4.4	2.1	93.4	22.7
Jul 08	57.3	60.1	432	10.2	7.1	3.1	98.7	23.0
Aug 08	54.2***	56.7***	272	8.5	8.2	0.3	100.0	23.7

Growth

Moulting individuals or shed exuviae were observed from May to late summer in both study years at surface water temperatures of greater than 15 °C – no moulting activities were detected below this temperature threshold. Because *P. clarkii* grows best at temperatures > 21°C (Huner 2002), it is reasonable to assume that no growth occurred during the cold winter months. The *WP* of the seasonalized VBGF was therefore set to mid-winter, and *C* was set arbitrarily to 1, which allowed for no growth in winter (Gayanilo and Pauly 1997). The resulting seasonal growth pattern and the size-frequencies of the study population in LR are shown in Figure 2 for each sex. The obtained Von Bertalanffy parameters (L_{∞} , k and t_0), their derivatives (t_{\max} , ϕ' , Z , M and F) and the calculated mean lifetime ($t_{1/2}$) are summarised per sex in Table 2.

Table 2. Von Bertalanffy's parameters (asymptotic CL , L_{∞} , curvature parameter, k , and initial condition parameter, t_0), longevity (t_{\max}), mean lifetime ($t_{1/2}$), growth performance index (ϕ'), total mortality index (Z), natural mortality (M) and mortality resulting from fishing (F) of the *P. clarkii* population in LR, distinguished per sex.

	L_{∞}	k	t_0	t_{\max}	$t_{1/2}$	ϕ'	Z	M	F
males	74.6	0.49	-0.022	6.1	3.5	3.44	2.26	2.26	0.00
females	79.8	0.45	-0.027	6.6	4.0	3.46	2.79	2.55	0.24

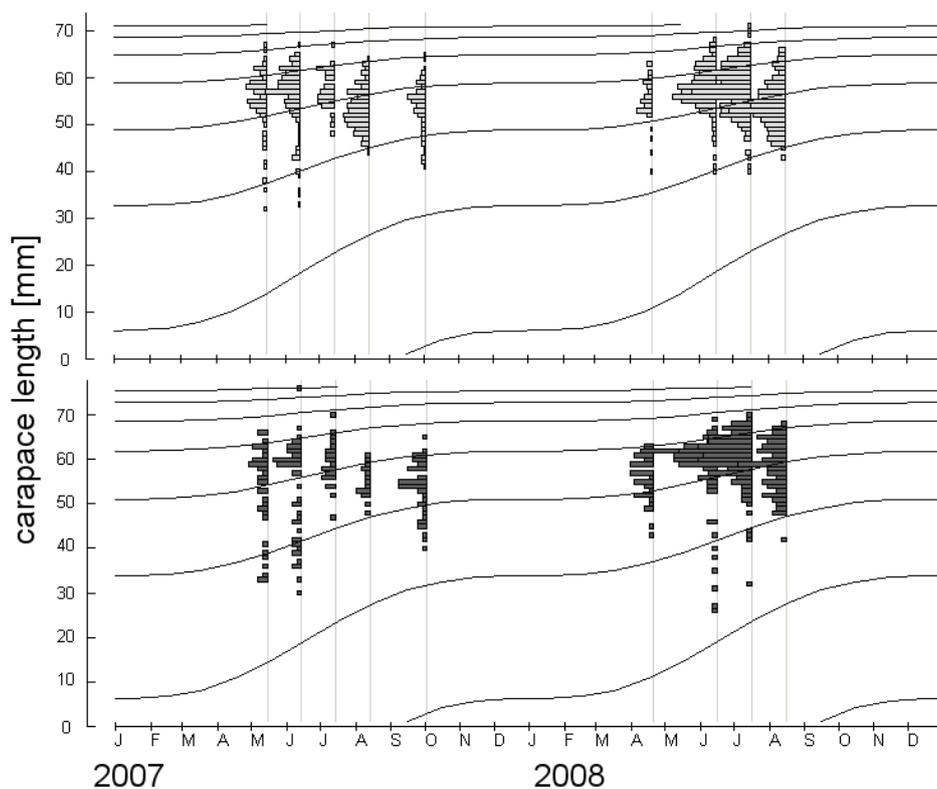


Figure 2. Growth models for *Procambarus clarkii* males (light grey) and females (dark grey) in LR, southern Germany.

Female reproductive pattern

The GSI of female crayfish in LR was consistently low in spring and increased until late summer in both years (Figure 3, Table 3). The values differed significantly from those of the previous sample month in April and July, 2008 (Mann-Whitney rank sum test: $P < 0.05$) and highly significantly in July and October, 2007, and June, 2008 (Mann-Whitney rank sum test: $P < 0.001$; Table 3).

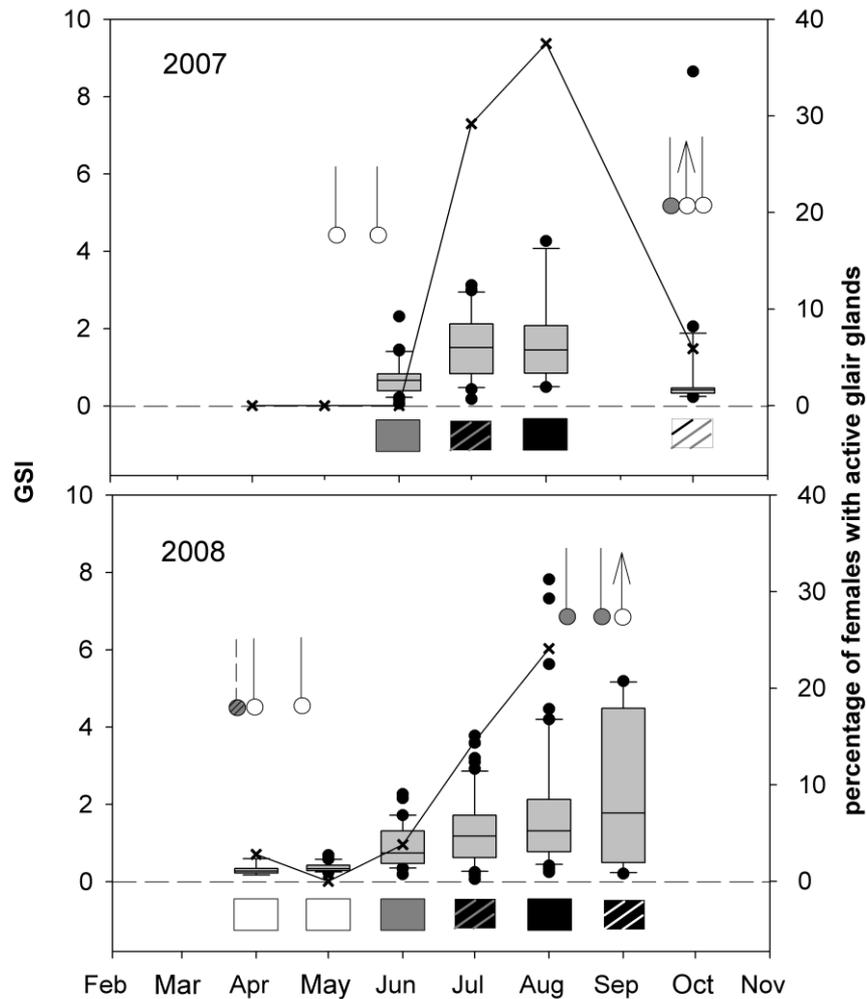


Figure 3. Reproductive traits of *P. clarkii* females in LR. The GSI is depicted with box plots (left axis; see Table 3 for statistics), and the frequency of females with active glair glands is depicted with a solid line (right axis). Boxes below the plots show the ovary development stages based on their colour: white - ovary at rest, dark grey - maturing ovary, black - mature ovary; hatched boxes indicate a mixture of ovary stages (dominant stage as background). Circle symbols depict females with eggs (solid grey), juveniles (open & upwards-pointing arrow) or egg remains (open); hatched symbol = single record with very few, dead eggs.

In 2008, there were significant relationships between female *CL* and the GSI (Figure 4): on July 22, the relationship was significantly positive (Pearson product moment correlation: $N = 33$, corr. coeffic. = 0.44, $P = 0.01$), whereas it was significantly negative on September 11, 2008 (Pearson product moment correlation: $N = 14$, corr. coeffic. = -0.57, $P = 0.04$). The relationship was not significant on the three intermediate capture occasions (August 6, 26 and 29, 2008; $P > 0.05$; Figure 4).

Females with active glair glands were captured as early as June (2008) and accounted for up to 38 % of the females captured in August (Figure 3, Table 3). After August, the proportion of captured females with active glair glands decreased strongly in both years (Figure 3, Table 3). A small proportion of the females captured in April 2008 also had active glair glands. Females with eggs or juveniles were found in October 2007, August 2008 and September 2008 (Figure 3). On April 16, 2008, one female with very few and apparently dead eggs was captured. Egg remains on the pleopods could be found in spring 2007 and 2008 as well as in October 2007 and September 2008 (Figure 3).

Table 3. Female reproductive traits in LR (*cf.*, Figure 3). GSI stands for gonadosomatic index and SE for standard error. N refers to the female sample size used for the GSI calculation (including trap captures and additional hand captures). Asterisks indicate significant differences from the previous month, given by a Mann-Whitney rank sum test (* = $P < 0.05$; *** = $P < 0.001$).

	% females w glair glands	% females w eggs/ juveniles	% females w egg remains	Mean GSI (N)	SE GSI
Apr 07	0.0	0.0	0.0	x	
May 07	0.0	0.0	12.0	x	
Jun 07	0.0	0.0	9.1	0.76 (27)	0.08
Jul 07	29.2	0.0	0.0	1.54 *** (24)	0.17
Aug 07	37.5	0.0	0.0	1.72 (16)	0.28
Oct 07	5.9	5.9	0.0	0.89 *** (21)	0.40
Apr 08	2.8	2.8	11.1	0.31 * (9)	0.04
May 08	0.0	0.0	27.3	0.38 (23)	0.03
Jun 08	3.8	0.0	0.0	0.89 *** (34)	0.09
Jul 08	14.5	0.0	0.8	1.32 * (58)	0.12
Aug 08	24.1	0.0	0.0	1.82 (53)	0.22
Sep 08	x	0.0	14.3	2.3 (14)	0.53

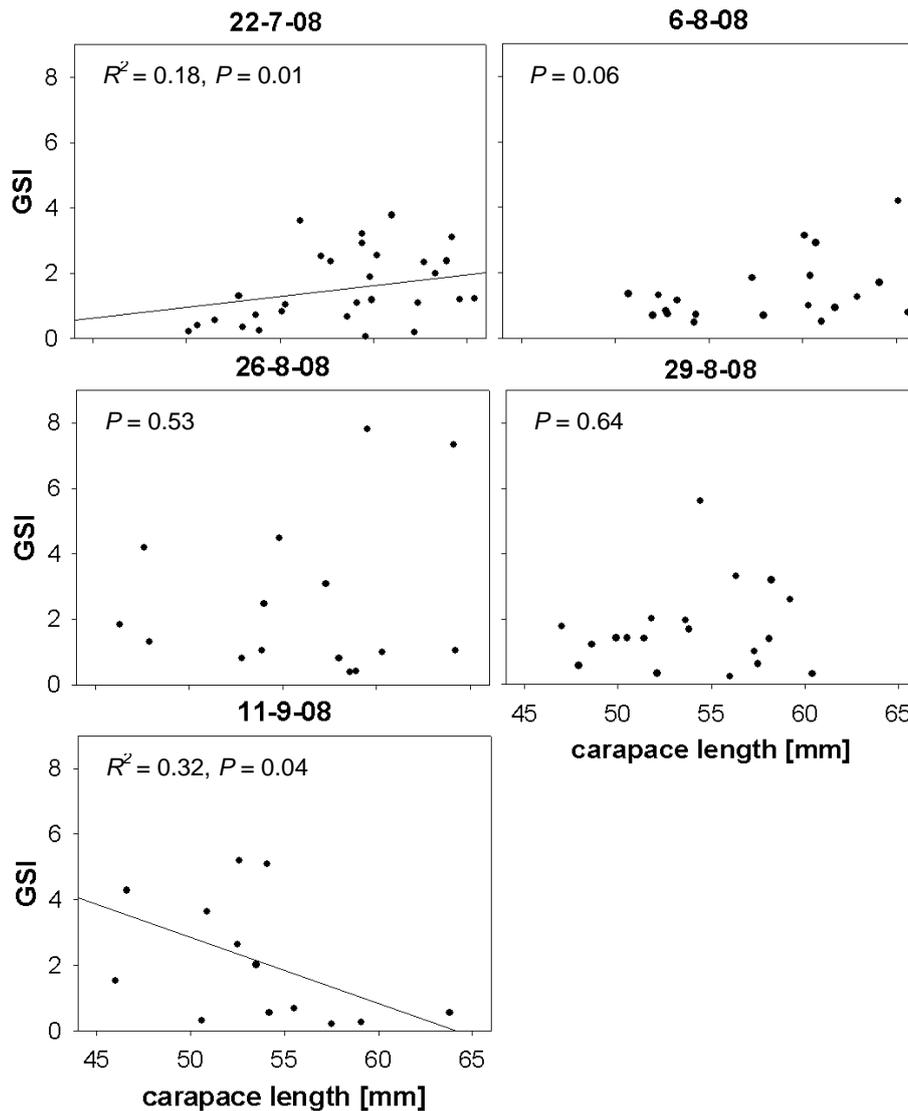


Figure 4. Relationship between female CL and the GSI in LR from late July to mid-September 2008. *P* values are given by a Pearson correlation, and R^2 values were calculated by linear regression.

Female reproductive traits were followed closely in LB from autumn, 2008 to spring, 2009 on seven trapping occasions (Figure 5). In mid-September, 64 % of the captured females had active glair glands, but the proportion dropped to 3 % in late November. The proportion of females with active glair glands correlated significantly with water temperature (Pearson product moment correlation: $N = 7$, corr. coeffic. = 0.97, $P < 0.001$; Figure 5). Egg-bearing females were most abundant on September 25 and still accounted for 13 % of the captured females in late November, when water temperatures had fallen to well below 5 °C. The first females with egg remains were noted on September 25, and their proportion accounted for 19 % of the total in mid-November (Figure 5). The appearance of egg remains was notably linked to female size: on September 25, only females with a CL larger than 60 mm showed egg remains (18 %). It was not until mid-October that females with a CL between 50 and 59 mm were found to carry egg remains (33 %). Females smaller 50 mm CL did not carry egg remains before mid-November (17 %).

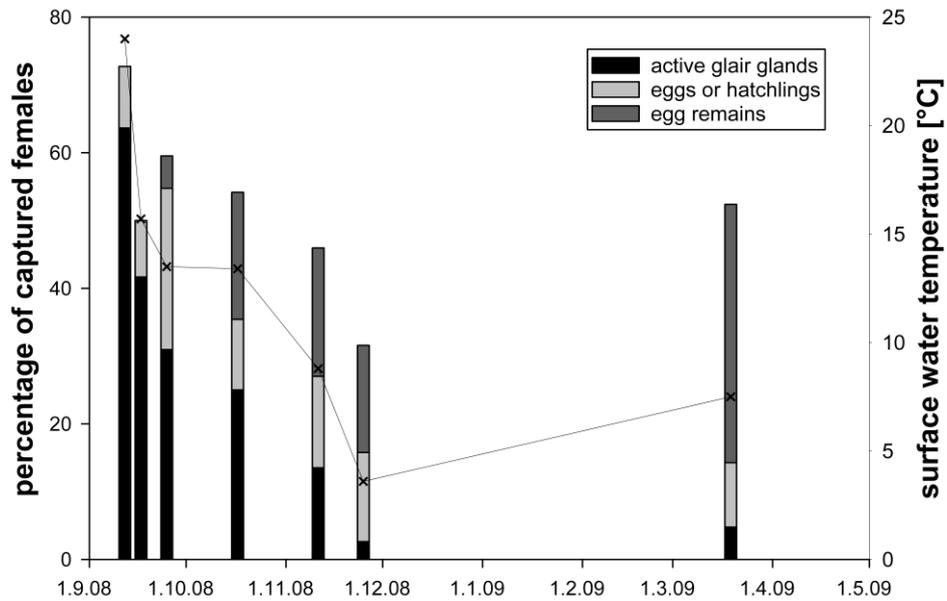


Figure 5. Female reproductive traits and surface water temperature in LB from autumn, 2008 to spring, 2009.

Fecundity

The size of the sampled egg-bearing females ranged from 44.9 to 56.1 mm *CL*, and the mean *CL* was 50.9 mm. The number of pleopodal eggs averaged 285 per female (Figure 6). The largest clutch (597 eggs) had a female with 51.8 mm *CL*, the smallest (7 eggs) a female with 44.9 mm *CL*. There was no significant correlation between carapace length and the number of attached eggs (Pearson product moment correlation: $N = 10$, corr. coeffic. = 0.31, $P = 0.39$; Figure 6). The highest number of eggs per pleopod was 105, and the mean egg count was 44 (pleopods without eggs excluded).

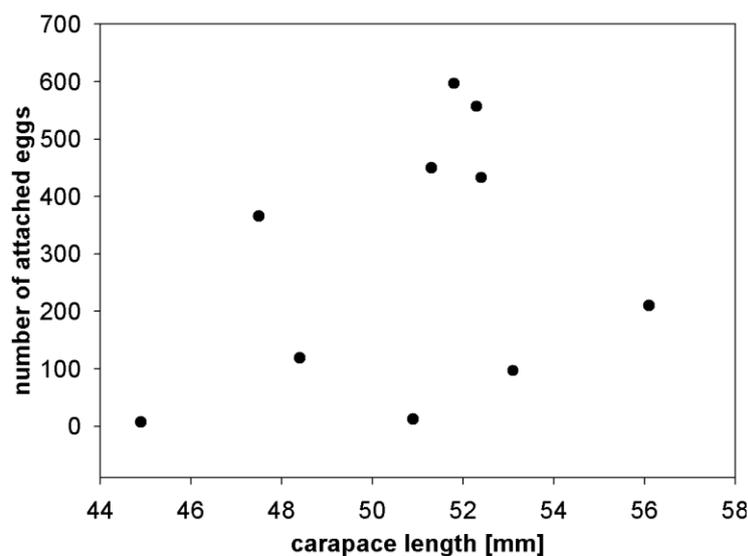


Figure 6. Fecundity of *Procambarus clarkii* females captured from LB in Summer 2008.

Size at maturity

The smallest captured form I male had a *CL* of 37.1 mm, the smallest female with active glair glands had a *CL* of 39.8 mm and the smallest gravid female had a *CL* of 44.6 mm. The CL_{50} of male *P. clarkii* was estimated at 44.5 mm (SE = 0.17; logistic regression analysis, $R^2 = 0.981$), and the CL_{95} was estimated at 50.1 mm (SE = 0.47; Figure 7). No estimates for CL_{50} and CL_{95} were derived for females as a result of a low sample size of the small size classes and because of the seasonal influence on the presence of active glair glands and external eggs (*i.e.*, it was often impossible to determine maturity in small females in the spring and summer; *cf.*, Figure 2).

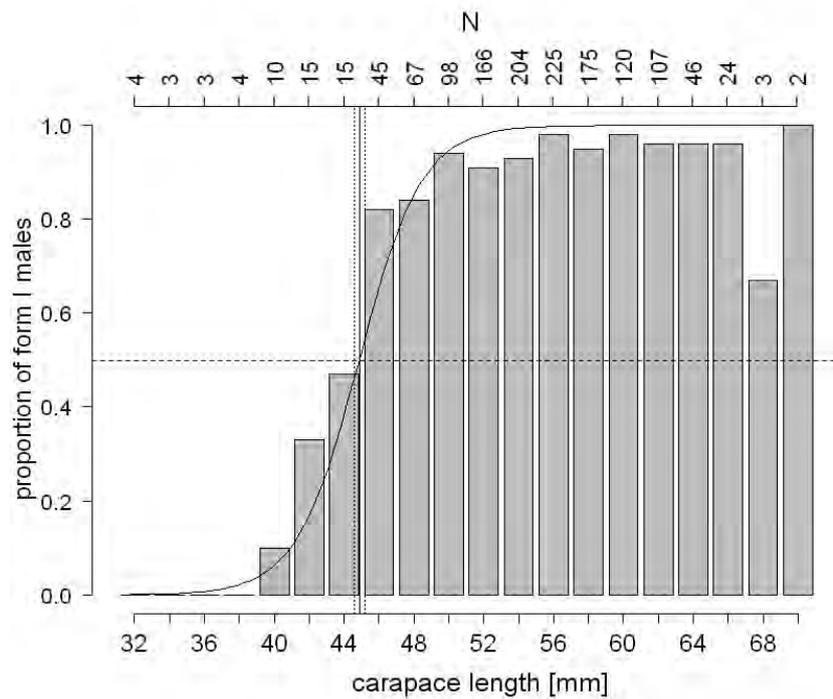


Figure 7. Size at maturity in male *P. clarkii*. Logistic curve was fitted to the proportion of form I males (regression analysis, $R^2 = 0.981$). The horizontal dashed line indicates a proportion of 0.5. The vertical solid line depicts the estimated carapace length at which 50 % of the males matured (SE as dotted lines). The sample size of each size class is given at the top of each column.

The influence of latitude on life-history parameters

Longevity (t_{\max}) and L_{∞} of *P. clarkii* populations correlated significantly positive with latitude (Pearson product moment correlation: $N = 11$, corr. coeffic. = 0.85, $P < 0.001$ and $N = 11$, corr. coeffic. = 0.76, $P = 0.006$, respectively), whereas k correlated significantly negative with latitude (Pearson product moment correlation: $N = 11$, corr. coeffic. = -0.79, $P = 0.004$; Figure 8).

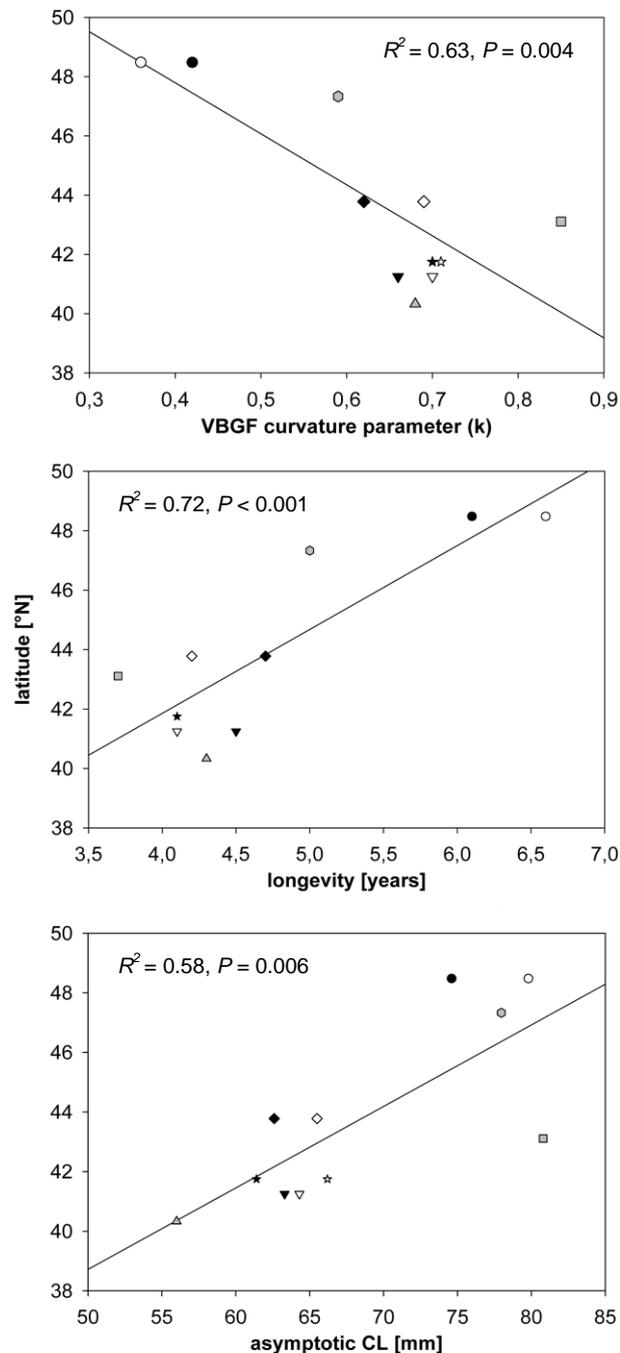


Figure 8. Life-history parameters of *P. clarkii* populations in Europe in relation to latitude. Symbol colour indicates the sex (white – female, black – male, grey – pooled) and symbol type depicts the data source (circle – present study; hexagon – Frutiger *et al.* 1999; diamond – Scalici and Gherardi 2007; rectangle – Dörr *et al.* 2006; asterisk – Chiesa *et al.* 2008; triangle down – Chiesa *et al.* 2006; triangle up - Anastácio and Marques 1995). P values are given by a Pearson correlation, and R^2 values were calculated by linear regression.

Discussion

The life-history analysis was based on a large ample number of captured crayfish and includes data from two consecutive years. Most data were obtained by baited traps, which can produce a bias in size and sex (France *et al.* 1991; Dorn *et al.* 2005) and are, therefore, a potential source of error. However, the comparison between trap and SCUBA data showed that the actual size bias was very small and, therefore, probably had no effect on the life-history analysis. The obtained growth performance indices are remarkably consistent with published values from Italian and Iberian populations (Anastácio and Marques 1995; Chiesa *et al.* 2006; Scalici and Gherardi 2007), indicating that the growth parameter estimates are unbiased (Pauly and Monroe 1984). The trappable population was probably composed of crayfish at least one-year-old, which becomes apparent from the growth model as shown in Figure 2, where smaller size classes (probably the 0+ and 1+ cohorts) are largely missing from the length frequency diagrams. As expected, the sex ratio of the trap data was considerably biased towards males. The SCUBA data suggest a balanced sex ratio of the actual population as proposed by Gherardi *et al.* (1999) and Scalici and Gherardi (2007) for populations in central Italy. The natural mortality (M) was lower than that of a *P. clarkii* population in central Italy (Scalici and Gherardi 2007), which coincides with the suggestion of Frutiger *et al.* (1999) that M is lower at higher latitudes. Fishing mortality (F) was very low in females and not detectable in males, confirming that the study population was not subject to substantial fishing (*cf.*, Chucholl 2011). The selective removal of female crayfish from the lake for the calculation of the GSI during the present study probably contributed to F in females.

Growth

The growth of crayfish is temperature dependant and decreases with temperature. Below a certain temperature threshold, it may cease completely (Reynolds 2002). Huner and Barr (1991) stated that optimal growth of *P. clarkii* occurs at temperatures between 21 and 27 °C. Surface water temperature in LR rarely exceeded 24 °C, and optimal growth conditions were reached only in the midsummer for three to four months. Low temperatures in the autumn, winter and spring restricted the growing season markedly and probably induced the comparatively slow growth rate (curvature parameter of the VBGF, $k = 0.45$ and 0.49). Typical curvature parameters (k) from populations in southern Europe are consistently higher and range between 0.62 and 0.85 (Anastácio and Marques 1995; Chiesa *et al.* 2006; Dörr *et al.* 2006; Scalici and Gherardi 2007). The slow growth was attended by a marked increase in longevity (up to 6.6. years), mean lifetime (3.5–4 years) and size. For comparison, longevity at lower latitudes was estimated to be 3.7 to 4.7 years (Huner 2002; Chiesa *et al.* 2006; Scalici and Gherardi 2007), and mean lifetime was estimated to be only 12 to 18 months (Huner 2002; Scalici and Gherardi 2007). The estimated asymptotic CL in southern Europe typically ranges between 56 and 66 mm (Anastacio and Marques 1995; Chiesa *et al.* 2006; Scalici and Gherardi 2007;) as opposed to the CL of 75 and 80 mm obtained by the present study. Increased mean lifetime, longevity and size were also reported from an introduced *P. clarkii* population in Switzerland (Frutiger *et al.* 1999), which gives rise to the hypothesis that an increase in those life-history traits represents a general phenomenon at colder environments

and, thus, a latitudinal cline. This idea is strongly supported by the fact that longevity, asymptotic CL and curvature parameter (k) were found to correlate with latitude (*cf.* Figure 8).

An increase in size at colder environments is known to occur in many ectotherms (Angilletta and Dunham 2003), including latitudinal clines in aquatic macro-invertebrates, such as the freshwater pearl mussel [*Margaritifera margaritifera* (L., 1758); Bauer 1992]. Although this relationship between temperature and body size has been widely confirmed, a general explanation for this phenomenon has remained elusive (Atkinson and Sibly 1997; Angilletta and Dunham 2003; Stillwell 2010). However, the increase in lifetime can be intuitively explained by the close relationship between growth rate and metabolism. At lower temperatures, metabolic rate declines and growth rate decreases (Reynolds 2002). Consequently, more time is needed to attain the asymptotic length and longevity and mean lifetime increase (Bauer 1992).

Reproduction

Procambarus clarkii has a very plastic life cycle and is capable of surviving in tropical, subtropical and temperate climates worldwide. Data from its introduced tropical range (*e.g.*, Costa Rica, Hawaii, Kenya and Uganda) suggest year-round breeding with at least two generations per year (Huner 1977; Oluoch 1990). Within its natural subtropical-temperate range (Louisiana, USA), *P. clarkii* also breeds year round, with distinct recruitment peaks from the late summer to early winter and again in the spring (Figure 9A). In populations in favourable conditions, there are up to three recruitment periods per year (Huner 2002). In California, recruitment peaks in January and February, followed by smaller recruitment events in March, August and September (Sommer 1984). In southern Spain, *P. clarkii* was shown to reproduce from April to late October, with ovigerous females found to be either continuously present from May to October (Gutiérrez-Yurrita *et al.* 1999) or peaking in September and May (Cano and Ocete 1997). Introduced populations in Portugal may reproduce year round, with a single recruitment peak from October to November (Fidalgo *et al.* 2001) or several recruitment periods from mid-April to mid-June, in August and from October to mid-January (Figure 9D; Anastácio and Marques 1995). In central Italy, populations exhibit breeding peaks in the spring and late summer/autumn (Figure 9C; Gherardi *et al.*, 1999; Scalici and Gherardi 2007). Similarly, naturalised *P. clarkii* populations in Japan show distinct reproduction peaks in May–June and September–October, but egg-bearing females may be present in every season of the year, except in the deep winter (Suko 1956, 1958).

In the present study, reproduction was shown to take place from the midsummer to late autumn, and the recruitment period lasted from autumn to winter. Single ovigerous females were also caught in the spring, but those females had most likely laid their eggs at the end of the previous autumn breeding period in late October and November (Figure 9B). Because egg development is effectively arrested at temperatures below 10 °C, it may take up to five months until those winter eggs hatch (Suko 1956; Huner and Barr 1991). The time of egg laying was notably related to female size, with larger females breeding earlier than smaller ones: GSI values were positively correlated with female size in late July, 2008 and were negatively correlated with female size in mid-September. This correlation suggests that

the eggs of larger females matured earlier and that those larger females had already spawned by mid-September (their GSI values were, therefore, lower than those of smaller individuals that had not yet laid their eggs). The presence of egg remains on female pleopods was also remarkably linked to female size and was first observed in larger females. A similar pattern was observed by Smith (1981) in *Orconectes limosus* (Rafinesque, 1817). Form I males peaked in the midsummer, and form II males were abundant only in the spring and autumn, which coincides with data from higher latitudes in North America (Pine Lake, Washington; Müller 2007) but is diametrically opposed to the pattern at lower latitudes, where form II males peak in the summer and form I males are abundant in the spring, autumn and winter (Souty-Grosset *et al.* 2006).

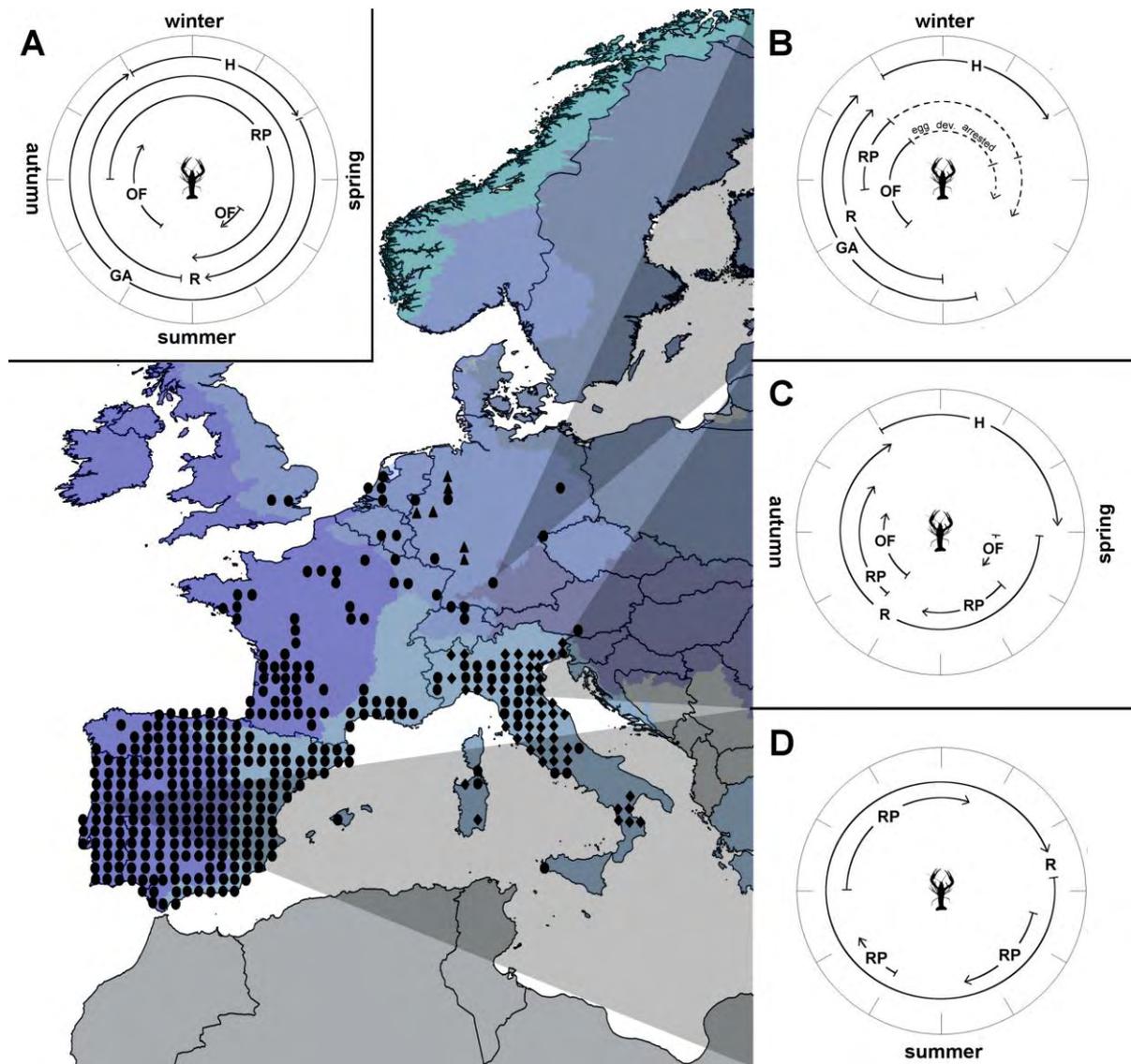


Figure 9. Life cycle of *Procambarus clarkii* in its native range (A, Huner 2002) and across different latitudes of its introduced range in Europe (B: southern Germany, present study; C: central Italy, Gherardi *et al.* 1999; Scalici and Gherardi 2007; D: Portugal, Anastácio and Marques 1995). OF – ovigerous females, GA – great activity, R - reproduction, RP – recruitment period, H – hibernation. Distribution is shown as presence in CGRS squares (data source: dot – Souty-Grosset *et al.* 2006; diamond – Gherardi *et al.* 2009; triangle – Dümpelmann *et al.* 2009; Chucholl 2011; Gross 2011).

When comparing the reproduction and recruitment patterns across different latitudes, two phenomena become apparent: first, there is a general trend from multi-voltine life cycles with year-round breeding (Figure 9A) at tropical climates to a univoltine life cycle with a seasonal recruitment pattern at higher latitudes (Figure 9B). Second, there is a latitudinal shift in the onset of the recruitment period in the annual cycle. At lower latitudes, the first recruitment wave usually starts in the spring months (Figure 9A, C, D), whereas at higher latitudes, recruitment does not occur before the late summer (Figure 9B).

Both phenomena, *i.e.*, the switch from a multi-voltine to a univoltine life cycle and the seasonal shift in the timing of reproductive events, are most likely driven by the latitudinal gradient in water temperature. Ovarian development in *P. clarkii* is induced by increases in both photoperiod and temperature (Daniels *et al.* 1994), and eggs are rarely laid at temperatures below 15 °C (Suko 1958). At higher latitudes, most females are not able to attain breeding condition before midsummer, which is stressed by the consistently low GSI values in the spring and by the positive correlation between the proportion of females with active glair glands and water temperature. Because egg development at optimal temperatures takes at least 2–3 weeks and because the egg maturation cycle takes at least six weeks (Huner and Barr 1991), female *P. clarkii* can reproduce at most every two months. In colder environments, with low average temperatures during the winter half-year, this means that females can reproduce only once per year, from the late summer until the winter.

Contrary to the plastic recruitment pattern and timing of reproductive events, other reproduction-related life-history traits of *P. clarkii* seem to vary little across different latitudes. The estimated size at maturity for male *P. clarkii* and female fecundity data are comparable to values reported from permanent lake habitats in its natural and introduced ranges (Huner and Romaine 1978; Olouch 1990; Huner 2002; Stucki 2002).

Overall, *P. clarkii* adopted some *K*-selected life-history traits (slow growth, high longevity, large size), while retaining some of its typical *r*-selected characteristics (early maturation, high fecundity). Although the changes in the reproductive pattern most likely trace back to life cycle plasticity, it is unclear whether the large size can also be attributed to (phenotype) plasticity or whether it has a genetic basis and is adaptive (Stillwell 2010). More studies are necessary to address this question and to confirm whether the observed life-history changes indeed represent latitudinal clines as proposed here.

The presented findings demonstrate that *P. clarkii* is able to cope well with a new cold habitat by modulating its life history and reproductive pattern. This is worrying, as new *P. clarkii* populations are increasingly discovered at temperate zones (Müller 2007; Dümpelmann *et al.* 2009; Gross 2011; *cf.*, Figure 9). The prevention of any new introductions should therefore be of high priority.

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References

- Anastácio, P.M. and Marques, J.C., 1995. Population biology and production of the red swamp crayfish *Procambarus clarkii* (Girard) in the lower Mondego river valley, Portugal. *J. Crust. Biol.*, 15,156–168.
- Angilletta, M.J.Jr. and Dunham, A.E., 2003. The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general. *Am. Naturalist*, 162, 332–342.
- Atkinson, D. and Sibly, R.M., 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol.*, 12, 235-239.
- Bauer, G., 1992. Variation in the life span and size of the freshwater pearl mussel. *J. Anim. Ecol.*, 61, 425-436.
- Begon, M., 1979. Investigating animal abundance: capture-recapture for biologists. University Park Press, Baltimore, 97 p.
- Beatty, S., Morgan, D. and Gill, H., 2005. Role of life history strategy in the colonisation of Western Australian aquatic systems by the introduced crayfish *Cherax destructor* Clark, 1936. *Hydrobiologia*, 549, 219-237.
- Cano, E. and Ocete, M.E., 1997. Population Biology of red swamp crayfish, *Procambarus clarkii* (Girard, 1852), in the Guadalquivir River marshes, Spain. *Crustaceana*, 70, 553-561.
- Chiesa, S., Scalici, M. and Gibertini, G., 2006. Occurrence of allochthonous freshwater crayfishes in Latium (Central Italy). *Bull. Fr. Peche. Piscic.*, 380–381, 883–902.
- Chucholl, C., 2011. Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquat. Inv.*, 6, doi: 10.3391/ai.2011.6.1.
- DAISIE European Invasive Alien Species Gateway, 2010. One hundred of the worst. Available from: <http://www.europe-aliens.org/speciesTheWorst.do> [Accessed 1st August 2010]

- Daniels, W.H., D'Abramo, L.R. and Graves, K.F., 1994. Ovarian development of female red swamp crayfish (*Procambarus clarkii*) as influenced by temperature and photoperiod. *J. Crust. Biol.*, 14, 530-537.
- Dehus, P., Phillipson, S., Bohl, E., Oidtmann, B., Keller, M. and Lechleiter, S., 1999. German conservation strategies for native crayfish species with regard to alien species. *Crustacean Issues*, 11, 149-159.
- Dorn, N.J., Urgelles, R. and Trexler, J.C., 2005. Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *J. N. Am. Benthol. Soc.*, 24, 346-356.
- Dörr, A.J.M., La Porta, G., Pedicillo, G. and Lorenzoni, M., 2006. Biology of *Procambarus clarkii* (Girard, 1852) in Lake Trasimeno. *Bull. Fr. Peche. Piscic.*, 380-38, 1155-1170.
- Dümpelmann, C., Bonacker, F. and Häckl, M., 2009. Erstnachweis des Rotem Amerikanischen Sumpfkrebse *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia*, 67, 39-47.
- Fidalgo, M.R.A., Carvalho, P. and Santos, P., 2001. Population dynamics of the red swamp crayfish, *Procambarus clarkii* (Girard, 1852) from the Averio Region, Portugal (Decapoda, Cambaridae). *Crustaceana*, 74, 69-375.
- France, R., Holmes, J. and Lynch, A., 1991. Use of Size-Frequency data to estimate the age composition of crayfish populations. *Can. J. Fish. Aquat. Sci.*, 48, 2324-32.
- Frutiger, A., Borner, S., Büsser, T., Eggen, R., Müller, R., Müller, S. and Wasmer, H.R., 1999. How to Control unwanted Populations of *Procambarus clarkii* in Central Europe? *Freshwater Crayfish*, 12, 714-726.
- Gayanilo, FC.Jr. and Pauly, D., 1997. The FAO ICLARM stock assessment tools. FiSAT reference manual. FAO Computerized Information Series (Fisheries), Rome, FAO.
- Gherardi, F., 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. *Mar. Freshwater Behav. Physiol.*, 39, 175-191.
- Gherardi, F., Raddi, A., Barbaresi, S. and Salvi, G., 1999. Life history patterns of the red swamp crayfish (*Procambarus clarkii*) in an irrigation ditch in Tuscany, Italy. *Crustacean Issues*, 12, 99-108.

- Gherardi, F., Aquiloni, L., Tricarico, E. and Morpurgo, M., 2009. Süßwasserkrebse in Italien. In: Füreder, L. (ed.), *Flusskrebse: Biologie, Ökologie, Gefährdung.*, Folio, Wien/Bozen, 53-65.
- Gross, H., 2011. Edelkrebsprojekt NRW – Flusskrebsverbreitung. Available from: http://www.edelkrebsprojekt nrw.de/verbreitung_frame.htm [Accessed January 12, 2011].
- Gutiérrez-Yurrita, P.J., Martínez, J.M., Bravo-Utrera, M.Á., Montes, C., Ilhéu, M. and Bernardo, J.M., 1999. The status of crayfish populations in Spain and Portugal. *Crustacean Issues*, 11, 161-192.
- Henderson, P.A., 2003. *Practical Methods in Ecology*. Blackwell Science, Oxford.
- Henttonen, P. and Huner, J.V., 1999. The Introduction of alien species of crayfish in Europe: A historical introduction. *Crustacean Issues*, 11, 13-22.
- Hobbs, H.H., Jass, J.P. and Huner, J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana*, 56, 299–316.
- Huner, J.V., 1977. Introductions of the Louisiana red swamp crayfish, *Procambarus clarkii* (Girard); an update. *Freshwater Crayfish*, 3, 193-202.
- Huner, J.V., 2002. *Procambarus*. In: Holdich, D.M., (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, 541–574.
- Huner, J.V. and Barr, L.E., 1991. *Red Swamp Crawfish: Biology, Culture, and Exploitation*. Louisiana State University Sea Grant College System, Louisiana State University, Baton Rouge, Louisiana, 128 p.
- Huner, J.V. and Romaine, R.P., 1978 Size at maturity as a means of comparing populations of *Procambarus clarkii* (Girard) (Crustacea, Decapoda) from different habitats. *Freshwater Crayfish*, 4, 53-64.
- Ligas, A., 2008. Population dynamics of *Procambarus clarkii* (GIRARD, 1852) (Decapoda, Astacidea, Cambaridae) from southern Tuscany (Italy). *Crustaceana*, 81, 601-609.
- Lindqvist, O.V. and Huner, J.V., 1999. Life history characteristics of crayfish: What makes some of them good colonizers? *Crustacean Issues*, 11, 23-30.
- Lodge, D.M., Taylor, C.A., Holdich, D.M. and Skurdal, J., 2000. Non indigenous crayfishes threaten North American Freshwater Biodiversity. *Fisheries*, 25, 7-19.

- McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J. and Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity Distrib.*, 16, 95–108.
- Müller, K.W., 2007. Reproductive Habits of Non-native Red Swamp Crayfish (*Procambarus clarkii*) at Pine Lake, Sammamish, Washington. *Northwest Sci.*, 81, 246-250.
- Nyström, P., 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. *Crustacean Issues*, 11, 63-85.
- Nyström, P., 2002. Ecology. *In*: Holdich, D.M., (ed.) *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, 192-224.
- Oluoch, A.O., 1990. Breeding biology of the Louisiana red swamp crayfish *Procambarus clarkii* Girard in Lake Naivasha, Kenya. *Hydrobiologia*, 208, 85–92.
- Pauly, D., 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. *J. Cons. CIEM*. 39, 175–192.
- Pauly, D. and David, N., 1981. ELEFAN I, a BASIC program for the objective extraction of growth parameters from length frequency data. *Ber. Dtsch. Wiss. Kommission Meeresforschung*, 28, 205-211.
- Pauly, D. and Munro J.L., 1984. Once more on growth comparison in fish and invertebrates. *Fishbyte, Newsletter Network Trop. Fish. Sci.*, 2, 21.
- Pauly, D. and Morgan, G.R., 1987. *Length Based Methods in Fisheries Research*. ICLARM, Manila, Philippines, and KIRSI, Safat, Kuwait, 468 p.
- Price, J.E. and Welch, S.E., 2009. Semi-quantitative methods for crayfish sampling: sex, size, and habitat bias. *J. Crust. Biol.*, 29, 208–216.
- Reynolds, J.D., 2002. Growth and Reproduction. *In*: Holdich, D.M., (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, 152–191.
- Rodríguez, C.F., Bécáres, E., Fernández-Aláez, M. and Fernández-Aláez, C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biol. Inv.*, 7, 75–85.

- Sala, O.E., Chapin, III F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L., Jackson, R.B., Kinzig, A., Leemanns, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, B.H., Walker, B.H., Walker, M. and Wall, D.H., 2000. Biodiversity scenario for the year 2100. *Science*, 287, 1770-1774.
- Scalici, M. and Gherardi, F., 2007. Structure and dynamics of an invasive population of the red swamp crayfish (*Procambarus clarkii*) in a Mediterranean wetland. *Hydrobiologia*, 583, 309-319.
- Scalici, M., Chiesa, S., Scuderi, S., Celauro, D. and Gibertini, G., 2010. Population structure and dynamics of *Procambarus clarkii* (Girard, 1852) in a Mediterranean brackish wetland (Central Italy). *Biol. Invasions*, 12, 1415-1425.
- Smith, D.G., 1981. Life History parameters of the crayfish *Orconectes limosus* (Raf.) in Southern New England. *Ohio. J. Sci.*, 81, 169-172.
- Soes, M. and van Eekelen, R., 2006. Rivierkrefeten, een oprukkend probleem ? *De Levede Natuur*, 107, 56-59.
- Somers, K.M. and Stetchy, D.P.M., 1986. Variable trappability of crayfish associated with bait type, water temperature and lunar phase. *Am. Naturalist*, 116, 36-44.
- Sommer, T., 1984. The biological response of the crayfish *Procambarus clarkii* to transplantation into California ricefields. *Aquaculture*, 41, 373-384.
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D. and Haffner, P., (eds.) 2006. Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris (Patrimoines naturels, 64), 187 p.
- Stillwell, R.C., 2010. Are latitudinal clines in body size adaptive? *Oikos*, 119, 1387-1390.
- Stucki, T.P., 2002. Differences in life history of native and introduced crayfish species in Switzerland. *Freshwater Crayfish*, 13, 463-476.
- Suko, T., 1956. Studies on the development of the crayfish. IV. The development of winter eggs. *Sci. Rep. Saitana Univ. (Jpn.) Ser. B*, 2, 213-219.
- Suko, T., 1958. Studies on the development of the crayfish. VI. The reproductive cycle. *Sci. Rep. Saitana Univ. (Jpn.) Ser. B*, 3, 79-9.

Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae)

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Abstract

In the present study, the life history and diet of the highly successful North American invader *Orconectes immunis* was assessed for the first time in its introduced European range. In 2007, *O. immunis* population dynamics were monitored in a typical backwater habitat using unbaited funnel traps, and its life history was analysed using Von Bertalanffy's growth function. Juveniles hatched as early as March and may attain sexual maturity at the end of their first summer. The adult population moulted up to four times during the summer months, with the non-breeding form (II) lasting for a remarkably short time period. The high growth rate of *O. immunis* was combined with a short longevity, which was estimated at 2.5 years. The fecundity ranged from 119 to 495 pleopodal eggs. The stomach contents were dominated by detritus, followed by macroinvertebrates and macrophytes, and no ontogenetic shift in diet was observed. The ability to prey on a wide array of invertebrate taxa presumably supports the sustained high growth rate of *O. immunis*. The presented data provide evidence that *O. immunis* exhibits a strongly *r*-selected life history and omnivorous feeding habits. These ecological properties have often been linked to successful invaders and enhance the invasiveness of *O. immunis*.

Key words: *r*-selected species and life history; non-indigenous crayfish; polytrophic omnivore

Introduction

Invasive alien species (IAS) are one of the greatest threats to global biodiversity (Clavero and García-Berthou 2005; McGeoch *et al.* 2010). IAS can perturb the functioning of indigenous ecosystems and compete with or displace indigenous species, resulting in a global homogenisation of biota (Lockwood *et al.* 2007). Freshwater crayfish are important members of littoral food webs. They affect freshwater communities at different trophic levels, alter physical habitat characteristics and are considered to be keystone species (Momot 1995;

Nyström 2002). Non-indigenous freshwater crayfish species (NICS) may out-compete indigenous crayfish species (ICS) and severely disturb indigenous ecosystem functioning. For instance, the invasive crayfish *Procambarus clarkii* degraded shallow, macrophyte-dominated lakes in Spain within a few years (Rodríguez *et al.* 2005).

In Europe, only four to five species of crayfish are indigenous, whereas ten NICS have become established during the last 130 years (Souty-Grosset *et al.* 2006; Holdich *et al.* 2009; Chucholl and Pfeiffer 2010). One of the 'new' NICS is the calico crayfish, *Orconectes immunis* (Hagen, 1870), which was first reported in Europe in the mid-1990s from two locations in the Upper Rhine system (Dehus *et al.* 1999; Gelmar *et al.* 2006; *cf.* Figure 1). *Orconectes immunis* originates from north-eastern and central North America, where it is widely distributed in 26 US states and three Canadian provinces (Hobbs 1989). The pathway of introduction into Europe remains unknown; it was probably introduced either as fishing bait by Canadian soldiers (Gelmar *et al.* 2006) or as an ornamental pet (Dehus *et al.* 1999). The calico crayfish is popular as fishing bait in North America and is cultivated in ponds to supply the bait market (Forney 1957; Brown and Gunderson 1997). It was introduced into the states of New York and Colorado and parts of Canada (summarised by Hobbs *et al.* 1989; Jansen *et al.* 2009), but there are no records of the calico crayfish outside of the American continent except for the present occurrence along the Upper Rhine River in Europe.

In the past fifteen years, the species has rapidly colonised the Upper Rhine system over a stretch of more than 98 km (Gelmar *et al.* 2006; Chucholl and Dehus 2011; Collas *et al.* 2011; Figure 1). *Orconectes immunis* has successfully invaded several types of lentic and lotic habitats, including gravel pit lakes, small canals, temporary backwaters, small brooks and the main river channel. It has become abundant in the natural, slow-flowing or lentic backwaters and floodplains along the main river channel, of which most reaches are nature reserves and harbour a variety of rare plant and animal species (Chucholl 2006; Gelmar *et al.* 2006; Chucholl and Dehus 2011). Unlike the indigenous European crayfish, *Orconectes immunis* digs deep burrows, which allows it to also inhabit shallow and temporary water bodies (Tack 1941; Bovbjerg 1970), a niche formerly not occupied by any ICS in central Europe (Souty-Grosset *et al.* 2006).

Orconectes immunis is the second alien *Orconectes* species to invade the Rhine River; its predecessor *Orconectes limosus* (Rafinesque, 1817) arrived approximately 50 years before but is now widely displaced by the newcomer where their range overlaps (Chucholl 2006; Gelmar *et al.* 2006; Chucholl *et al.* 2008). *Orconectes immunis* was shown to be dominant in direct interactions and superior in competition for shelter. Furthermore, preliminary field observations have suggested that life-history differences between the two species may also contribute to the observed species displacement (Chucholl 2006; Chucholl *et al.* 2008), whereas only minor differences have been found in the behavioural reaction to and learning of predatory cues (Schlenker 2009).

To assess the risk that IAS represent for indigenous biota and ecosystems, it is important to understand their life history and feeding ecology. Furthermore, life-history data are crucial to develop population management or eradication strategies (*cf.* Scalici and Gherardi 2007), especially when an IAS has become well established and early eradication has failed (Simberloff 2003; Bufford and Daehler 2011).

Filipová *et al.* (2011) recently demonstrated a considerable genetic divergence between European and North American cytochrome c oxidase subunit I (COI) gene fragment sequences of *O. immunis*. The authors suggested that *O. immunis* might represent a cryptic species complex, making the forecasting of ecological properties of European *O. immunis* populations based on data from populations in its indigenous North American range difficult because the latter might comprise different cryptic species. Moreover, the ecological properties of an IAS can markedly differ between its indigenous and introduced range, as a result of genetic changes that accompany the founding process and different abiotic (*e.g.*, climate) and biotic conditions (*e.g.*, competitors, predators) (Lockwood *et al.* 2007; Chucholl 2011).

The primary aim of the present study was to report the life-history data, population dynamics and feeding habits of a European *O. immunis* population in a representative backwater habitat near the Rhine River and, thus, to provide a better understanding of its invasion success. Specifically, the population dynamics were monitored using unbaited funnel traps, and the obtained length-frequency data were used to assess life-history parameters, such as growth performance, mean lifetime, longevity, asymptotic length, and mortality, using Von Bertalanffy's growth function. To assess the feeding habits of *O. immunis*, stomach contents of juvenile and adult crayfish were analysed and the prey electivity was calculated. With respect to the previously observed displacement of *O. limosus* (Chucholl *et al.* 2008), fecundity and hatch data of both *O. limosus* and *O. immunis* were collected to assess whether those life-history traits may contribute to the species displacement.

Methods

Study site and sampling

To assess the population ecology and life history of *O. immunis*, an abundant population of calico crayfish was monitored in a typical backwater habitat, Lake Bärensee (LB; 15.7 ha, 48°54'4.72" N, 8°9'16.74" E). LB is a former branch of the Rhine River that has retained a shallow downstream connection to the main river channel and is part of the nature reserve 'Rastatter Rheinaue' and the special area of conservation 'Rheinniederung zwischen Wintersdorf und Karlsruhe' (SAC 7015-341). The lake is flooded during high-water events, usually several times per year. The lake water was turbid (Secchi depth < 1.25 m; Table 1), and macrophytes were scarce (mostly represented by *Ceratophyllum demersum* and *Elodea nutallii*). The bottom sediment was soft, and the water depth rarely exceeded 2 m. The lake had featured a large population of the endangered water chestnut (*Trapa natans*), but the species had recently disappeared from the lake, accompanied by a decline of other macrophyte species. The macro-zoobenthos was dominated by IAS, *e.g.*, *Corbicula fluminea*, *Dreissena polymorpha*, *Gammarus tigrinus*, *Dikerogammarus villosus*, and *Chelicorophium curvispinum*. *Orconectes immunis* occurred syntopic with *O. limosus* in LB; however, the latter was very rare.

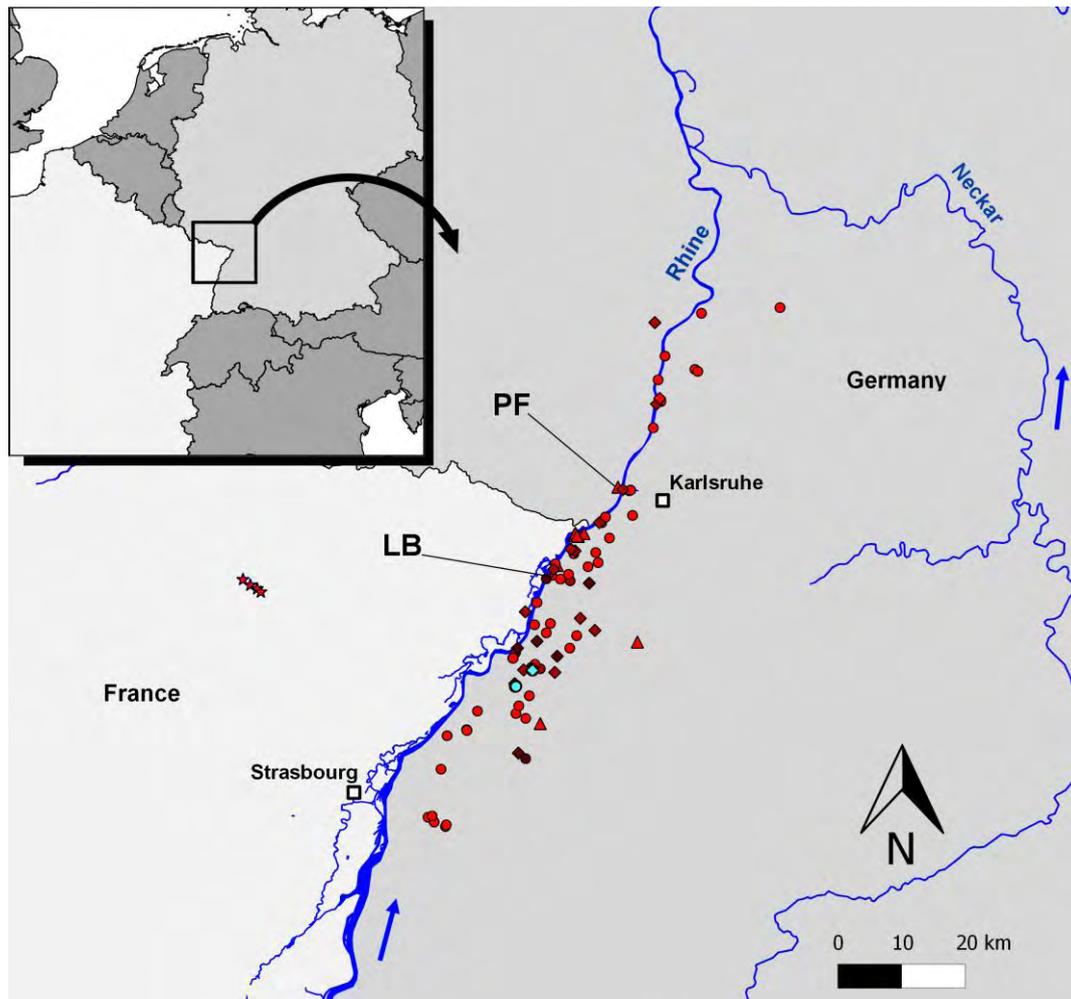


Figure 1. Currently known distribution of *O. immunis* in Europe. The symbol shape indicates the data source (circle: Chucholl and Dehus 2011; diamond: Gelmar *et al.* 2006; triangle: Chucholl, unpubl. data; star: Collas *et al.* 2011), and the symbol colour indicates the year of the record (black: 1993-1999; dark red: 2000-2005; red: 2006-2010). Light blue symbols indicate the two sites where *O. immunis* was first discovered; PF and LB denote the two study populations.

The crayfish were captured using unbaited, cylindrical funnel traps (0.4 m diameter opening, 3 m long, with 4- to 6-mm mesh). Two traps at a time were connected by a 2-m long net, with the openings facing towards each other. The pairs of traps were set along the shoreline at a depth of between 0.4 and 1.5 m and were exposed for seven days (one unit effort is equivalent to one trap set for seven days). On each sampling occasion, the surface water temperature, conductivity, and Secchi depth were measured at a distance from shore of approximately 20 m using a conductometer, LF 191, and a pH sensor, pH 197-S, from WTW (Weilheim, Germany).

The trapping lasted from early April to early August in 2007 (eight trapping occasions). Because of the high capture per unit effort (CPUE) in the late spring and summer (with a CPUE of up to 70 specimens in late July), only the contents of two randomly selected traps were sampled. The total number of traps varied between eight and 16. After a severe flooding event in August 2007, the CPUE decreased dramatically, and the trapping was ceased in October. An additional trapping occasion was performed in spring 2009 (four traps) to compare the reproductive pattern across years.

To assess juvenile growth, small crayfish were captured using manual net sampling on May 31, 2007. A hand net (with a mesh size of 1 mm) was pulled gently over the substrate perpendicular to the shoreline twelve times. Upon each pull, the net content was carefully searched for crayfish by hand.

An additional crayfish population in a backwater downstream of LB (“Pforzer Alrtheim”, PF; 49°1'26.14" N, 8°17'31.45" E) was trapped on three occasions from April 10 to April 24, 2007. *Orconectes limosus* occurred in PF at a noticeably higher density than in LB, making it possible to compare the reproductive pattern of both of the species within the same habitat. The trapping method was the same as in LB, but the traps were only exposed for three to four days on each sampling occasion. The trapping at PF was ceased after all of the captured *O. immunis* females had released their offspring.

Measurements

The species identity (*O. immunis* or *O. limosus*), carapace length (*CL*; measured from the tip of the rostrum to the dorsal posterior margin of the cephalothorax with a digital slide calliper to the nearest 0.1 mm), sex and reproductive traits (*i.e.*, active glair glands, attached eggs, hatchlings or egg remains) were noted from all of the crayfish in the sampled traps. Mature cambarid crayfish show a cyclic dimorphism between a reproductively active (I) and a reproductively inactive form (II). The reproductive form, *i.e.*, form I or form II, was distinguished in both sexes and also noted. The form changes usually with every moult, *i.e.*, an individual in form I moults into form II and vice versa. The cyclic dimorphism (form alteration) occurs in mature males of all cambarid genera and was also reported from *Orconectes* spp. females (Wetzel 2002; Buřič *et al.* 2010). In *O. immunis* and *O. limosus*, form I males (hereafter m I) differ from form II (hereafter m II) in having distinctly larger chelae (Chucholl 2006) and more slender and pronounced gonopod tips. The mesial gap between the terminal elements of the 1st gonopod is also conspicuously wider. Form I females (hereafter f I) have a distinctly wider pleon than form II females (hereafter f II; Wetzel 2002; Buřič *et al.* 2010) and usually show the above-mentioned reproductive traits. Moreover, form I individuals of *O. immunis* often have conspicuously purple-coloured chelae.

To assess the length-weight relationship, intact specimens (with no missing or regenerated chelae) of *O. immunis* ($N = 139$ and 98 for males and females, respectively) were transported to the laboratory and weighed to the nearest 0.01 g using a Kern balance (type 822-67). Prior to the weight measurement, the specimens were wiped with blotting paper and gently shaken ten times to remove adherent water drops.

Life-history analysis

The obtained size-frequency data were analysed using the program FiSAT 2 (v. 1.2.2; Gayanilo and Pauly 1997). The growth was described per sex using Von Bertalanffy’s growth function (VBGF), adjusted for seasonal growth in summer and no growth in winter (Pauly and Morgan 1987): $L(t) = L_{\infty} (1 - e^{-k(t-t_0)+Ck/2\pi\{\sin 2\pi(t-t_s)-\sin 2\pi(t_0-t_s)\}})$, where L is the CL at age t , L_{∞} is the asymptotic CL , k is the curvature parameter (*i.e.*, the rate at which L_{∞} is approached) and t_0 is the initial condition parameter (*i.e.*, the hypothesised age at which the CL is zero). T_s was substituted with WP , using the formula $WP = t_s + 0.5$, which represents the winter point

at which the growth rate is slowest in the annual cycle. The *WP* was set to mid-winter, and the parameter expressing the amplitude of the seasonal growth oscillation (*C*) was set arbitrarily to 1, allowing for no growth in winter, as suggested by Tack (1941). L_{∞} and k were estimated within the ELEFAN module of FiSAT 2 (non parametric scoring), using the size frequencies obtained from the juvenile hand captures in late May 2007 as starting points, and t_0 was assessed using non-linear regression analysis.

Longevity (t_{\max}) was estimated using the equation $t_{\max} = (3/k) + t_0$, and mean lifetime ($t_{1/2}$) was estimated using the equation $t_{1/2} = \{\sum[n(t) \cdot t]\}/N$, where n is the number of individuals at time t and N the total number of individuals (Gayanilo and Pauly 1997; Scalici and Gherardi 2007; Chucholl 2011). The growth performance index (ϕ') was derived from k and L_{∞} by applying the equation $\phi' = \log(k) + 2\log(L_{\infty})$ (Pauly and Munro 1984).

The total mortality (Z) was estimated in FiSAT2 from the Powell–Wetherall plot equation, which allows for the calculation of L_{∞} and the ratio Z/k using length-frequency data. The natural mortality (M) was computed by solving the equation $\log(M) = -0.0066 - 0.279\log(L_{\infty}) + 0.6543\log(k) - 0.463\log(T)$, where T is the mean environmental temperature during the study period (Pauly 1980). Because Z is the sum of M and the fishing mortality (F), the fishing mortality was obtained by subtracting M from Z .

Fecundity

The pleopodal fecundity was determined from 38 gravid *O. immunis* females captured from LB and 25 gravid *O. limosus* females captured from PF. Upon capture, the specimens were transported carefully to the laboratory, and all attached eggs were stripped off using forceps and counted.

Feeding ecology

Active crayfish were captured from the eastern littoral zone of LB on July 24 and August 6, 2007, beginning 30 min after sunset. The crayfish were captured with a hand net within a 10-m radius of the four transects used to assess the prey abundance (see below). Upon capture, the crayfish were immediately put on crushed ice to halt their digestion. The specimens were subsequently transported to the laboratory and dissected. The stomach was carefully removed and placed into 70 % ethanol. To assess a possible ontogenetic shift in diet, the specimens were grouped into two size classes, roughly corresponding to juvenile and adult crayfish ($CL \leq 27$ mm and $CL > 27$ mm).

The stomach content was washed into a petri dish and analysed qualitatively and quantitatively under a binocular. Invertebrates were identified by characteristic hard parts (*e.g.*, head capsule, legs, abdomen, and shell fragments) and counted. The relative volume proportion of the food categories (detritus, macroinvertebrates and macrophytes) was estimated on an ordinal scale (0-5). The percentage of the stomachs containing prey i (OcP_i) and the percentage of the total number of organisms representing prey i (AbP_i), and the percentage of the stomachs containing food category i (OcF_i) and the estimated volume proportion of food category i ($VolF_i$) were used to calculate a relative importance index (RI) for each prey item and food category using the following formula (modified from Pérez-Bote 2005): $RI_i = AI_i \cdot 100 / \sum(AI_{i=1-n})$, where $AI_i = OcP_i \cdot AbP_i$ or $AI_i = OcF_i \cdot VolF_i$ for prey items or

food categories, respectively, and n is the number of prey items or food categories. The RI incorporates the information derived from two methods of stomach content analysis and thereby reduces the biases produced by using a single measure of stomach content (Windell 1971; Pérez-Bote 2005).

To assess the prey electivity, benthic macroinvertebrates were sampled in the eastern littoral zone of LB on July 19 and 30, 2007, and on August 3 and 6, 2007. A total of 12 samples were taken with a Birge-Ekman grab (15 x 15-cm opening) at 1, 5 and 10 m shore distance along four transects perpendicular to the shore line (with a maximum depth of 1.6 m). The samples were transported to the laboratory and searched for macroinvertebrates. The sediment fraction was rinsed two times through a 600- μm sieve. The collected macroinvertebrates were identified as far as feasible and counted. The prey electivity of *O. immunis* was then assessed using Jacobs (1974) index, $D_{ji} = (RI_i - r_i)/(r_i + RI_i - 2r_iRI_i)$, where r_i is the relative abundance of prey item i in the environment. Values of D_j between 0 and 1 indicate positive prey selection, while values between 0 and -1 indicate negative selection.

Statistics

All statistical analyses were performed using SigmaPlot 10.0 (with SigmaStat 3.5 Integration) except for the generalised linear models (GLMs; see below), which were run in the software package R 2.14 (R Development Core Team 2011). The correlations between the abiotic factors and CPUE, CPUE and the proportion of form II individuals, and fecundity and CL were assessed using Pearson correlations. The mean CL was compared for each sex between consecutive sampling occasions using Mann-Whitney rank sum tests, and the proportions of form II individuals and females with active glair glands, eggs, hatchlings or egg remains were compared using Chi-square tests with Yates correction. The sex ratio of the total catch and the sex ratio of the catch of each sampling occasion were tested for statistical differences from 1:1 using Chi-square tests with Yates correction.

The relationship between the CL and body weight (W) was described for each sex by means of the power equation $y = 10^a \cdot x^b$, with $x = CL$ [mm] and $y = W$ [g]. The parameters a and b were estimated using a linear regression after log transformation of the data.

GLMs were used to assess whether sex had a significant effect on W and whether species (*O. immunis* or *O. limosus*) had a significant effect on the fecundity (*cf.* McCullagh and Nelder 1989; Jones *et al.* 2009). W and the number of pleopodal eggs were used as response variables, and CL was used as predictor variable. The sex and species were entered as factors. Both of the GLMs allowed for interactions between the factor and the CL .

To assess ontogenetic shifts in the diet of *O. immunis*, the RI values of prey items and food categories were compared between the two crayfish size classes using z-tests with Yates correction. Differences in the RI of prey items and food categories within each crayfish size class were also assessed using z-tests with Yates correction, followed by a Bonferroni adjustment of the P level. The AbP_i of prey items was compared within each crayfish size class using a Kruskal-Wallis one-way ANOVA on ranks, followed by Tukey's post-hoc test. A Chi-square test was used to assess significant differences between the RI of prey items and r_i .

Results

Population dynamics

In total, 1216 *O. immunis* specimens were captured and measured during the study period in 2007, and 283 additional crayfish were captured on April 3, 2009. The largest male had a *CL* of 49 mm, while the *CL* of the largest captured female measured 48 mm. The minimum size at maturity was 25.6 mm *CL* and 26.7 mm *CL* in males and females, respectively. The sex ratio of the total catch did not differ significantly from 1:1 (Chi-square-test with Yates correction: $P = 0.08$). The sex ratio was also balanced on each sampling occasion (Chi-square-tests with Yates correction: $P > 0.05$), except for April 4 and August 1, 2007, when it was significantly biased towards males (Chi-square-tests with Yates correction: $P < 0.001$ and $P < 0.05$, respectively; Table 1).

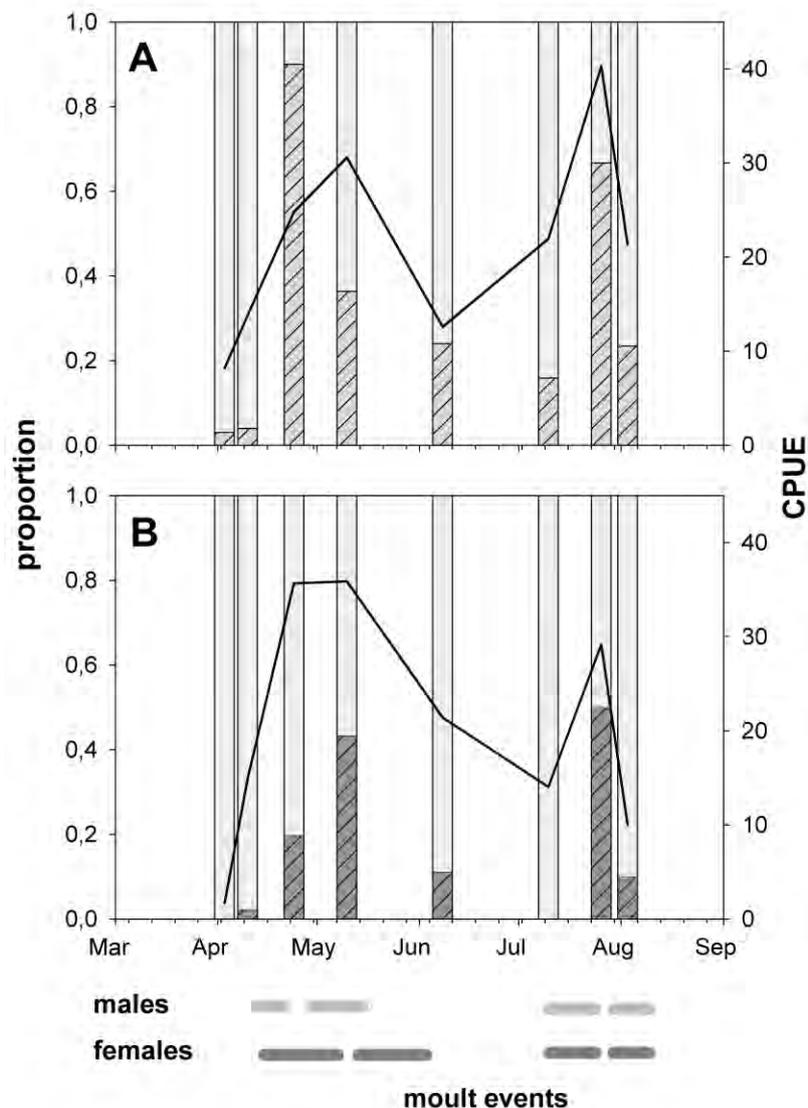


Figure 2. Proportion of form II individuals within the total catch (hatched bars), CPUE (solid line; right axis) and supposed moult events (bottom) of the *O. immunis* population in LB, according to sex (A, males; B, females).

The surface water temperature at LB ranged from 14 °C in early April to 27 °C in September and dropped below 10 °C in October (Table 1). There was no significant correlation between the surface water temperature and CPUE (Pearson correlation: $p = 0.155$). The proportion of form II individuals within the catch tended to correlate with the CPUE in males (Pearson correlation: $R = 0.69$, $P = 0.058$; Figure 2) and correlated significantly with the CPUE in females (Pearson correlation: $R = 0.77$, $P = 0.025$; Figure 2). The highest proportion of m II occurred in late April, whereas the proportion of f II peaked later in mid-May (Table 1, Figure 2). A second peak of form II individuals occurred simultaneously in both sexes in late July (Table 1, Figure 2). Freshly moulted crayfish were abundant in the catch from mid-April to June and again from mid-July to August (Figure 2).

The mean size of the captured crayfish ranged between 32 mm *CL* in mid-July and 40 mm *CL* in early June, 2007. The mean *CL* decreased significantly in both sexes in mid-July, 2007 (Chi-square tests with Yates correction: $P < 0.001$; Table 1), when the largest size classes mostly disappeared from the catch (Figure 3). By early August, 2007, the mean *CL* had significantly increased to values comparable to the previous months (Chi-square tests with Yates correction: $P < 0.001$; Table 1, Figure 3).

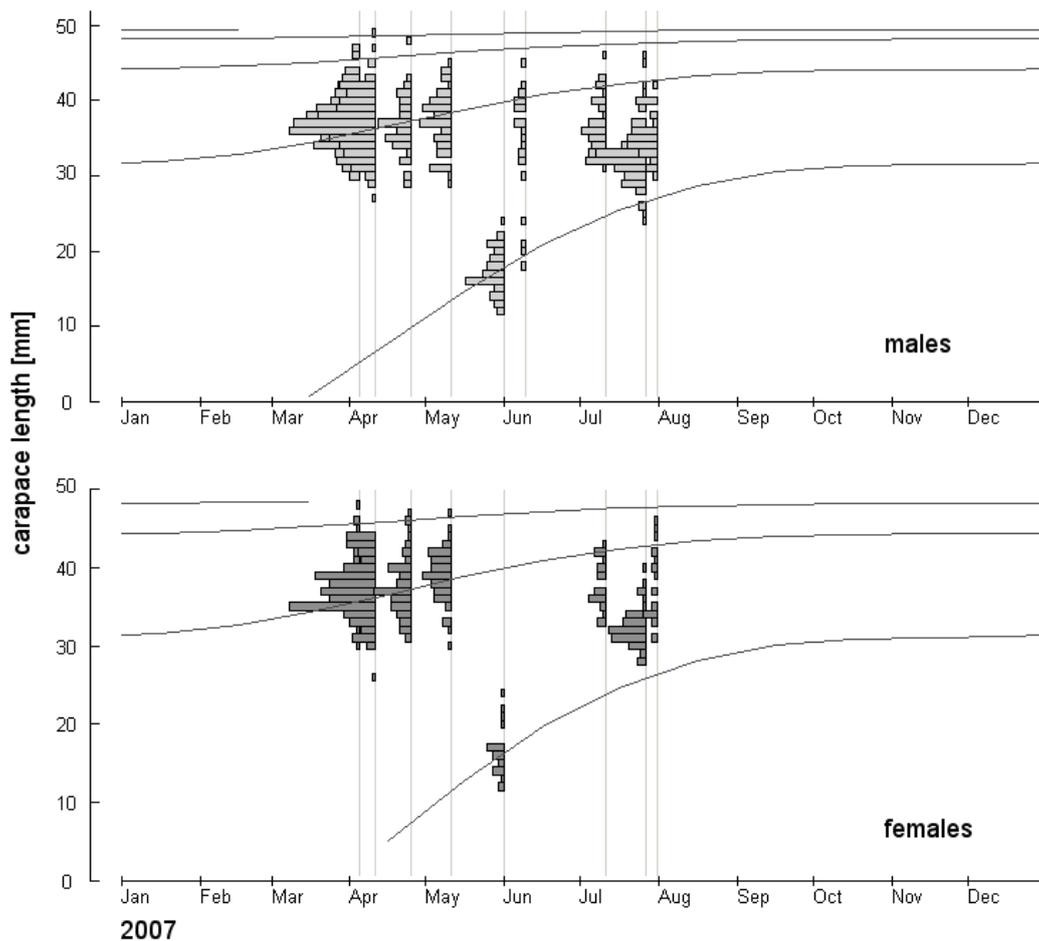


Figure 3. Seasonal growth model for *O. immunis* males (light grey) and females (dark grey). YoY may attain sexual maturity within their first summer.

Life-history parameters

The length frequencies of the study population in LR and the resulting seasonal growth patterns are shown in Figure 3 for each sex. The obtained VBGF parameters (L_{∞} , k , and t_0), their derivatives (longevity, t_{\max} , and the growth performance index, ϕ'), the calculated mean lifetime ($t_{1/2}$), and the mortality estimates (the total mortality, Z , the natural mortality, M , and the fishing mortality, F) are summarised according to sex in Table 2.

Table 2. VBGF parameters (L_{∞} , k , and t_0), their derivatives (longevity, t_{\max} , and the growth performance index, ϕ'), mean lifetime ($t_{1/2}$), and mortality (the total mortality, Z , the natural mortality, M , and the fishing mortality, F) of the *O. immunis* population in LB, according to sex.

	L_{∞}	k	t_0	t_{\max}	$t_{1/2}$	ϕ'	Z	M	F
males	50.0	1.15	-0.09	2.52	2.09	3.46	3.68	1.30	2.38
females	49.8	1.22	-0.09	2.37	2.06	3.48	3.95	1.36	2.59

Length-weight relationship

There was a significant positive relationship between CL and W in both sexes (Pearson correlation of log-transformed data: $P < 0.001$). The obtained parameters for the power equation are summarised per sex in Table 3, and the relationship between CL and W is shown for each sex in Figure 4. The GLM analysis showed that sex had a significant effect on W (analysis of deviance: $F_{1,233} = 18.05$, $\eta^2 = 0.236$, $P < 0.001$). The GLM included also a significant interaction between sex and CL (analysis of deviance: $F_{1,233} = 58.40$, $P < 0.001$), indicating that W increased faster in males than in females (*cf.* Figure 4).

Table 3. Parameters of the length-weight relationship in *O. immunis* for each sex (form I in adults). R was determined using linear regression of the log-transformed data. The equation to derive the weight is as follows: $W [g] = 10^a CL [mm]^b$.

Sex	N	R	a	b
males	133	0.98	-4.198	3.465
females	85	0.98	-3.840	3.203

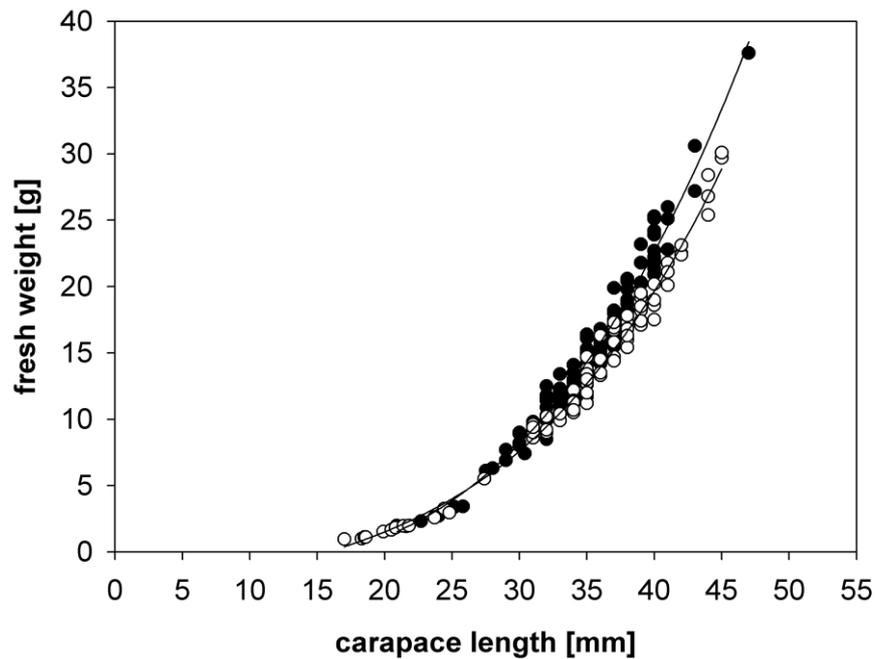


Figure 4. Length-weight relationship in *O. immunis*. Males are depicted as solid symbols, and females are depicted as open symbols. Adult crayfish were in form I. See Table 3 for the regression parameters.

Reproduction

On April 10, 2007, approximately half of the captured *O. immunis* females from LB ($N = 181$) carried eggs, whereas eight per cent and 30 per cent already carried hatchlings and egg remains, respectively (Table 1). By late April 2007, none of the captured females ($N = 71$) carried eggs, and hatchlings were present on the pleopods of six per cent of the females. Egg remains were found on 18 per cent of the captured females (Table 1). The last egg-bearing female was captured on May 10, 2007 (one out of 67 captured females), together with the last female with egg remains on the pleopods. The proportion of females with active glair glands increased significantly on the same sampling occasion (Chi-square test with Yates correction: $P < 0.001$; Table 1), and by early August, 83 per cent of the sampled females had active glair glands (Table 1). On April 3, 2009, 165 females were trapped from LB to compare reproductive the pattern across years: 84 per cent carried eggs, seven per cent carried hatchlings, and one per cent carried egg remains (Table 1).

Orconectes immunis and *O. limosus* occurred together in PF, where reproductive patterns were analysed from April 10 to April 24, 2007, on three capture occasions (Figure 5). During this time period, the proportion of captured *O. immunis* females with attached eggs dropped from 44 to zero per cent, while the proportion of females with egg remains increased from eight to 100 per cent. Females with attached hatchlings were captured on the first two capture occasions (Figure 5). None of the captured *O. limosus* females carried hatchlings or egg remains, but up to 98 per cent of the females that were sampled carried eggs (Figure 5).

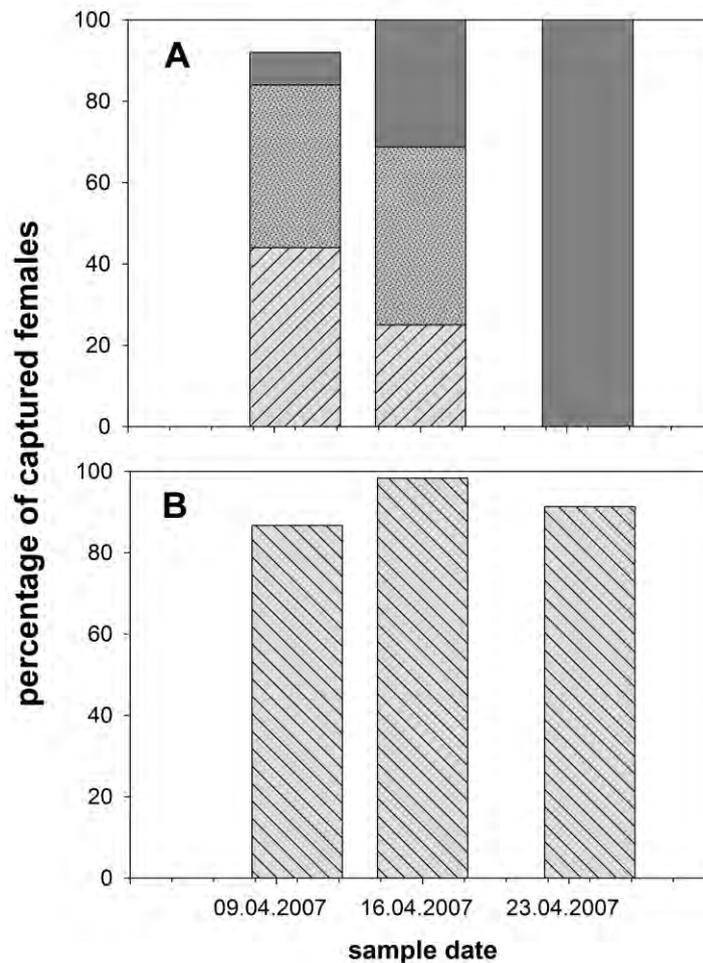


Figure 5. Reproductive pattern of *O. immunis* (A; top) and *O. limosus* (B; bottom) in PF in spring 2007. The hatched bars represent the proportion of females with attached eggs; dotted bars represent the proportion of females carrying hatchlings. The proportion of females with egg remains is depicted in dark grey. Note that *O. limosus* females did not carry hatchlings or egg remains within the study period, whereas all of the sampled *O. immunis* females had released their offspring by late April.

The pleopodal fecundity of the sampled *O. immunis* females ranged from 119 to 495 eggs and averaged 277.7 eggs (± 94.8 SD) per female, whereas the pleopodal fecundity of the *O. limosus* females averaged 261.3 eggs (± 128.6 SD) per female. The number of pleopodal eggs and the female *CL* were significantly correlated in both of the species (Pearson correlation: $R = 0.62$, $P < 0.001$ and $R = 0.79$, $P < 0.001$, for *O. immunis* and *O. limosus*, respectively; Figure 6). The GLM analysis showed that there was no significant effect of species on the number of pleopodal eggs (analysis of deviance; main effect: $F_{1,58} = 2.222$, $P = 0.142$; interaction with *CL*: $F_{1,58} = 0.038$, $P = 0.847$), indicating that the fecundity did not differ between the two species (Figure 6).

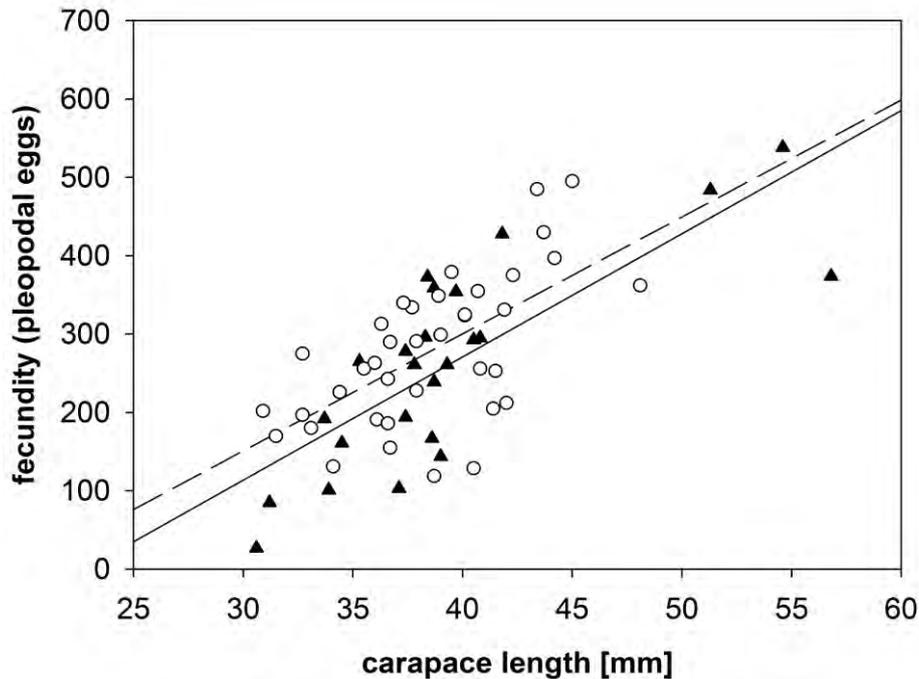


Figure 6. Fecundity of *O. immunis* (open circles) and *O. limosus* (solid triangles). The trend lines were determined using linear regression (*O. immunis*, dashed line; *O. limosus*, solid line; see the results section for the statistics).

Feeding ecology

The stomach contents of 58 *O. immunis*, evenly grouped into two size classes, were analysed. The juvenile size class was made up of crayfish with a mean *CL* of 24.3 mm (± 2.3 mm SD), whereas the specimens of the adult size class averaged 32.3 mm *CL* (± 2.9 mm SD).

In both of the size classes, detritus was found to be the most important food category, followed by macroinvertebrates and macrophytes (Figure 7); however, the *RI* of the food categories did not significantly differ from each other (based on z-tests with Yates correction, followed by a Bonferroni adjustment of the *P* level). The consumed macroinvertebrate prey largely consisted of Chironomidae larvae and Cladocera, followed by unidentified insects and Ephemeroptera larvae (Figure 7). Chironomidae larvae and Cladocera attained significantly higher *RI* values than the other 15 prey items in both of the crayfish size classes (according to z-tests with Yates correction, followed by a Bonferroni adjustment of the *P* level) and were also consumed in significantly higher numbers than the other prey items (Kruskal-Wallis one-way ANOVA on ranks: $H_{11} = 221.0$, $P < 0.001$, and $H_{14} = 232.0$, $P < 0.001$, for the juvenile and adult size class, respectively; Figure 8). The *RI* of the prey items and food categories did not significantly differ between the two crayfish size classes (z-tests with Yates correction: $P > 0.05$; Figure 7).

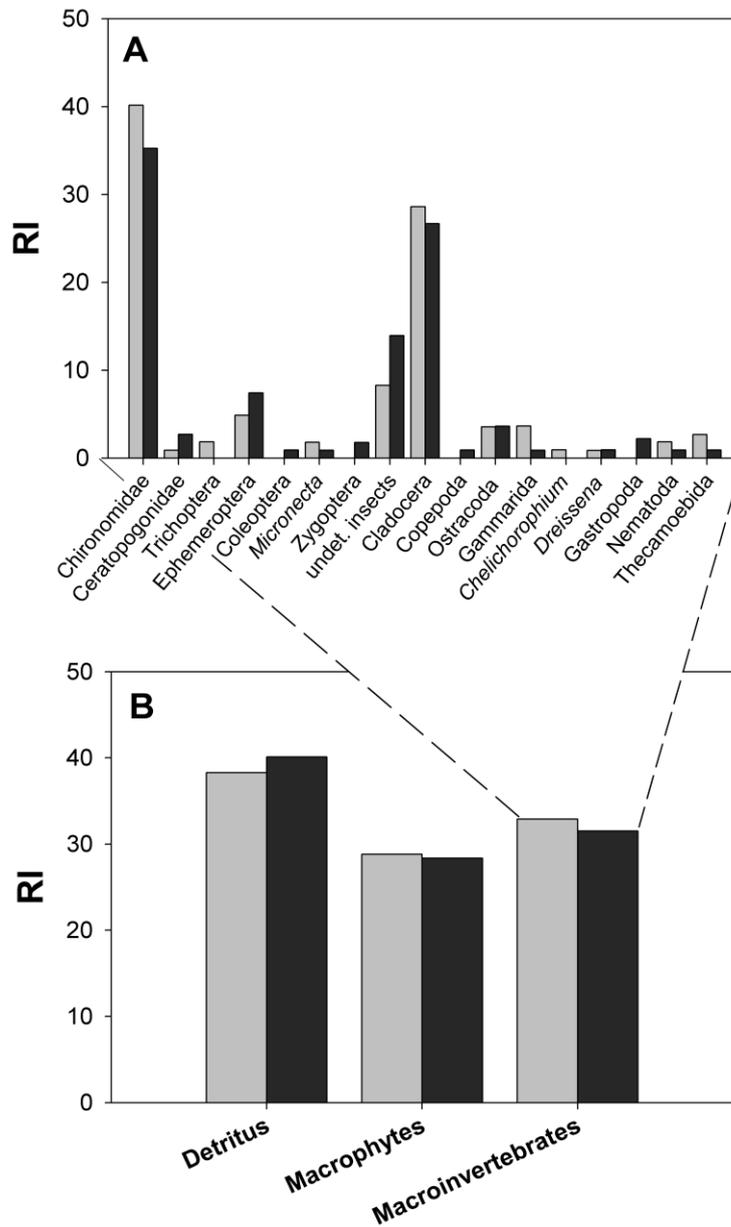


Figure 7. Relative importance (*RI*) of prey items (A; top) and food categories (B; bottom) in the diet of *O. immunis*. Crayfish shorter than 27 mm *CL* (juveniles) are depicted in light grey, crayfish longer than 27 mm *CL* (adults) are shown in dark grey.

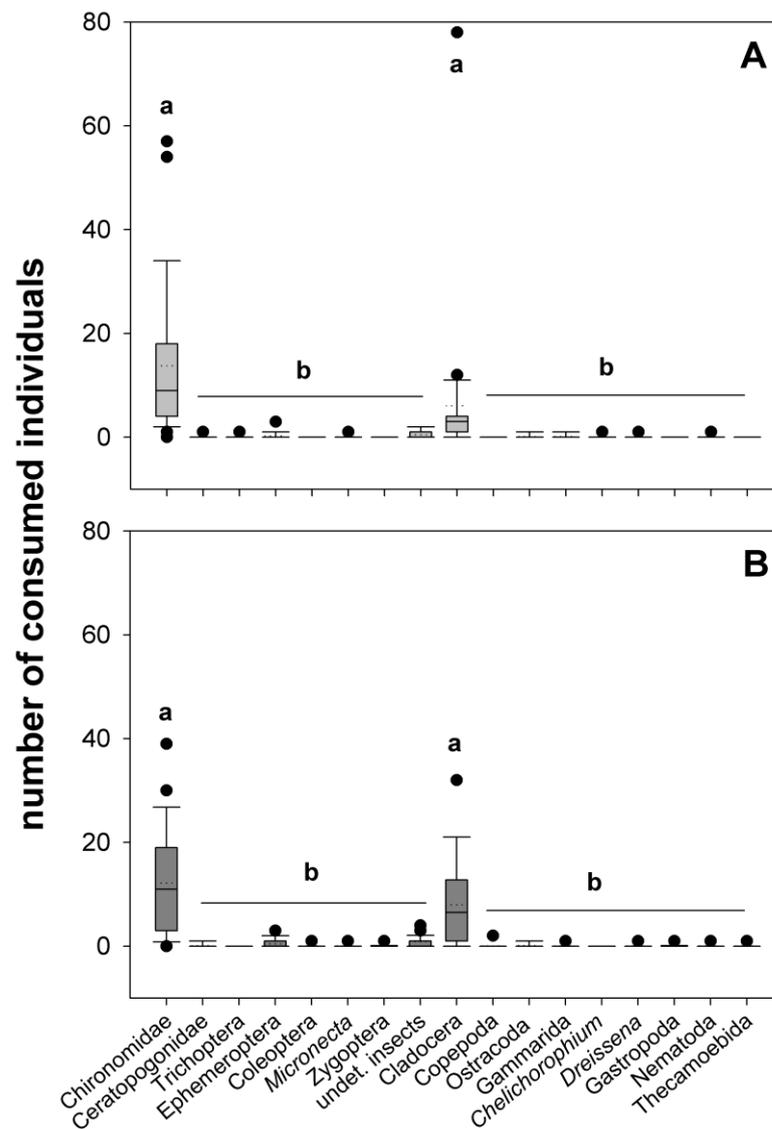


Figure 8. Number of consumed prey individuals per crayfish stomach. Juvenile crayfish are depicted on top (A; light grey); adult crayfish are shown below (B; dark grey). The dashed lines in the boxes indicate the mean value; the solid lines indicate the median. Different letters show significant differences, determined using a Kruskal-Wallis one-way ANOVA on ranks, followed by a Tukey's post-hoc test ($P < 0.05$).

To calculate the prey electivity, the stomach content data of the two crayfish size classes were pooled because they showed no significant statistical differences. D_j was found to be positive for Trichoptera larvae, Ephemeroptera larvae and *Dreissena polymorpha*, whereas D_j reached distinct negative values ($D_j < -0.5$) for Gammarida and Oligochaeta (Figure 9). The RI of *Dreissena polymorpha* and Oligochaeta differed significantly from r_i (Chi-square test: $P < 0.05$; Figure 9).

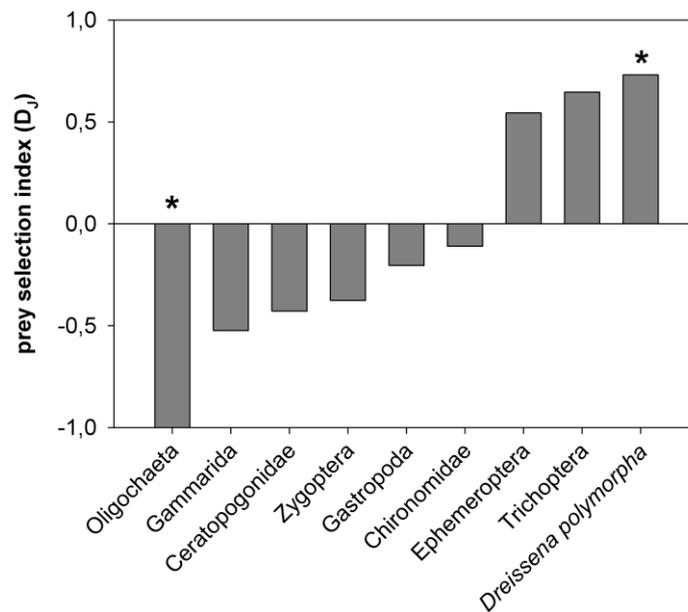


Figure 9. Prey electivity index (D_{ji}) in *O. immunis*. Values between 0 and 1 indicate positive selection; values between 0 and -1 indicate avoidance relative to the abundance of prey taxa in the littoral zone of LB. The asterisks indicate significant differences between r_j and RI , determined using Chi-square tests ($P < 0.05$).

Discussion

Life history

The life-history analysis was based on an ample number of captured crayfish and was carried out in a typical *O. immunis* habitat. The traps used were not baited and produced, presumably, less bias in crayfish size and sex than baited traps, which typically show a bias towards more competitive and vagile individuals, *i.e.*, males and larger crayfish (France *et al.* 1991; Olsen *et al.* 1991; Chucholl 2011). The sex ratio of the total catch was balanced, which is in accordance with the sex ratio suggested for other Cambaridae, including *Orconectes* spp. (Olsen *et al.* 1991; Hamr 2002 and citations therein). The trappable crayfish population was largely composed of crayfish larger than 27 mm *CL* and probably included individuals of the 0+ cohort in late summer and spring (*cf.* Figure 3). The observed total mortality resulted largely from fishing mortality, demonstrating the capture-effort to which the population was subjected in LB. In total, approximately 70 kg of crayfish were removed from LB during the 2007 study period, corresponding to approximately 3500 specimens (estimated from the obtained length-weight relationships and size-frequency distributions).

The growth rate (curvature parameter k) of *O. immunis* was estimated at 1.22 and 1.15, which indicates very rapid growth, especially when compared to the curvature parameters of ICS, which typically range between 0.25 and 0.50 (summarised by Scalici *et al.* 2008). The obtained values of the growth performance index (ϕ') were also considerably higher than the ϕ' values reported for ICS (Scalici *et al.* 2008) but were remarkably similar to values known from other fast-growing NICS, such as *P. clarkii* (Scalici *et al.* 2009 and citations therein; Chucholl 2011; Table 4). The k values of *O. immunis* obtained in the present study exceed

those of *P. clarkii* (Scalici *et al.* 2009 and citations therein), suggesting that *O. immunis* approaches its asymptotic length at a faster rate than *P. clarkii* and attains its sexual maturity earlier. In fact, young-of-the-year (YoY) *O. immunis* in LB may attain sexual maturity at the end of their first summer, a finding that coincides with data reported from permanent habitats in its indigenous range, where one third to three quarters of the YoY mature by autumn (Tack 1941; Caldwell and Bovbjerg 1969). This quick maturation probably represents a unique life-history trait because no other ICS or NICS in central Europe is known to mature within its first summer (Stucki 2002; Souty-Grosset *et al.* 2006; Chucholl 2011; Table 4), with the possible exception of *O. juvenilis*, for which no data are available (*cf.* Chucholl and Daudey 2008). As a consequence of the high growth rate, adult *O. immunis* also moult more frequently than ICS. The observed occurrence of freshly moulted individuals in the traps and the temporal variation in the ratio of form I to form II individuals (Figure 2) suggest concordantly that the adult population probably moulted up to four times during the summer months. The form II condition prevailed only for a short time period, and high occurrences of form II individuals coincided with phases of great activity, resulting in a high CPUE. The form II condition may have lasted only a few weeks in individual crayfish, a finding that is supported by a casual aquarium observation: one form I male captured in May 2007 moulted into form II and back into form I within two weeks. A similar fast sequence of moults, resulting in a comparably short form II condition, was also recently reported for *O. limosus* (Buřič *et al.* 2010). The fact that most adult *O. immunis* in LB had moulted back into form I by mid-June may reflect an adaptation to seasonally drying habitats, where mating in late summer and autumn may be hampered as a result of drying out (*cf.* Caldwell and Bovbjerg 1969).

The fast growth rate of *O. immunis* was combined with a relatively small asymptotic *CL* and a short mean lifetime and longevity. ICS live markedly longer and attain larger sizes than *O. immunis*, with *Austropotamobius torrentium* being an exception concerning size (Souty-Grosset *et al.* 2006; Scalici *et al.* 2008; Table 4). Tack (1941) estimated the longevity of *O. immunis* in its indigenous range at 2-3 years, which is in good accordance with the estimates obtained in the present study. The mean lifetime of the study population in LB was close to the maximum lifespan, indicating a high mortality within the third year, possibly influenced by the high fishing mortality. Within the estimated mean lifetime, *O. immunis* individuals may reproduce two to three times, depending on whether they attain sexual maturity at the end of their first summer. For comparison, most ICS do not even attain sexual maturity within the mean lifetime of *O. immunis* (Stucki 2002; Souty-Grosset *et al.* 2006; Table 4).

The collected fecundity data of *O. immunis* are consistent with values reported from its indigenous range (Gunderson and Kapuscinski 1992) and are remarkably close to the data collected for *O. limosus*. Both species are very fecund, especially when compared to ICS, which carry on average less than 200 eggs (Stucki 2002; Table 4) in contrast to the 240 eggs carried on average by *O. immunis*. In 2007, *Orconectes immunis* eggs hatched in LB as early as March 17 (based on a preliminary net sampling prior to the main study period), and hatching continued throughout April until the first half of May. The early hatching time was confirmed in April 2009, when eight per cent of the sampled females were found to already

carry hatchlings or egg remains, and is consistent with data from the native range of *O. immunis* (Tack 1941; Caldwell and Bovbjerg 1969). The early hatching time suggests that most *O. immunis* females in LB had laid their eggs in autumn and carried them throughout winter, as was reported by Caldwell and Bovbjerg (1969) for populations in permanent habitats. This autumnal egg-laying is a distinct difference to other *Orconectes* species, such as *O. limosus*, which typically lay their eggs in spring (Souty-Grosset *et al.* 2006). In PF, all of the captured *O. immunis* females had released their offspring by late April 2007, whereas *O. limosus* females were still breeding at this time. The egg incubation of *O. immunis* throughout the winter presumably favours the unusual early hatching period.

Overall, *O. immunis* is a strongly *r*-selected species, exhibiting typical *r*-selected life-history traits, such as rapid growth, a small asymptotic *CL*, a short life cycle, and high fecundity. The life history of *O. immunis* contrasts markedly with the life history of ICS, which are more *K*-selected, *i.e.*, live longer, grow slower but larger, and are less fecund (Stucki, 2002; Neveu, 2006; Souty-Grosset *et al.*, 2006; Table 4). The strong *r*-selection of *O. immunis* probably represents an adaptation to its natural ecological niche; in its indigenous range, *O. immunis* often inhabits temporary backwaters or pools, where it is released from the competition pressure of other, larger *Orconectes* species, such as *O. virilis*, that prefer permanent habitats (Bovbjerg 1970). The temporary habitats colonised by *O. immunis* feature seasonal dry-downs, during which crayfish retreat into burrows (Tack 1941; Caldwell and Bovbjerg 1969; Bovbjerg 1970). While reproduction may take place in burrows, growth is most likely restricted to the flooding periods, favouring a fast growth rate and a short life cycle with an early sexual maturation.

Feeding ecology

Freshwater crayfish are generally considered to be omnivorous, but species-specific differences in feeding habits likely exist. Ontogenetic shifts in diet were reported for several larger species, suggesting interdependencies between growth rate, size and feeding preferences (Momot 1995; Nyström 2002; Alcorlo *et al.* 2004). A relatively small species like *O. immunis* may therefore exhibit other feeding preferences than larger NICS or ICS. The results of the present study clearly indicate that *O. immunis* is a polytrophic omnivore. It was found to feed on detritus and primary producers (algae and macrophytes) as well as on higher trophic levels, *e.g.*, predatory insect larvae (*cf.* Figure 7). Detritus was found to be the most important food category, followed by macroinvertebrates and macrophytes. A prevalence of detritus in the stomach content is a common phenomenon in crayfish and has been observed in many species (*cf.* Momot 1995). No ontogenetic shift in the diet of *O. immunis* was observed in the present study, perhaps as a result of its rather small size and the sustained high growth rate throughout its life. Both a slower growth rate in adult crayfish and an incremental size difference between larger crayfish and their prey have been previously suggested as explanations for ontogenetic shifts in crayfish diets (Nyström 2002; Alcorlo *et al.* 2004).

Orconectes immunis was found to prey on a wide spectrum of macroinvertebrates. The main prey taxa were Chironomidae larvae, Cladocera and Ephemeroptera larvae, of which the latter tended to be positively selected. A similar high importance of Chironomidae larvae in the diet of *Orconectes luteus* and *O. punctimanus* was reported by Whitley and Rabeni

(1997), and a preference for Chironomidae and Ephemeroptera larvae is also known for *P. clarkii* (Alcorlo *et al.* 2004). *Orconectes immunis* positively selected for slow-moving or immobile prey that could be easily captured, such as *Dreissena polymorpha* and Trichoptera, whereas sediment-dwelling prey, such as Oligochaeta, were significantly underrepresented in the *O. immunis* stomachs. A similar under-representation of sediment-dwelling prey was reported in the diet of *P. clarkii* (Alcorlo *et al.* 2004), suggesting that crayfish might generally select against sediment-dwelling taxa, probably because sediment dwellers are hard to capture and the handling is not profitable for crayfish. However, sediment-dwelling *Ephemera* sp. larvae were occasionally consumed by *O. immunis*, presumably because the larvae are fairly large and energy-rich.

The relatively high importance of zooplankton in the diet of *O. immunis* shown in the present study is in accordance with the results of Tack (1941), who found that *Daphnia* constituted up to 25 per cent of the stomach content of *O. immunis* in ponds in New York. Budd *et al.* (1978) provided evidence that *O. immunis* is able to filter feed on phytoplankton using a filter apparatus formed by the first maxillipeds and the maxillae. The authors concluded that juvenile *O. immunis* might be obligatory filter feeders, whereas adult *O. immunis* might be facultative filter feeders. However, it is unclear whether *O. immunis* is able to catch relatively large and mobile zooplankton (*e.g.*, Cladocera) using this filter apparatus. During the present study, it was occasionally observed that *O. immunis* rapidly formed a ‘trap basket’, using the larger 2nd and 3rd maxillipeds and the subsequent pereopods, to catch smaller prey.

The relatively high importance of Cladocera in its diet, along with the positive selection of *Dreissena*, indicates that *O. immunis* is able to access the plankton pool as an energy resource, in addition to benthic macroinvertebrates, macrophytes and detritus. The relatively high importance of energy-rich macroinvertebrate prey in its diet probably supports the sustained high growth rate of *O. immunis* and underscores the importance of crayfish as functional predators, as suggested by Momot (1995) in his seminal paper.

Conclusion

An *r*-selected life history has often been linked to successful invaders, including many freshwater macroinvertebrates, such as *Dikerogammarus villosus* and *Corbicula fluminea* (Füreder and Pöckl 2007 and citations therein), although it is not an exclusively predictive or consistent trait among IAS (Bufford and Daehler 2011). Specifically, a fast life cycle and high reproductive output are beneficial throughout most stages of the invasion process, *i.e.*, initial introduction, establishment and spread. *Orconectes immunis* is no exception, in that it is a markedly *r*-selected species that has quickly become invasive. Moreover, its omnivorous feeding habits allow for a high flexibility in the allocation of nutrients needed for growth and reproduction. Both ecological properties, *i.e.*, the strongly *r*-selected life history and the omnivorous feeding habits, enhance its invasiveness and make *O. immunis* a perfect invader in the Upper Rhine plain. In contrast to other *r*-selected ‘new’ NICS in central Europe that originate from warmer, subtropical climates, such as *Procambarus clarkii* and Marmorkrebs (Souty-Grosset *et al.* 2006; Chucholl and Pfeiffer 2010; Chucholl 2011), *Orconectes immunis*

is perfectly adapted to cold habitats in temperate zones and will most likely continue to spread along the Rhine plain.

Within the invaded central European range, *Orconectes immunis* was observed to displace its invasive congener *O. limosus* from many habitats, probably as a result of niche overlap (Chucholl *et al.* 2008). *Orconectes immunis* has been found to be dominant in direct aggressive interactions and competition for shelter (Chucholl *et al.* 2008) and exhibits a faster life cycle and earlier hatching period than *O. limosus* (present study). Additionally, Chucholl (2006) found that the specific growth rate and relative moult increment of adult crayfish was significantly higher in *O. immunis* than in *O. limosus*, when held in the laboratory for 200 days. To date, *O. immunis* has not come into contact with ICS because ICS stocks have largely vanished from the upper Rhine plain during the last two centuries, as a result of habitat degradation, water pollution, crayfish plague and the invasion by *O. limosus* (Souty-Grosset *et al.* 2006; Chucholl and Dehus 2011). However, based on the observed displacement of *O. limosus*, the author anticipates that *O. immunis* has the potential to out-compete ICS. Additionally, *O. immunis* was recently shown to be a carrier of the crayfish plague (Maiwald *et al.* 2009). Any contact of *O. immunis* with ICS stocks will therefore most likely result in the loss of the latter. On the basis of its omnivorous feeding habits, extensive burrowing behaviour, and high abundance in many habitats, it is likely that *O. immunis* also has a pronounced ecological effect on indigenous biota and ecosystems. For instance, the decline of macrophyte species in LB coincided with the first observations of *O. immunis* in LB, suggesting that *O. immunis* might negatively affect macrophyte biomass, as was experimentally shown by Letson and Makarewicz (1994). However, explicit evidence for the ecological impact of *O. immunis* is currently lacking, and should be targeted in future research.

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References

- Alcorlo, P., Geiger, W. and Otero, M., 2004. Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. *Crustaceana*, 77, 435–453.
- Bovbjerg, R.V., 1970. Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology*, 51, 225–236.
- Brown, P. and Gunderson, J., (eds.) 1997. Culture potential of selected crayfishes in the North Central region. *Tech. Bull. Ser.*, 112, 26 p.
- Budd, T.W., Lewis, J.C. and Tracey, M.L., 1978. The filter-feeding apparatus in crayfish. *Can. J. Zool.*, 56, 695–707.
- Bufford, J.L. and Daehler, C.C., 2011. Life history strategies. In: Simberloff, D. and Rejmánek, M., (eds.) *Encyclopedia of Biological Invasions*. University of California Press, Los Angeles, 437–441.
- Buřič, M, Kouba, A. and Kozák, P., 2010. Intra-sex dimorphism in crayfish females. *Zoology*, 113, 301–307.
- Caldwell, M.J. and Bovbjerg, R.V., 1969. Natural history of the two crayfish of northwestern Iowa, *Orconectes virilis* and *Orconectes immunis*. *Iowa Aca. Sci.*, 76, 463–472.
- Chucholl, C., 2006. Konkurrenz zwischen zwei Neozoen: Verdrängungs-Mechanismen zwischen Kamberkrebs (*Orconectes limosus*, Crustacea: Decapoda) und Kalikokrebs (*O. immunis*). Diploma thesis, University of Ulm, Germany, 97 p.
- Chucholl, C., 2011. Population ecology of an alien 'warm water' crayfish (*Procambarus clarkii*) in a new cold habitat. *Knowl. Manag. Aquat. Ec.*, DOI: 10.1051/kmae/2011053.
- Chucholl, C. and Daudey, T., 2008. First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). *Aquat. Inv.*, 3, 105–107.
- Chucholl, C., Stich, H.B. and Maier, G., 2008. Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. *Fund. Appl. Limnol. / Arch. Hydrobiol.*, 172, 27–36.

- Chucholl, C. and Pfeiffer, M., 2010. First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquat. Inv.*, 5, 405–412.
- Chucholl, C. and Dehus, P., 2011. Flusskrebse in Baden-Württemberg. Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen, Germany, 92 p.
- Clavero, M. and García-Berthou, E., 2005. Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.*, 20, 110.
- Collas, M., Beinstainer, D., Fritsch, S. and Morelle, S., 2011. Première observation en France d'*Orconectes immunis* (Hagen, 1870) l'ecrevisse calicot. Office National de l'Eau et des Milieux Aquatiques, La Petite Pierre, France, 22 p.
- Dehus, P., Dussling, U., and Hoffmann, C., 1999. Notes on the occurrence of the calico crayfish (*Orconectes immunis*) in Germany. *Freshwater Crayfish*, 12, 786–790.
- Filipová, L., Grandjean, F., Chucholl, C., Soes, D.M. and Petrusek, A., 2011. Identification of exotic North American crayfish in Europe by DNA barcoding. *Knowl. Manag. Aquat. Ec.*, DOI: 10.1051/kmae/2011025.
- Forney, J.L., 1957. Raising bait fish and crayfish in New York ponds. *Cornell Extension Bull.*, 986, 3-30.
- France, R., Holmes, J. and Lynch, A., 1991. Use of Size-Frequency data to estimate the age composition of crayfish populations. *Can. J. Fish. Aquat. Sci.*, 48, 2324-32.
- Füreder, L. and Pöckl, M., 2007. Ecological traits of aquatic NIS invading Austrian fresh waters. In: Gherardi, F., (ed.) Biological invaders in inland waters: profiles, distribution and threats. Springer, Dordrecht, 233–257.
- Gayanilo, FC.Jr. and Pauly, D., 1997. The FAO ICLARM stock assessment tools. FiSAT reference manual. FAO Computerized Information Series (Fisheries), Rome, FAO.
- Gelmar, C., Pätzold, F., Grabow, K. and Martens, A., 2006. Der Kalikokrebs *Orconectes immunis* am nördlichen Oberrhein: ein neuer amerikanischer Flusskrebs breitet sich rasch in Mitteleuropa aus (Crustacea: Cambaridae). *Lauterbornia*, 56, 15–25.
- Gunderson, J.L. and Kapuscinski, A., 1992. Crayfish Aquaculture Demonstration in Minnesota Rice Paddies. Legislative Commission on Minnesota Resources Report, 4 p.

- Hamr, P., 2002. *Orconectes*. In: Holdich, D.M., (ed.) *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, 585-608.
- Hobbs, H.H. Jr., 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian C. Zool.*, 480, 236 p.
- Hobbs, H.H. Jr., Jass, J.P. and Huner, J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana*, 56, 299–316.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C. and Sibley, P.J., 2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Manag. Aquat. Ec.* 394–395, 11, DOI: 10.1051/kmae/2009025
- Jacobs, J., 1974. Quantitative measurement of food selection. *Oecologia*, 14, 413–417.
- Jansen, W., Geard, N., Mosindy, T., Olson, G. and Turner, M., 2009. Relative abundance and habitat association of three crayfish (*Orconectes virilis*, *O. rusticus*, and *O. immunis*) near an invasion front of *O. rusticus*, and long-term changes in their distribution in Lake of the Woods, Canada. *Aquat. Inv.*, 4, 627–649.
- Jones, J.P.G., Rasamy, J.R., Harvey, A., Toon, A., Oidtmann, B., Randrianarison, M.H., Raminosoa, N. and Ravoahangimalala, O.R., 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biol. Inv.*, 11, 1475–1482.
- Letson, M.A. and Makarewicz, J.C., 1994. An Experimental Test of the Crayfish (*Orconectes immunis*) as a Control Mechanism for Submersed Aquatic Macrophytes. *Lake Reserv. Manage.*, 10, 127–132.
- Lockwood, J.L., Hoopes, M.F. and Marchetti, M.P., 2007. *Invasion Ecology*. Blackwell Publishing, Oxford, 304 p.
- Maiwald, T., Vralstad, T., Jarausch, W., Schulz, H.K., Smietana, P. and Schulz, R., 2009. Kamberkrebs und Edelkrebs zwischen Koexistenz und Krebspest. Abstracts volume, 4. Internationales Flusskrebsforum, Gersfeld/ Röhn, Germany, 43–48.
- McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J. and Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity Distrib.*, 16, 95–108.
- McCullagh, P., and Nelder, J.A., 1989. *Generalized Linear Models*. Chapman and Hall, London, 532 p.

- Momot, W.T., 1995. Redefining the Role of Crayfish in Aquatic Ecosystems. *Rev. Fish. Sci.*, 3, 33–63.
- Neveu, A., 2006. Les écrevisses étrangères sont elles invasives? Quelles caractéristique expliquent leur développement? *L'Astacuculteur de France*, 86, 2–11.
- Nyström, P., 2002. Ecology. In: Holdich, D.M., (ed.) *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, 192-224.
- Olsen, T.M., Lodge, D.M., Capelli, G.M. and Houlihan, R.J., 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails and macrophytes. *Can. J. Fish. Aquat. Sci.*, 48, 1853–1861.
- Pauly, D., 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. *J. Cons. Ciém.*, 39, 175–192.
- Pauly, D. and Munro J.L., 1984. Once more on growth comparison in fish and invertebrates. *Fishbyte, Newsletter Network Trop. Fish. Sci.*, 2, 21.
- Pauly, D. and Morgan, G.R., 1987. *Length Based Methods in Fisheries Research*. ICLARM, Manila, Philippines, and KIRSI, Safat, Kuwait, 468 p.
- Pérez-Bote, J.L., 2005. Feeding Ecology of the exotic Red Swamp Crayfish, *Procambarus clarkii* (Girard, 1852) in the Guadiana River (SW Iberian Peninsula). *Crustaceana*, 77, 1375–1387.
- Rodríguez, C.F., Bécares, E., Fernández-Aláez, M. and Fernández-Aláez, C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biol. Inv.*, 7, 75–85.
- Scalici, M. and Gherardi, F., 2007. Structure and dynamics of an invasive population of the red swamp crayfish (*Procambarus clarkii*) in a Mediterranean wetland. *Hydrobiologia*, 583, 309–319.
- Scalici, M., Belluscio, A. and Gibertini, G., 2008. Understanding population structure and dynamics in threatened crayfish. *J. Zool.*, 275, 160–171.
- Scalici, M., Chiesa, S., Scuderi, S., Celauro, D. and Gibertini, G., 2009. Population structure and dynamics of *Procambarus clarkii* (Girard, 1852) in a Mediterranean brackish wetland (Central Italy). *Biol. Inv.*, 12, 1415–1425.

- Schlenker, M.A., 2009. Verhaltensweisen der invasiven Flusskrebsarten *Orconectes immunis* (Crustacea: Decapoda) und *O. limosus* bei Anwesenheit von Futter-, Artgenossen- und Räuberduftstoffen. Diploma thesis, University of Ulm, Germany, 31 p.
- Simberloff, D., 2003. How much information on population biology is needed to manage introduced species? *Conserv. Biol.*, 17, 83–92.
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D. and Haffner, P., (eds.) 2006. Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris (Patrimoines naturels, 64), 187 p.
- Stucki, T.P., 2002. Differences in life history of native and introduced crayfish species in Switzerland. *Freshwater Crayfish*, 13, 463-476.
- Tack, I.P., 1941. The life history and ecology of the crayfish *Cambarus immunis* Hagen. *Am. Midl. Nat.*, 25, 420–446.
- Wetzel, J.E., 2002. Form Alteration of Adult Female Crayfishes of the Genus *Orconectes* (Decapoda: Cambaridae). *Am. Midl. Nat.*, 147, 326–337.
- Windell, J.T., 1971. Food analysis and rate of digestion. In: Ricker, W.E. (ed.), *Methods for assessment of fish production in freshwaters*. Blackwell Scientific Press, Oxford, 197–203.
- Whitledge, G.W. and Rabeni, C.F., 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analyses. *Can. J. Fish. Aquat. Sci.*, 54, 2555–2563.

Table 1. *Orconectes immunis* population dynamics, surface water temperature, Secchi depth and conductivity in LB. *M* means males and *f* females. The asterisks indicate significant differences from the previous sampling occasion based on Mann-Whitney rank sum tests (*CL*) or Chi-square tests with Yates correction (other parameters), except for the sex ratio, for which the asterisks show significant differences from 1:1. N.t. = not tested due to sample size < 10. The number of asterisks indicates the *P*-level: * ≤ 0.05, ** ≤ 0.01, and *** ≤ 0.001.

Sample date	<i>N</i>	CPUE	Mean <i>CL</i> <i>m</i>	Mean <i>CL</i> <i>f</i>	Prop. <i>f</i>	Sex ratio balanced	Prop. <i>m</i> II	Prop. <i>f</i> II	Prop. active glair gl.	Prop. eggs	Prop. hatchlings	Prop. egg remains	T [°C]	Secchi depth [m]	Conductivity [μS/cm]
04.04.2007	158	10	37.4	38.6	0.17	no ***	0.03	0.0	0.04	0.12	0.19	0.31	13.6	0.7	416
10.04.2007	346	29	36.7	37.1	0.52 ***	yes	0.04	0.02	0.05	0.51 ***	0.08	0.30	17.9	0.6	402
24.04.2007	121	61	36.6	37.4	0.59	yes	0.90 ***	0.20 ***	0.01	0.0 ***	0.06	0.18	24.3	1.0	370
10.05.2007	133	67	37.2	39*	0.54	yes	0.36 ***	0.43 **	0.39 ***	0.02	0.0	0.02 **	19.0	0.5	379
08.06.2007	34	34	34.6	40 n.t.	0.26 n.t.	n.t.	0.24	0.11 n.t.	0.0 n.t.	0.0 n.t.	0.0 n.t.	0.0 n.t.	24.0	1.1	412
10.07.2007	72	36	36.7	37.9	0.39	yes	0.16	0.0	0.96	0.0	0.0	0.0	18.3	1.1	356
26.07.2007	139	70	32.5***	32.3***	0.42	yes	0.67 ***	0.50 ***	0.09 ***	0.0	0.0	0.0	21.5	1.3	405
01.08.2007	94	31	34.9***	36.7***	0.32	no *	0.23***	0.10 **	0.83 ***	0.0	0.0	0.0	21.6	1.1	401
03.04.2009	283	71	32.7***	34***	0.58 ***	yes	0.03	0.04	0.02	0.84 ***	0.07	0.01 ***	12.6	x	x

Table 4. Comparison of the life-history traits of ICS and selected NICS in western and central Europe. NA = not available.

Species	Status	Maximum <i>CL</i> [mm]	Longevity [a]	Age at maturity [a]	ϕ'	Maximum fecundity	References
<i>Astacus astacus</i>	ICS	88	7.9-13.0	2+ (3+)	0.6-1.3	282	Stucki 2002; Scalici <i>et al.</i> 2008; Souty-Grosset <i>et al.</i> 2006
<i>Austropotamobius (p.) pallipes</i>	ICS	55	7.7-14.3	2+ (1+)	0.4-1.1	200	Stucki 2002; Scalici <i>et al.</i> 2008; Souty-Grosset <i>et al.</i> 2006
<i>Austropotamobius torrentium</i>	ICS	50	10.7-12.0	2+ (1+)	0.8	120	Stucki 2002; Scalici <i>et al.</i> 2008; Souty-Grosset <i>et al.</i> 2006
<i>Pacifastacus leniusculus</i>	NICS	78	4-11	1+ (2+)	NA	242	Stucki 2002; Souty-Grosset <i>et al.</i> 2006; Füreder and Pöckl 2007
<i>Procambarus clarkii</i>	NICS	76	6.1-6.6	1+ (2+)	3.4-3.5	597	Stucki 2002; Chucholl 2011
<i>Orconectes limosus</i>	NICS	55	3-4	1+	NA	538	Hamr 2002; Stucki 2002; present study
<i>Orconectes immunis</i>	NICS	50	2.4-2.5	0+ (1+)	3.5	495	present study

Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*

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Abstract

Orconectes immunis, a crayfish species from North America, has been recorded first from habitats along the Rhine river (Germany) in the 1990th. Coincidental with the arrival of *O. immunis* resident populations of *O. limosus*, also a non-native crayfish species from North America, declined. We studied in laboratory experiments aggressive interactions and competition for shelter between the two invasive crayfish species as these interactions can result in species replacements. Agonistic behaviour was monitored in heterospecific 1 : 1 combinations of both species in 20-L aquaria. *Orconectes immunis* was strongly aggressively dominant over *O. limosus* when size-matched form I males or females were combined. Even 4 mm smaller *O. immunis* (carapace length) were still dominant over larger *O. limosus* and males of *O. limosus* were not dominant over similar-sized females of *O. immunis*. *Orconectes immunis* was also highly superior in competition for shelter. Shelter occupancy was approximately 6 times higher in *O. immunis* than in *O. limosus*. We conclude that one of the reasons for the observed decline of *O. limosus* coincidental with the arrival of *O. immunis* in some stretches of the Rhine catchment may be the inferiority of the former in aggressive contests. Inferiority in aggressive interactions may force *O. limosus* to leave refuges, making them vulnerable to predators.

Key words: non-indigenous crayfish, interspecific contests, shelter usage, species displacement

Introduction

The invasion of ecosystems by non-indigenous species (NIS) can pose a threat to the native fauna (Lodge *et al.* 2000; Sala *et al.* 2000). In particular native species that overlap in niche with invasives can be threatened or even replaced by the latter. Freshwater crayfish are of great ecological and economic importance. Many crayfish species are kept in aquaculture, used as live food, for bait fishing and, more recently, have become popular in the aquarium

trade (e.g., Lodge *et al.* 2000 and citations therein). Several crayfish species, predominantly from North America, escaped aquacultures or came into European waters by legal or illegal stocking and are now important components of native food webs (cf. Holdich 2002; Machino and Holdich 2006). Crayfish are polytrophic omnivores and prey for a wide array of predators. They often dominate the macro-invertebrate biomass and can have profound effects on freshwater littoral community structure. They are therefore considered keystone species (Momot 1995; Dorn and Wodjak 2004; Rodríguez *et al.* 2005). Ecological impact and thus, ecosystem functioning can differ significantly with crayfish species identity (cf. Rodríguez *et al.* 2006). Changes in the crayfish fauna should therefore be monitored carefully.

In order to predict further range expansions of invasive crayfish species, it is important to understand displacement mechanisms between them. North American species are vectors of the crayfish plague that has caused dramatic mortality in non-resistant European crayfish species (e.g., Dehus *et al.* 1999a; Evans and Edgerton 2002; Taylor 2002). Besides diseases, direct aggressive interactions between different crayfish species are regarded as an important mechanism for species replacement (Penn and Fitzpatrick 1963; Capelli and Munjal 1982; Tierney *et al.* 2000; Klocker and Strayer 2004). Inferiority in aggressive interactions may force a crayfish species to leave refuges making it vulnerable to predators (Garvey *et al.* 1994; Hill and Lodge 1994; Söderbäck 1994).

In this paper we tested the aggressive ability of two invasive crayfish species, *Orconectes limosus* (Rafinesque) and *O. immunis* (Hagen) in laboratory experiments. Both test species originate from North America. *Orconectes limosus* was introduced into German waters in the 1880s or 1890s (Pieplow 1938; Holdich 2002); since that time it has spread into almost all German river systems and many still water habitats, such as the Rhine, the Danube and Lake Constance (Schweng 1973; Nesemann 1987; Holdich 2002). Meanwhile, *O. limosus* has become one of the most common crayfish species in Central European waters. *Orconectes immunis* was first recorded in Germany in the 1990s in a small canal in the Rhine catchment near Baden-Baden; to our knowledge this was its first record in Europe (Dussling and Hoffmann 1998; Dehus *et al.* 1999b; Gelmar *et al.* 2006). Approximately 10 years later the species had spread in the upper Rhine region over a distance of 100 km (Gelmar *et al.* 2006). Coincidental with the arrival of *O. immunis*, a decline of resident *O. limosus* populations was observed. Several reaches along the Rhine formerly inhabited by *O. limosus* are now populated by *O. immunis* (Gelmar *et al.* 2006). Based on these observations, we expected *O. immunis* to be aggressively dominant over *O. limosus* and superior in the competition for shelter.

Material and methods

Morphological distinction of species, sampling sites and capture of test animals

Both species have similar habitat requirements (Tack 1941; Aiken 1965; Crocker 1979), attain approximately the same size (Brown and Gunderson 1997; Hamr 2002; Duriš *et al.* 2006), look similar but can clearly be distinguished by several morphological structures (Fig.

1). For example, hepatic spines are prominent in *O. limosus* but absent in *O. immunis*. Hair tufts on chelae (and on the second pereopod – not depicted in Fig. 1) are present in *O. immunis* but absent in *O. limosus*. The acumen is prominent in *O. limosus* and small in *O. immunis*. More details concerning differences between the two species are given in Gelmar *et al.* (2006).

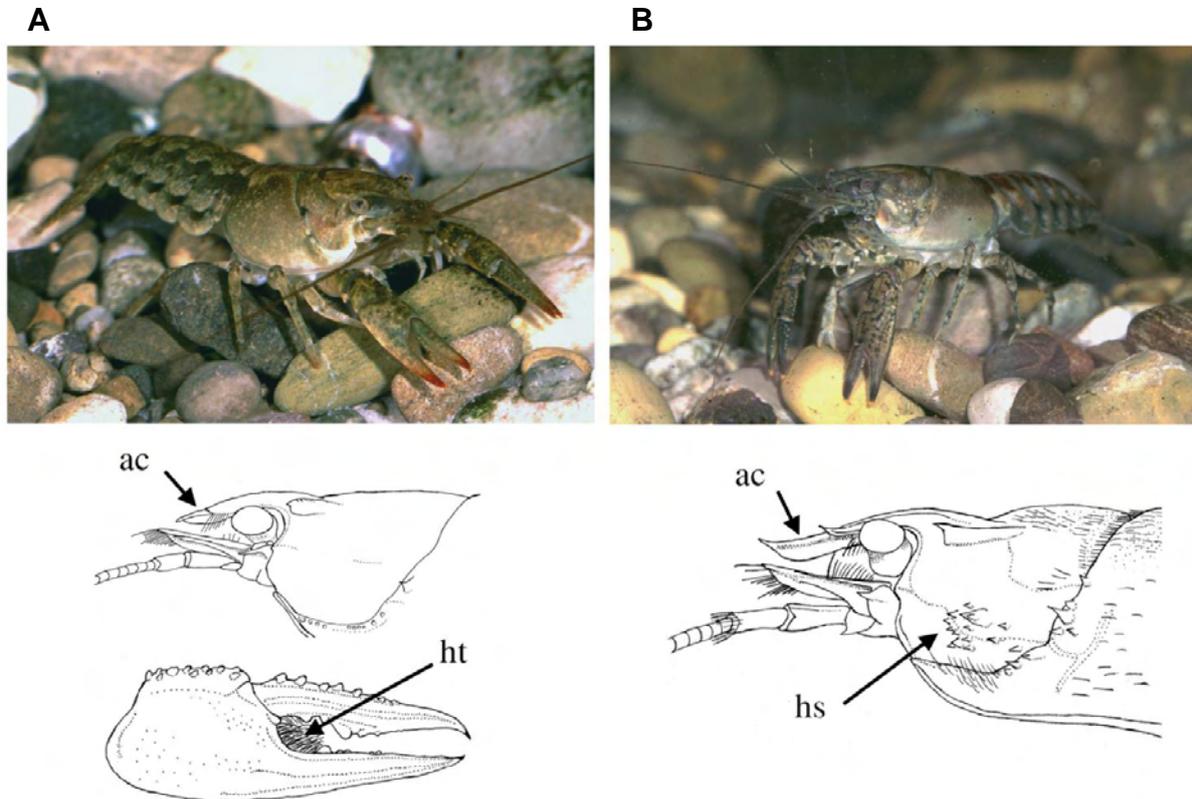


Figure 1. Photographs of *Orconectes immunis* (A) and *O. limosus* (B) and below line drawings with arrows showing some important morphological peculiarities of the two species: ac = acumen; hs = hepatic spines; ht = hair tufts.

Experimental animals were collected by hand or by a pond net at 3 sites. One site was a tributary of the river Rhine (48° 43.807' N, 8° 04.412' E to 48° 44.194' N, 8° 04.604' E), approximately 1 km apart from the locality where *O. immunis* was recorded first in Germany (*cf.* Dehus *et al.* 1999b). One site was in the Rhine itself and in an adjacent backwater (48° 57.377' N, 8° 11.970' E to 48° 57.455' N, 8° 12.000' E) and a third sample site was located in the river Kocher (49° 14.420' N, 9° 15.153' E to 49° 14.441' N, 9° 15.194' E). Conductivity at the sites ranged between 550 and 1260 $\mu\text{S cm}^{-1}$ and pH was 7.3–8.0. The substrate at the collection sites varied from larger stones and boulders (Rhine river) to sand and mud (Rhine tributary). Macrophytes (*Callitriche* sp. L., *Myriophyllum spicatum* L., *Elodea canadensis* Michx. and *Potamogeton pectinatus* L.) were only present in the Rhine tributary. Upon capture, animals were divided by species, sex and reproductive form (either form I or II; *cf.* Hamr 2002). Only animals with complete chelae and legs and a carapace length ≥ 25 mm were taken to the laboratory at the Institute of Lake Research, Lake Constance.

Chelae size

Dominance, *i.e.* the Resource Holding Potential (RHP), of crayfish can be governed by chelae size, favouring the individual with the bigger chelae (Gherardi 2002). To test whether *O. immunis* has bigger claws than *O. limosus* at a given carapace length, we measured the total length (from the tip of the rostrum to the end of the pleon), the carapace length (from the tip of the rostrum to the posterior end of the carapace) and the length of the chelae (propodus length) of all test animals with a slide calliper ($N = 125$). Additional measurements were taken from specimens captured in spring 2007 ($N = 121$).

Maintenance of test animals

Crayfish were marked individually with fingernail varnish (see Abrahamsson 1965 for marking system) and housed in single-species community tanks. All tanks were of the same size and contained similar crayfish densities (22–25 per tank) at an even sex ratio. All tanks were supplied with a 5 cm gravel layer, abundant clay pot halves, plastic tubes and large stones as shelters and with constantly flowing water from Lake Constance. Additionally, each aquarium was aerated and the water was filtered by self-constructed, air-driven sponge filters. During their maintenance (August 2005 until March 2006) crayfish experienced a water temperature of 19 to 8 °C. Natural light came from a nearby window and the photoperiod was between 13:10 h and 8:15 h L:D. Crayfish were fed 1–3 times per week with a mixture of carrots, peas and food pellets from the aquarium trade (most important components: 45 % raw protein, 6 % raw fat and 11 % raw ash, Tetra Company). Dried oak leaves were provided *ad libitum*. Mortality of crayfish was low under these conditions; only 3 specimens of 125 (2.4 %) disappeared during the maintenance time of 7 months.

Interspecific contests

The experimental setup was similar as in previous crayfish studies (*e.g.* Bovbjerg 1952; Söderbäck 1991; Vorburger and Ribí 1999; Maiwald *et al.* 2006) to produce largely comparable results. The experiments on aggressive interactions were carried out in 30 × 20 × 20 cm aquaria filled with aged tap water to a height of 15 cm. Conductivity and pH of the water were as at the collection sites. The aquaria were rounded with a plastic glass to avoid submissive animals being trapped in the corners, and their sides (except the front) were shaded with brown cardboard to reduce disturbances from outside. The remaining bottom area of the aquaria was 0.06 m². All aquaria were supplied with a 2 cm gravel layer. Light was provided by Osram coolwhite fluorescent lamps at low intensity since crayfish are night-active. Water was renewed after each experiment to avoid possible effects of semiochemicals on crayfish behaviour (*cf.* Acquistapace *et al.* 2003; Delgado-Morales *et al.* 2004; Moore and Bergmann 2005). No flow-through was established as both species often occur under still-water conditions. Pairs of crayfish were introduced 30 min. before the start of an experiment. During this acclimatization time, the test animals were separated by a removable glass divider. After removing the divider, *i.e.*, the start of an experiment, the behavioural interactions were recorded following the terminology of Bovbjerg (1952). Tension contacts were defined as head on head encounters between two crayfish individuals. Four behavioural tension contact types were distinguished: (1) Avoidance was noted when one individual

retreated without any offensive act of the other. (2) Threat was noted when one individual raised his chelae causing the retreat of the other. (3) Strike was noted when one individual retreated upon the struck of the other. (4) Fight was noted when one individual retreated after locking the chelae with the other. When more than one behavioural type (tension contact type) occurred during one interaction, only the tension contact type with the highest priority was noted. Priority was (from low to high): Avoidance, threat, strike and fight. A tension contact ended when the distance between crayfish was more than approximately 1.5 body lengths and the dominant individual did not chase the submissive one. The winner of a tension contact was the individual that did not retreat or change its initial moving direction (*cf.* Bovbjerg 1952). Crayfish were observed for 30 min. during which the tension contact types and the winners/losers of the tension contacts were noted. If less than 5 tension contacts occurred during the observation period, the experiment was prolonged until five contacts were observed, but no experiment lasted longer than 4 h. Experiments in which less than five tension contacts were recorded during 4 h were excluded from the analysis (Vorburger and Ribí 1999). Each particular crayfish pair was tested only once, with each crayfish being used at most three times. For individuals used more than once, time intervals between consecutive experiments were at least seven days so that previous fight experiences were unlikely to effect future fight behaviours (*cf.* Bergmann *et al.* 2003; Gherardi and Daniels 2004).

The following pairs were tested: Form I males of *O. limosus* vs. form I males of *O. immunis* (carapace length of 28–35 mm). Form I means that males were in the reproductive active condition. Females of *O. limosus* vs. females of *O. immunis* (carapace length 26–37 mm). Form I males of *O. limosus* vs. females of *O. immunis* (carapace length 27–40 mm). Form I males of *O. immunis* vs. females of *O. limosus* (carapace length 27–33 mm). All pairs were size matched, *i.e.* differences in carapace length were ≤ 1 mm. Chelae lengths did not differ more than 1 mm (females) or 4.5 mm (males and combinations with different sex; *cf.* Fig. 2). An additional experiment was carried out with unequal size combinations, *i.e.* in which *O. limosus* were larger than *O. immunis* (difference in carapace length 4 and 8 mm, respectively). This experiment was carried out as, based on the observations in the nature, we expected superiority in direct aggressive interactions of *O. immunis* in spite of size disadvantage. The number of replicate trials was 48, 44, 27, 12, 16 and 13, respectively. The uneven replicate numbers were due to different numbers of matching crayfish pairs available (*e.g.*, because of molts and the associated reproductive form alteration).

Shelter usage

In the experiments testing competition for shelter, we offered a single shelter (a plastic tube of 3.7 cm diameter and 11 cm length) to size-matched heterospecific pairs of either form I males or females of *O. limosus* and *O. immunis*. To test for independent shelter use (shelter affinity), 2 specimens of the same species and sex (carapace lengths of males and females were 27–37 and 28–35 mm in *O. limosus*, and 27–37 and 28–35 mm in *O. immunis*, respectively) were introduced into one aquarium containing 2 tubes and their shelter occupancy was recorded. The experimental design, *i.e.*, size and equipment of aquaria was as in the experiments for interspecific aggressive interactions. Test animals were introduced into the aquaria one day before the start of the experiment and allowed to acclimatize to experimental conditions for

13 h. The next day, shelter occupancy was recorded hourly for 9 h. A crayfish was regarded to be in a shelter when more than 3 quarters of its length was covered by the shelter tube (view angle from straight above); otherwise an individual was regarded to be outside of the shelter. In total 74 experiments were carried out (54 experiments for competition for shelter and 20 experiments for independent shelter use).

Statistics

Statistical analyses were performed with SigmaStat 9.0. Data were examined for normal distribution and homogeneity of variances. To test for differences between the chelae lengths at a given carapace length we performed linear regressions and compared them with an ANCOVA. Additionally, we tested for chelae size differences at a carapace length of 32 mm using the regression equations (modified T-Test, for details see Zar 1996). This was done because testing for differences in regression equations is not sufficient to exclude overlapping chelae sizes (*e.g.*, near the intersection of two regression lines). A carapace length of 32 mm was chosen because it is a frequent size class in both *O. limosus* and *O. immunis* populations in mid summer (unpublished data) and matches within the carapace lengths of the test animals. We used the number of tension contacts won by each crayfish per trial and the observations in shelter as response variables for the statistical analyses of the direct aggressive interaction trials and shelter usage trials, respectively. Possible differences between species were assessed by employing Wilcoxon Signed Rank Tests or Mann-Whitney Rank Sum Tests (only shelter affinity). The frequency of tension contact types was compared with Kruskal-Wallis ANOVAs within treatments and with Mann-Whitney Rank Sum Tests between different treatments. ANOVAs were followed by Dunn's post hoc test for pairwise comparison. To test whether shelter affinity can explain shelter use in competition experiments, we ran a contingency table. The expectation values were derived from the shelter affinity experiments.

Results

Chelae size

Orconectes immunis males have larger chelae than male *O. limosus*, while interspecific differences in females are less pronounced and not statistically significant at smaller carapace lengths. Chelae lengths were positively correlated with carapace lengths (Fig. 2, Table 1). At a given carapace length males have bigger chelae than females in both *O. immunis* and *O. limosus* (Table 1; ANCOVA: $P < 0.001$). In males of *O. immunis* chelae size augment more rapidly than in male *O. limosus* (Fig. 2a). Differences between regression equations were significant (ANCOVA: $P < 0.001$). At a carapace length of 32 mm, *O. immunis* males have significantly larger chelae than *O. limosus* males (T-Test: $P < 0.001$). Regression equations also differed between females of *O. immunis* and *O. limosus* (Table 1; ANCOVA: $P = 0.001$), with chelae size increasing faster in *O. immunis*. However, chelae size did not differ significantly between *O. immunis* and *O. limosus* females at a carapace length of 32 mm (T-Test: $P = 0.551$).

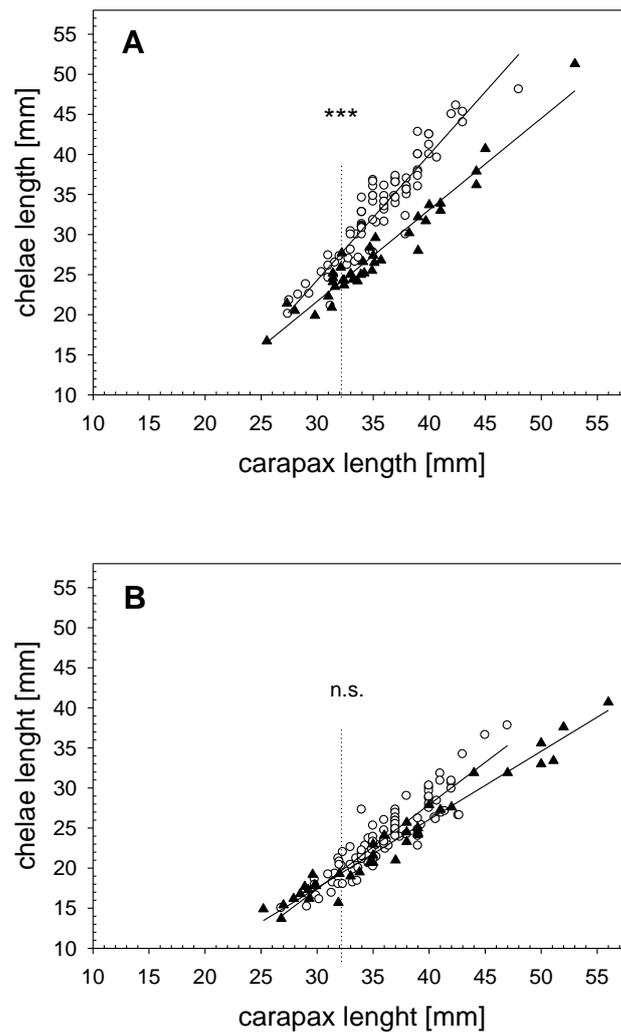


Figure 2. Relation between carapace length and chelae length in form I males (A) and females (B) of *Orconectes limosus* (triangles) and *O. immunis* (circles). See Table 1 for *N*, *R*, *P* values and regression equations. Asterisks indicate significant differences ($P < 0.001$) in chelae sizes at a carapace length of 32 mm (dotted line) given by modified T-Test (cf. Zar 1996); n.s. means not significant.

Table 1. Linear regressions between carapace-length (*CL*) and chelae-length (*PL*) in males and females of *Orconectes limosus* and *O. immunis*, respectively. All measurements were taken from form I specimens (sexual active condition).

Species	Sex (form I)	<i>N</i>	<i>R</i>	<i>P</i>	Regression equation
<i>O. immunis</i>	males	78	0.934	< 0.001	$PL = -21.8 + (1.5 \times CL)$ (1)
<i>O. immunis</i>	females	92	0.913	< 0.001	$PL = -14.2 + (1.1 \times CL)$ (2)
<i>O. limosus</i>	males	39	0.969	< 0.001	$PL = -12.6 + (1.1 \times CL)$ (3)
<i>O. limosus</i>	females	37	0.982	< 0.001	$PL = -8.0 + (0.9 \times CL)$ (4)

Interspecific aggression

In sex-matched combinations, *O. immunis* won more interactions than *O. limosus* and smaller *O. immunis* dominated even over 4 mm larger *O. limosus*. Form I males of *O. immunis* were highly dominant over size-matched form I males of *O. limosus* (Wilcoxon: $W = 737.0$, $N = 48$, $P < 0.001$; Fig. 3). Females of *O. immunis* were also aggressively dominant over females of *O. limosus* (Wilcoxon: $W = 919.0$, $N = 44$, $P < 0.001$; Fig. 3). Frequency of tension contact types differed significantly, both, in males and females (Kruskal Wallis ANOVA: $H_{(3/192)} = 32.8$, $P < 0.05$ and $H_{(3/172)} = 104.8$, $P < 0.05$). Strike and avoidance were the most important tension contact types between heterospecific form I males (Fig. 4A) and avoidance was the most frequently observed behavioural interaction between heterospecific females (Fig 4B). Males performed threats, strikes and fights more often than females (Mann-Whitney: $T = 1557$, $N = 44$; $P < 0.001$, $T = 1340$, $N = 44$, $P < 0.001$ and $T = 1652$, $N = 44$, $P < 0.001$, respectively) whereas females experienced more frequently avoidance behaviour than males (Mann-Whitney: $T = 2801$, $N = 44$, $P < 0.001$). Four mm smaller (carapace length) *O. immunis* specimens were still strongly dominant over larger *O. limosus* (Wilcoxon: $W = -109.0$, $N = 16$, $P = 0.003$; Fig. 5). Only when size difference between species was 8 mm, *i.e.* when *O. immunis* were 8 mm smaller in carapace length than *O. limosus*, *O. limosus* dominated over *O. immunis* (Wilcoxon: $W = 67.0$, $N = 13$, $P = 0.005$).

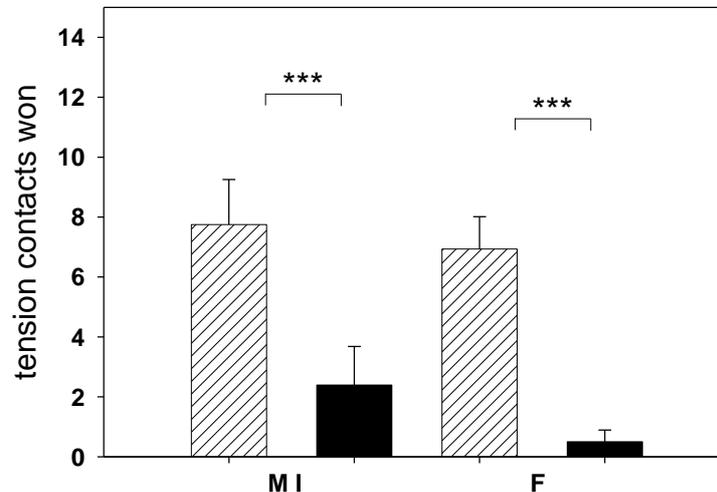


Figure 3. Mean number of tension contacts ($\pm 95\%$ CL) won by *Orconectes immunis* (hatched bars) and *O. limosus* (black bars), respectively, in size-matched combinations of form I males (M I) and females (F). Asterisks indicate P level (***) = $P < 0.001$ given by Wilcoxon Test.

In combinations of different sexes *O. immunis* males won more interactions than *O. limosus* females while *O. limosus* males were not superior to *O. immunis* females. Interactions between the latter combatants involved more intense tension contact types. Form I males of *O. limosus* were not dominant over size-matched females of *O. immunis* (Wilcoxon: $W = -3.0$, $N = 27$, $P = 0.931$; Fig. 6A). However, form I males of *O. immunis* were strongly dominant over size-matched females of *O. limosus* (Wilcoxon: $W = -78.0$, $N = 12$, $P < 0.001$; Fig. 6B). In encounters between *O. limosus* form I males and size-matched *O. immunis* females fight behaviour was less frequent than strike, threat and avoidance behaviour (Kruskal Wallis ANOVA: $H_{(3/108)} = 38.6$, $P < 0.05$; Fig. 7A). In encounters between form I males of *O. immunis* and size-matched females of *O. limosus* avoidance behaviour occurred more often than strikes or fights (Kruskal Wallis ANOVA: $H_{(3/44)} = 19.5$, $P < 0.05$; Fig. 7B). Interactions between *O. limosus* males and size-matched *O. immunis* females comprised more strikes and less avoidance behaviour than interactions between *O. immunis* males and size-matched *O. limosus* females (Mann-Whitney: $T = 147$, $N = 12$, $P = 0.005$ and $T = 326$, $N = 12$, $P = 0.009$, respectively).

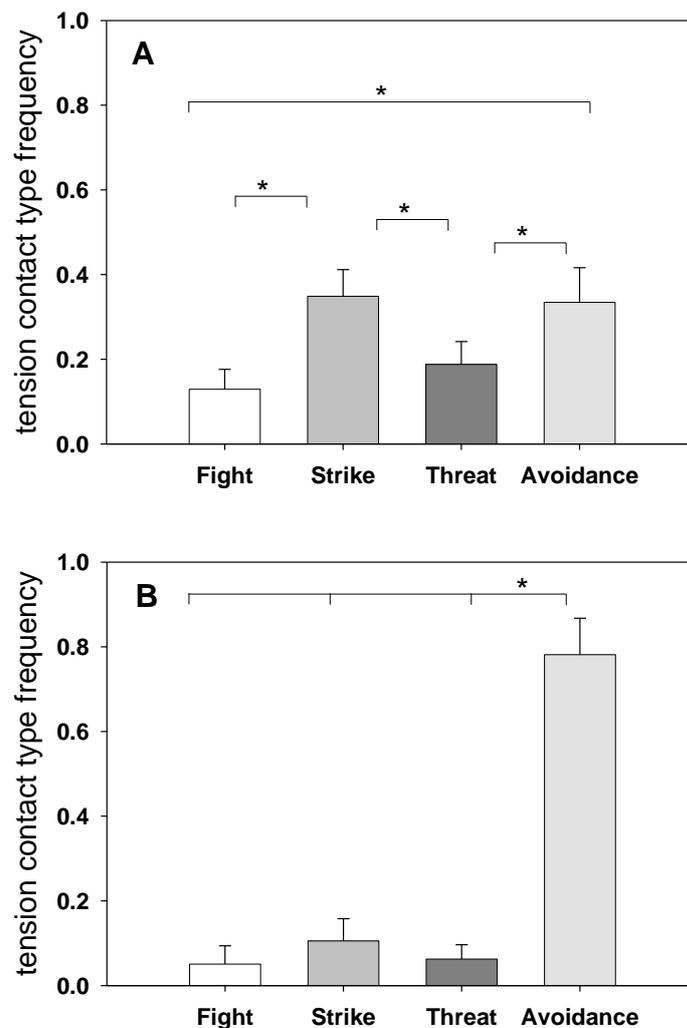


Figure 4. Mean frequency of tension contact types ($\pm 95\%$ CL) in combinations between size-matched form I males (A) and females (B) with results of Dunn's post hoc test showing differences in frequency between types. * means $P < 0.05$.

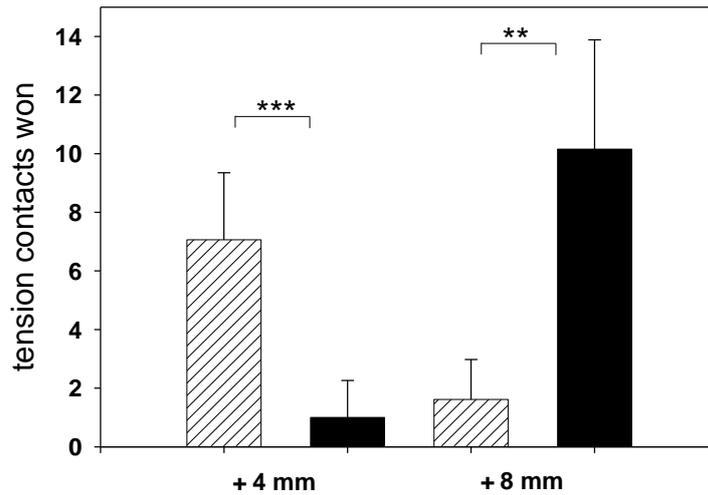


Figure 5. Mean number of tension contacts (\pm 95 % CL) won by *Orconectes immunis* (hatched bars) and *O. limosus* (black bars) in unequal size but sex-matched combinations. Carapace lengths of *O. immunis* was 4 and 8 mm smaller than carapace length of its opponent, respectively. *P* levels are given by Wilcoxon Test. ** means $P < 0.01$, *** means $P < 0.001$.

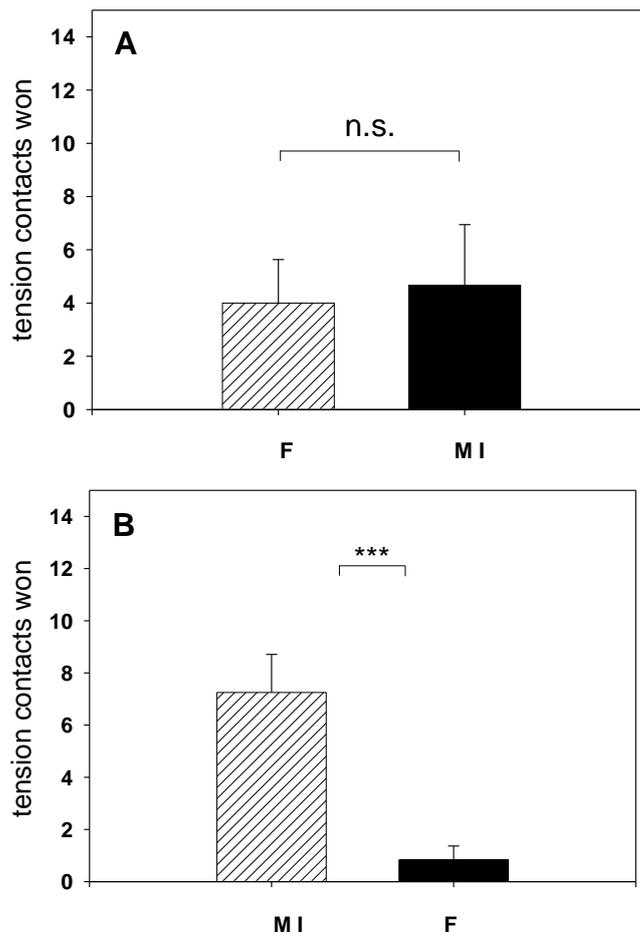


Figure 6. Mean number of tension contacts (\pm 95 % CL) won by (A) form I males (MI) of *O. limosus* (black bar) against size-matched females (F) of *Orconectes immunis* (hatched bar) and (B) form I males of *O. immunis* (hatched) against size-matched females of *O. limosus* (black), respectively; n.s. means not significant; *** means $P < 0.001$.

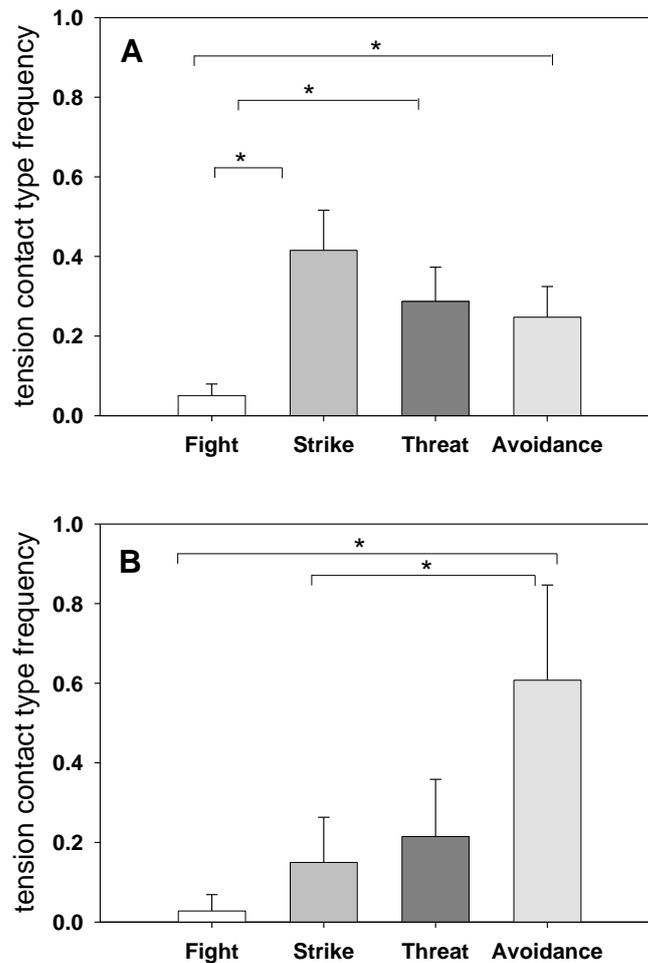


Figure 7. Mean frequency of tension contact types (\pm 95 % *CL*) in combinations of form I males of *Orconectes limosus* and size-matched *O. immunis* females (A) and form I males of *O. immunis* and size-matched *O. limosus* females (B) with results of Dunn's post hoc test showing differences in frequency between types. * means $P < 0.05$.

Competition for shelter

In the experiments in which shelter affinity was tested, *O. immunis* used shelters more often than *O. limosus* (Mann-Whitney: $T = 488$, $N = 20$, $P < 0.05$; Fig. 8A). Under conditions of competition, *O. immunis* clearly dominated *O. limosus* (Fig. 8B). *Orconectes immunis* were more often observed in shelters than *O. limosus* (Wilcoxon: $W = -1137.0$, $N = 54$, $P < 0.001$). The differences in shelter usage in the competition trails cannot be explained solely by the unequal shelter affinity of the two congeners (expectations were 60:40 in favour of *O. immunis*). The chi-square statistics produced a highly significant P -value ($P < 0.001$), thus we rejected the null hypothesis.

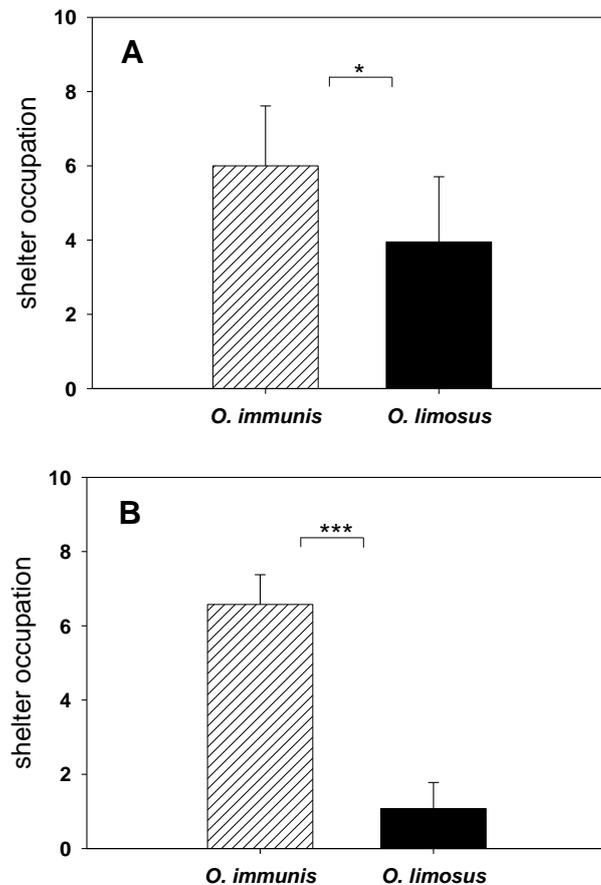


Figure 8. Mean shelter occupancy (\pm 95 % CL) in shelter affinity experiments (A) and shelter competition experiments (B) with P levels given by Mann-Whitney's Test and Wilcoxon Test, respectively. * means $P < 0.05$ and *** means $P < 0.001$. Hatched bars represent *O. immunis*, black bars *O. limosus*.

Discussion

The distribution of numerous crayfish species has been expanded by human activities (Hobbs *et al.* 1989; Machino and Holdich 2006). In Central Europe 3 to 5 species of crayfish (dependant on the author) are native and these species face competition with 10 introduced species predominantly from the American continent (*e.g.*, Albrecht 1982; Gherardi *et al.* 1999; Grandjean *et al.* 2002; Machino and Holdich 2006). Different crayfish species are known to fight each other when resources are limited. Crayfish perform aggressive interactions to gain access to food, shelter or mates. Aggressive interactions between combinations of crayfish species have been studied intensively and superiority in interspecific contests and competition for shelter are believed to be major reasons for crayfish replacements (Penn and Fitzpatrick 1963; Bovbjerg 1970; Guiasu *et al.* 1996; Vorburger and Ribí 1999). An aggressive crayfish species is generally able to expand its range at the expense of a less aggressive because the former is able to acquire more resources (Söderback 1991; Guiasu *et al.* 1996).

Our results show that *O. immunis* is clearly dominant over *O. limosus* in laboratory experiments. Males and females of *O. immunis* dominated over similar-sized males and

females of *O. limosus* and, even more remarkable was, that 4 mm smaller *O. immunis* were dominant over larger *O. limosus*. The latter fact is exceptional because dominance (i.e. Resource Holding Potential) is usually strongly size dependent, favouring the larger individual, given that ‘weapons’ (i.e. chelae) are of the same relative size (Gherardi 2002; Nakata and Goshima 2003). In male *O. immunis* chelae size augments more rapidly with increasing carapace length than in male *O. limosus*, resulting in a chelae size advantage of *O. immunis* males over carapace-length matched *O. limosus* males (cf. Fig. 2a). While this chelae size asymmetry may explain dominance patterns in combinations of males, chelae sizes overlap largely in females (cf. Fig. 2b). Given that, we assume that *O. immunis* is inherently more aggressive than *O. limosus*.

Another unexpected finding was that males of *O. limosus* - in spite of having bigger ‘weapons’ (i.e. chelae; Fig. 2) - were not dominant over similar-sized females of *O. immunis*. This was unexpected because males are generally dominant over similar-sized females (Gherardi 2002 and citations therein), which is also supported by the outcome of the combination *O. immunis* males vs. *O. limosus* females. The dominance of 4 mm smaller *O. immunis* over larger *O. limosus* and the inability of *O. limosus* males to dominate size-matched females of *O. immunis* (in spite of chelae size advantage), emphasize the higher competitive ability of *O. immunis*.

The fact that interactions between males were more intensive than between females with more fights, strikes and threats and less avoidance behaviour seems to be a commonplace in crayfish and was observed in several species (Bovbjerg 1952; Gherardi 2002). It is likely that the large proportion of avoidance behaviour in bouts between *O. limosus* females and size-matched *O. immunis* males and females, respectively, was caused by the weak competitive ability of *O. limosus* females (cf. Fig. 4b and Fig. 7b). *Orconectes limosus* females showed a tendency to avoid intense interactions and, unlike *O. immunis* females, often preferred to retreat from an approaching crayfish, even if the approaching individual did not display any agonistic behaviour.

In a comparable study on direct aggressive interactions between *Pacifastacus leniusculus* and *Austroptamobius torrentium*, Vorburger and Ribi (1999) reported a high frequency of fight behaviour. For instance, in size matched pairings of *P. leniusculus* and *A. torrentium*, fights occurred at a frequency of 31 %, whereas fight behaviour accounted only for 13 and 5 % of the interactions observed in our study (in size matched pairs of males and females, respectively). Tierney *et al.* (2000) showed *P. leniusculus* to be intraspecific more aggressive than several Orconectids (including *O. immunis*). Similar high frequencies of fight behaviour occurred in bouts between *Astacus astacus* and *O. limosus* (Maiwald *et al.* 2006). These studies, along with the low frequency of fights found in our study, might indicate that Astacidae (*Astacus*, *Pacifastacus*, *Austroptamobius*) perform intense interactions more readily than Orconectids. The fact that Noble Crayfish were strongly dominant over *O. limosus* and acquired shelters more often (Maiwald *et al.* 2006), stresses the weak competitive ability of *O. limosus* which is also reflected in the present study. The fact that *O. limosus* rapidly invaded large parts of Central European waters despite its weak performance in direct aggressive interactions gives rise to the hypothesis that *O. limosus* invaded vacant niches, i.e. native crayfish were wiped out by crayfish plague outbreaks (cf. Alderman 1997).

Shelter is one of the most important resources for crayfish (Hobbs 1991; Nakata and Goshima 2003; Davis and Huber 2007). Hiding in shelters reduces predation risk (Hill and Lodge 1994; Garvey *et al.* 1994). Söderbäck (1994), for example, showed that shelter usage increased in crayfish in the presence of predatory fish. *Orconectes immunis* and *O. limosus* are both known to burrow in river banks and along the shoreline of lakes and ponds (Hamr 2002). While *O. limosus* burrows are usually simple in construction and fairly short, *O. immunis* have a propensity to construct deep and complex burrows, sometimes with multiple openings. *Orconectes immunis* is also known to construct occasionally ‘crayfish chimneys’, similar to those of the primary burrowers of the genera *Cambarus* and *Procambarus* (Tack 1941; Hamr 2002). Overall, *O. immunis* seems to be more associated to burrows (*i.e.* shelters) than its relative which is in accordance with the results of our experiments on shelter affinity. Maiwald *et al.* (2006) also reported low shelter usage in *O. limosus* in comparison with *A. astacus*. The fact that differences in shelter affinity could not explain differences in shelter use in competition experiments highlights the higher competitive ability of *O. immunis* when shelters are limited and supports the results of the interspecific aggression experiments. That *O. immunis* excluded *O. limosus* from shelters may result in higher mortality of the latter due to predation. The habit to burrow deep holes into suitable substrates may protect *O. immunis* also from summer droughts and freezing during winter months, especially in shallow or temporary backwaters (Tack 1941; Gelmar *et al.* 2006).

We are aware that we worked under artificial laboratory conditions which differ from conditions in the field. However, aggressive encounters between crayfish species observed in the laboratory are similar to fight dynamics in nature (Bergmann and Moore 2003; Davis and Huber 2007) and results in aggressive dominance obtained in laboratory experiments are generally consistent with replacement patterns observed in the field (Penn and Fitzpatrick 1963; Bovbjerg 1970; Vorburger and Ribí 1999). The latter is supported by our study because *O. immunis* seems to supplant *O. limosus* where the ranges of both species overlap.

Apart from direct aggressive interactions one might consider other possible displacement mechanisms such as hybridisation (Perry *et al.* 2001), mating interference (Butler and Stein 1985), differences in life cycle (Hamr 2002) and different susceptibility to predation (Söderbeck 1994; Hazlett *et al.* 2003) or diseases (*e.g.* crayfish plague; Dehus *et al.* 1999a). Due to the different sculpture of form I gonopods of male *O. limosus* and male *O. immunis*, successful hybridisation appears to be unlikely. This suggestion is supported by the fact that no hybrids were found in mixed populations in the Rhine River. Mating interference may occur in the field, since copulations between *O. limosus* form I males and both *O. immunis* females and smaller *O. immunis* males were observed in the laboratory. However, it is unclear whether one species would benefit from mating interference since their fecundity is similar (*cf.* Butler and Stein 1985). On the other hand, we feel that a more rapid growth (Pieplow 1938; Tack 1941; Hamr 2002) and an earlier seasonal reproduction (unpublished data) may presumably support the success of *O. immunis*.

Finally, we stress that the further expansion of *O. immunis* should be carefully followed. We expect that *O. immunis* is a carrier of the crayfish plague which in addition to its aggressive behaviour can pose a threat to native species. Furthermore, nothing is known about the ecological impact of *O. immunis* on littoral communities in Europe.

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References

- Abrahamsson, S.A.A., 1965: A method of marking crayfish *Astacus astacus* Linné in population studies. *Oikos* 16: 228-231.
- Acquistapace, P., Hazlett, B.A., Gherardi, F., 2003: Unsuccessful predation and learning of predation cues by crayfish. *Journal of Crustacean Biology* 23: 364-370.
- Aiken, D.A., 1965: Distribution and ecology of three species of crayfish from New Hampshire. *The American Midland Naturalist* 73: 240-245.
- Albrecht, H., 1982: Das System der Europäischen Flusskrebse (Decapoda: Astacidae): Vorschlag und Begründung. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* 79: 187-210.
- Alderman, D.J., 1997: History of the spread of crayfish plague in Europe. Abbreviated from: Alderman, D.J., 1997: Crustaceans: Bacterial and Fungal Diseases, OIE Scientific and Technical Review 15.
- Bergmann, D. A., Moore, P. A., 2003: Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. *Biological Bulletin* 205: 26-35.
- Bergmann, D.A., Kozłowski, C.P., McIntyre, J.C., Huber, R., Daws, A.G., Moore, P.A., 2003: Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. *Behaviour* 140: 805-825.
- Bovbjerg, R.V., 1952: Dominance order in the crayfish *Orconectes virilis* (Hagen). *Physiological Zoology* 29: 127-136.
- Bovbjerg, R.V., 1970: Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* 51: 225-236.

- Brown, P., Gunderson, J., 1997: Culture potential of selected crayfishes in the North Central Region. *Technical Bulletin Series* 112: 1-26.
- Butler, M.J., Stein, R.A. 1985: An analysis of the mechanisms governing species replacements in crayfish. *Oecologia* 66: 168-177.
- Capelli, G.M., Munjal, B., 1982: Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. *Journal of Crustacean Biology* 2: 486-492.
- Crocker, D.W., 1979: The crayfishes of New England. *Proceedings of the Biological Society of Washington* 92: 225-252.
- Davis, K.M., Huber, R., 2007: Activity patterns, behavioural repertoires, and agonistic interactions of crayfish: A non-manipulative field study. *Behaviour* 144: 229-247.
- Dehus, P., Bohl, E., Oidtmann, B., Keller, M., Lechleiter, S., Phillipson, S., 1999a: Case studies of alien crayfish in Europe. German conservation strategies for native crayfish species with regard to alien species. In: Gherardi, F., Holdich, D.M. (eds): *Crayfish in Europe as alien species. How to make the best of a bad situation?* AA Balkema, Rotterdam, pp 149-159.
- Dehus, P., Dussling, U., Hoffmann, C., 1999b: Notes on the occurrence of the calico crayfish (*Orconectes immunis*) in Germany. *Freshwater Crayfish* 12: 786-790.
- Delgado-Morales, G., Hernández-Falcón, J., Ramón, F., 2004: Agonistic behaviour in crayfish: the importance of sensory inputs. *Crustaceana* 77: 1-24.
- Dorn, N.J., Wodjak, J.M., 2004: The role of omnivorous crayfish in littoral communities. *Oecologia* 140: 150-159.
- Duriš, Z., Drozd, P., Horká, I., Kozák, P., Polícar, T. 2006: Biometry and demography of the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Français de la Pêche et Pisciculture* 380-381: 1215-1228.
- Dussling, U., Hoffmann, C. 1998: First discovery of a population of *Orconectes immunis* in Germany. *IAA Newsletter* 20: 5.
- Evans, L.H., Edgerton, B.F., 2002: Pathogens, Parasites and Commensals. In: Holdich, D. M. (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford pp 377-438.
- Garvey, J.E., Stein, R.A., Thomas, H.M., 1994: Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. *Ecology* 75: 532-547.

- Gelmar, C., Pätzold, F., Grabow, K., Martens, A., 2006: Der Kalikokrebs *Orconectes immunis* am nördlichen Oberrhein: Ein neuer amerikanischer Flusskrebs breitet sich schnell in Mitteleuropa aus (Crustacea: Cambaridae). *Lauterbornia* 56: 15-25.
- Gherardi, F. 2002: Behaviour. In: Holdich, D. M. (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, pp 258-281.
- Gherardi, F., Baldacchini, G. N., Ercolini, P., Barbaresi, S., de Luise, G., Mazzoni, D., Mori, M., 1999: Alien crayfish: the situation of Italy. *Crustacean Issues* 11: 107- 128.
- Gherardi, F., Daniels, W. H., 2004: Agonism and shelter competition between invasive and indigenous crayfish species. *Canadian Journal of Zoology* 82: 1923-1932.
- Guiasu, R.C., Dunham, D.W., Barr, D.W., 1996: Interspecific agonistic contests between male *Cambarus bartonii bartonii* (Fabricius 1798) and *Cambarus robustus* Girard 1852 (Decapoda, Cambaridae) crayfish and the possible competition between the two species in Ontario. *Freshwater Crayfish* 11: 364-377.
- Grandjean, F., Frelon-Raimond, M., Souty-Grosset, C., 2002: Compilation of molecular data for the phylogeny of the genus *Austropotamobius*: one species or several? *Bulletin Français de la Pêche et Pisciculture* 367: 671-680.
- Hamr, P., 2002: *Orconectes*. In: Holdich, D.M. (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, pp 541-574.
- Hazlett, B.A., Burba, A., Gherardi, F., Acquistapace, P., 2003: Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biological Invasions* 5: 223-228.
- Hill, A.M., Lodge, D.M., 1994: Diel changes in resource demand: Competition and predation in species replacement among crayfish. *Ecology* 75: 2118-2126.
- Hobbs, H.H., Jass, J.P., Huner, J.V., 1989: A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56: 299-316.
- Hobbs, H.H. 1991: Adaptions and convergence in North American crayfishes. *Freshwater Crayfish* 2: 541-549.
- Holdich, D.M., 2002: Distribution of Crayfish in Europe and some adjoining countries. *Bulletin Français de la Pêche et Pisciculture* 367: 611-650.

- Klocker, K., Strayer, D. L., 2004: Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeridae and Unionidae). *Northeastern Naturalist* 11: 167-178.
- Lodge, D.M., Taylor, C.A., Holdich, D.M., Skurdal, J., 2000: Nonindigenous crayfishes threaten North American freshwater biodiversity. *Fisheries* 25: 7-19.
- Machino, Y.L., Holdich, D.M., 2006: Distribution of crayfish in Europe and adjacent countries: updates and comments. *Freshwater Crayfish* 15: 292-323.
- Maiwald, T., Schulz, H.K., Śmietana, P., Schulz, R., 2006: Aggressive interactions and interspecific competition between the indigenous crayfish *Astacus astacus* (Linne) and the non-indigenous *Orconectes limosus* (Rafinesque). *Freshwater Crayfish* 15: 203-211.
- Momot, W.T., 1995: Redefining the Role of Crayfish in Aquatic Ecosystems. *Reviews in Fisheries Science* 3: 33-63.
- Moore, P.A., Bergmann, D.A., 2005: The smell of success and failure: The role of intrinsic and extrinsic chemical signals on the social behaviour of crayfish. *Integrative and Comparative Biology* 45: 650-657.
- Nakata, K., Goshima, S., 2003: Competition for shelter of preferred sizes between the native crayfish species *Cambaroides japonicus* and the alien crayfish species *Pacifastacus leniusculus* in Japan in relation to prior residence, sex difference and body size. *Journal of Crustacean Biology* 23: 897–907.
- Nesemann, H., 1987: Erste Bestände des Amerikanischen Flusskrebsses *Orconectes limosus* in der Donau. *Senckenbergiana Biologica* 67: 397-399.
- Penn, G.H., Fitzpatrick, J.F. Jr., 1963: Interspecific competition between two sympatric species of dwarf crawfishes. *Ecology* 44: 793-797.
- Perry, W.L., Feder, J.L., Dwyer, G., Lodge, D. A., 2001: Hybrid zone dynamics and species replacement between *Orconectes* crayfishes in a northern Wisconsin Lake. *Evolution* 55: 1152-1166.
- Pieplow, U., 1938: Fischereiwissenschaftliche Monographie von *Cambarus affinis* Say. *Zeitschrift für Fischerei* 36: 349-440.
- Rodríguez, C.F., Bécares, E., Fernández-Aláez, M., Fernández-Aláez, C., 2005: Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions* 7: 75-85.

- Rodríguez, C.F., Bécares, E., Fuertes, B., 2006: Comparison of the impact of the freshwater decapod species *Austropotamobius pallipes* (indigenous) and *Procambarus clarkii* (non-indigenous), on the submerged vegetation of two mediterranean wetlands. *Freshwater Crayfish* 15: 166-175.
- Sala, O.E., Chapin, III F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L., Jackson, R.B., Kinzig, A., Leemanns, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, B.H., Walker, B.H., Walker, M., Wall, D.H., 2000: Biodiversity scenario for the year 2100. *Science* 287: 1770-1774.
- Schweng, E., 1973: *Orconectes limosus* in Deutschland, insbesondere im Rheingebiet. *Freshwater Crayfish* 8: 79-87.
- Söderbäck, B., 1991: Interspecific dominance relationship and aggressive interactions in the freshwater crayfishes *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). *Canadian Journal of Zoology* 69: 1321-1325.
- Söderbäck, B., 1994: Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* 100: 229-235.
- Statzner, B., Fièvet, E., Campagne, J.Y., Morel, R., Herouin, E., 2000: Crayfish as geomorphic agents and ecosystem engineers: Biological behaviour affects sand and gravel erosion in experimental streams. *Limnology and Oceanography* 45: 1030-1040.
- Tack, I. P., 1941: The life history and ecology of the crayfish *Cambarus immunis* HAGEN. *American Midland Naturalist* 25:420 – 446.
- Taylor, C.A., 2002: Taxonomy and conservation of native crayfish stocks. In: Holdich, D.M. (ed.): *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford pp 236-254.
- Tierney, A.J., Godleski, M.S., Massanari, J.R., 2000: Comparative analysis of antagonistic behaviour in four crayfish species. *Journal of Crustacean Biology* 20: 54-66.
- Vorburger, C., Ribi, G., 1999: Aggression and competition for shelter between a native and an introduced crayfish in Europe. *Freshwater Biology* 42: 111-119.
- Zar, J.H., 1996: *Biostatistical Analysis*. Prentice Hall, New Jersey, 662 pp.

Feeding ecology and ecological impact of an alien “warm water” omnivore in temperate lakes

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Abstract

The present study attempted to investigate the feeding ecology and ecological impact of *Procambarus clarkii*, the world's worst invasive crayfish and a recent invader in temperate lakes, by linking stomach-content analysis with an *in situ* enclosure experiment in temperate lakes in southern Germany. A stomach-content analysis showed that *P. clarkii* is a polytrophic omnivore that feeds on macrophytes, detritus and macroinvertebrates. The trophic diversity of its diet was highest in mid-summer and in smaller crayfish. Chironomidae larvae and *Dreissena polymorpha* were the most preferred prey, whereas sediment-dwelling taxa were rarely consumed. The number of consumed small and agile prey negatively correlated with the crayfish size, suggesting an ontogenetic shift in diet. A five-week enclosure experiment was used to determine the impact of *P. clarkii* on the basal levels of a typical littoral food web of temperate lakes at different crayfish densities (0, 2.5, and 5 crayfish m⁻²). The abundance of aquatic snails sharply decreased with increasing crayfish density and conditioned leaf breakdown was up to five times higher in the presence of crayfish than in the control treatment without crayfish. Crayfish also had a negative effect on macrophyte biomass, resulting from both consumption and uprooting. However, the impact mechanisms and outcomes differed among macrophyte species. In the crayfish treatments, the final biomass of the indigenous *Myriophyllum spicatum* and *Chara* sp. was significantly reduced relative to the initially stocked biomass, whereas the alien *Elodea nuttallii* was able to gain biomass. This finding is consistent with an invasional meltdown scenario, in that *P. clarkii* indirectly facilitated a dominance of *E. nuttallii*. Overall, the results concordantly suggest that *P. clarkii* is a keystone species that can profoundly alter recipient communities *via* direct trophic links and non-consumptive destruction, and may indirectly facilitate other invasive alien species.

Key words: non-indigenous crayfish, benthic omnivore, keystone species

Introduction

Invasive alien species (IAS) are one of the leading threats to biodiversity worldwide, apart from habitat destruction and climate change (Clavero and García-Berthou 2005; McGeoch *et al.* 2010). IAS impact all levels of biodiversity and can adversely affect ecosystem integrity, making the control and eradication of IAS one of the top priorities of environmental management in the present century. This fact holds especially true for freshwater ecosystems, which have been invaded by numerous IAS (Bernauer and Jansen 2006; Gherardi 2007) and which were estimated to be more prone to the effects of IAS than terrestrial ecosystems (Sala *et al.* 2000).

Freshwater crayfish have been frequently recognized as key members of littoral food webs (reviewed by Momot 1995; Nyström 1999, 2002). Crayfish are the largest mobile freshwater invertebrates, are long-lived, alter their habitats and act as polytrophic omnivores. Non-indigenous crayfish species (NICS) can therefore cause ecological havoc and eliminate plant or animal species from ecosystems through grazing, predation, competition and indirect cascading effects through food webs (Momot 1995; Nyström 2002; Rodríguez *et al.* 2005).

Procambarus clarkii (Girard, 1852) is the world's most successful NICS. This species exhibits considerable ecological plasticity and is one of the most important freshwater decapods farmed for food consumption (Huner 2002). For that reason, *P. clarkii* has been introduced into at least 25 countries beyond its natural range, which comprises the central southern region of the USA and northeastern Mexico, and is now established on every continent except Australia and Antarctica (Gherardi *et al.* 1999; Gherardi 2006, and citations therein). In Europe, the species was first introduced in 1973, when Spain intentionally imported the species for commercial production. The commercial success of *P. clarkii* in Spain led to a series of illegal introductions into France and Italy, and today, *P. clarkii* is widespread and abundant throughout southwestern Europe and northern and central Italy (Souty-Grosset *et al.* 2006). In addition, there are an increasing number of *P. clarkii* records from higher latitudes and altitudes, including Belgium, Swiss, the Netherlands, Austria and Germany in central Europe (Dümpelmann *et al.* 2009; Soes and Koese 2010; Chucholl 2011a, and citations therein). These recent records emphasize that *P. clarkii* is also able to thrive in colder climates, despite being often considered as a 'warm-water' species (Henttonen and Huner 1999; Dümpelmann *et al.* 2009; Chucholl 2011a).

The economic benefit in Spain notwithstanding, the majority of *P. clarkii* introductions have had negative consequences (Hobbs *et al.* 1989), and in Europe the species is listed among the "100 of the worst" IAS (DAISIE 2010). *Procambarus clarkii* is a known carrier of the oomycete *Aphanomyces astaci*, the causative agent of the crayfish plague, which inflicts devastating mass mortalities among the susceptible imperiled European crayfish (Souty-Grosset *et al.* 2006). Moreover, *P. clarkii* has become a pest in agricultural areas, such as rice fields, because it damages young rice plants, dams and irrigation systems (reviewed by Gherardi 2006). The feeding ecology and impact on indigenous biota of *P. clarkii* has been investigated in its introduced Iberian and Mediterranean range, and *P. clarkii* was found to have a more drastic ecological impact than the indigenous European crayfish (Rodríguez *et al.* 2006). *Procambarus clarkii* is an omnivorous generalist that may reduce the biodiversity of

the invaded community in a short time (Alcorlo *et al.* 2005; Pérez-Bote 2005; Gherardi and Acquistapace 2007). For instance, the invasion of a shallow lake in Spain by *P. clarkii* led to a switch from a clear water state to a turbid one within a few years, followed by a collapse of trophic chains dependent on macrophytes. As a consequence, the biodiversity was severely reduced, including 71 % losses of macroinvertebrate genera, 83 % losses of amphibian species and 75 % losses of duck species (Rodríguez *et al.* 2005). Similarly, *P. clarkii* may have driven indigenous amphibians to local extinction on the Iberian Peninsula (Cruz *et al.* 2006; Rodríguez *et al.* 2006).

Notwithstanding its documented effects on invaded communities on the Iberian peninsula and in Mediterranean habitats, the ecological role and impact of *P. clarkii* in colder climates received surprising little scientific attention. Recent studies revealed that the life history and population ecology of *P. clarkii* in colder climates is different from its life history at lower latitudes or in its indigenous range (Frutiger *et al.* 1999; Chucholl 2011a). Specifically, *P. clarkii* grows slower at high latitudes than at lower latitudes, whereas longevity and size markedly increase with latitude. Furthermore, the reproduction of *P. clarkii* at colder climates tends to be univoltine, which contrasts with its multivoltine life cycle at lower latitudes (Chucholl 2011a). Yet, very little is known regarding how *P. clarkii* impacts temperate communities, and because omnivorous crayfish do not fit neatly into trophic-chain models, it is difficult to predict their effects on new recipient communities. This lack of knowledge is unfortunate because the understanding and forecasting of the ecological consequences of *P. clarkii* are crucial to justify and prioritize NICS population management and eradication efforts. The fact that *P. clarkii* frequently occurs in nature reserves in Germany, and might cause significant ecological harm there, emphasizes the need for an understanding of its ecological role and impact on temperate lake communities (Dümpelmann *et al.* 2009; Chucholl and Dehus 2011).

The present study was designed to fill this gap of knowledge by linking two complementary approaches to analyze the ecological role and impact of *P. clarkii* in temperate lakes in southern Germany. First, the feeding ecology and prey electivity of *P. clarkii* was investigated using stomach-content analyses, and second, the impact of *P. clarkii* on its food resources and key components of a typical littoral community of temperate lakes was assessed at different population densities, using an *in situ* enclosure experiment. The results of both approaches were then used to discuss the ecological role and impact of *P. clarkii* in temperate lakes.

Methods

Study sites

The study was carried out in two small mesotrophic gravel pit lakes in southern Germany, which is within the northeastern range limit of *P. clarkii* in Europe. The lakes are part of an artificial lake system along the River Danube and were invaded by *P. clarkii* in the late 1990s or early 2000s (Chucholl 2011b). The feeding ecology of *P. clarkii* was assessed in Lake Riedheim (LR; 9.3 ha; 450 m above sea level; 48°27'32.4''N, 92 10°10'32.3''E) in June and

September, 2007, and the enclosure experiment was carried out in Lake Burlafingen (*LB*; 5.1 ha; 456 m above sea level; 48°25'28.7"N, 10°04'27.4"E), starting in mid-August, 2008, and lasting for five weeks. *LR* featured a dense *P. clarkii* population, low turbidity and easy accessibility from shore, which facilitated captures of active crayfish for stomach content analysis, whereas *LB* offered homogeneous shallow zones necessary to set up the enclosures. A more-detailed description of the study area was reported by Chucholl (2011b).

Trophic ecology

To assess the diet of *P. clarkii*, the stomach contents of 102 crayfish captured in June and September, 2007, were analyzed. Active crayfish were captured using hand nets from the western littoral zone of *LR* (maximum depth 1.5 m). Because crayfish are nocturnal, the sampling began one hour after sunset, which coincided with a phase of great activity. Crayfish about to molt and freshly molted individuals were not taken, because crayfish do not forage shortly before and after ecdysis (Reynolds 2002). Upon capture, the crayfish were immediately put on crushed ice to halt digestion and later transported to the laboratory. To assess potential ontogenetic shifts in the diet of *P. clarkii*, the captured crayfish were grouped into three size classes with a balanced sex ratio: carapace length (*CL*) < 50 mm (class I), *CL* of 50-58 mm (class II), and *CL* > 58 mm (class III).

In the laboratory, the crayfish were dissected and the stomach was carefully removed and placed into 70 % ethanol. The stomach content was then washed into a Petri-dish and qualitatively and quantitatively analyzed under a dissecting microscope. The invertebrates were identified as far as was feasible by characteristic hard parts (*e.g.*, head capsule, legs, abdomen or shell fragments), and the number of consumed individuals was counted. The volume proportion of the main food categories (detritus, macroinvertebrates, *Chara* sp., and *Elodea nuttallii*) was estimated on an ordinal scale with six ranks. To reduce the biases produced by a single measure of the stomach content, two response variables were used to calculate a relative importance index (*RI*) for each prey item and food category (Windell 1971; Pérez-Bote 2005):

$$RI_i = \frac{(AI_i 100)}{\sum_{i=1}^n AI_i},$$

where $AI_i = OcP_i AbP_i$ or $AI_i = OcF_i VolF_i$, for prey items or food categories, respectively, and *n* is the number of prey items or food categories. OcP_i is the percentage of the stomachs containing prey *i*, AbP_i is the percentage of the total number of organisms represented by prey *i*, OcF_i is the percentage of the stomachs containing food category *i*, and $VolF_i$ is the estimated volume proportion of food category *i* (modified from Pérez-Bote, 2005). The diet similarity among the crayfish size classes was calculated using the percentage similarity index (*PSI*; Matthews *et al.* 1988):

$$PSI = 1 - 0.5 \sum |RI_{Xi} - RI_{Yi}|,$$

where RI_{Xi} is the *RI* of prey item *i* of size class X, and RI_{Yi} is the *RI* of size class Y. The *PSI* ranges from 0 to 1, with 1 denoting complete similarity and 0 indicating no similarity (Chang *et al.* 1999).

The trophic diversity of the diet of *P. clarkii* was assessed for each size class using the diversity index (D) of Herrera (1976):

$$D = -\sum_{i=1}^n \log(OcP_i),$$

which attains values between 0 and $n \log(N)$, where N is the sample size. High values of D indicate either that individual diets are very different or that the sampled population is a generalist (cf. Alcorlo *et al.* 2004).

Prey electivity

To assess the prey electivity of *P. clarkii*, benthic macroinvertebrates in the western littoral zone of LR were sampled quantitatively with a Birge-Ekman grab (15 x 15 cm opening) in June and September 2007. In each sampling month, twelve samples were taken at depths of 0.1, 0.7 and 1.4 m along four transects perpendicular to the shoreline. The two outermost transects bordered the littoral area that was searched for active crayfish to assess the diet of *P. clarkii*, and the other two transects were situated within this area. The samples were carefully searched for macroinvertebrates at the laboratory and the sediment fraction was rinsed two times through a 600- μm sieve to retrieve sediment-dwelling taxa. The collected macroinvertebrates were identified as far as was feasible and counted. The prey electivity of *P. clarkii* was then calculated using the Jacobs (1974) index (Alcorlo *et al.* 2004):

$$P_i = \frac{RI_i r_i}{r_i + RI_i - 2r_i RI_i},$$

where r_i is the relative abundance of prey item i in the benthic macroinvertebrate samples. Values of P between 0 and 1 indicate positive prey selection, whereas values between 0 and -1 indicate negative selection.

Enclosure experiment

To evaluate the impact of *P. clarkii* on its food resources and key components of a typical littoral community of temperate lakes, a five-week *in situ* enclosure experiment was carried out in LB in the late summer of 2008. The experimental design included three treatments, with each treatment replicated five times. The treatments differed in the stocked crayfish density: control treatment (*CT*) – no crayfish, low-density treatment (*LT*) – two crayfish and high-density treatment (*HT*) – four crayfish. The treatments with crayfish corresponded to a crayfish density of 2.5 (*LT*) and 5 (*HT*) individuals m^{-2} (equal to $91 \pm 12 \text{ g m}^{-2}$ and $182 \pm 25 \text{ g m}^{-2}$), which is comparable to the crayfish density observed in *LR*.

The fifteen enclosures were made of 0.9 x 0.9 x 0.9 m aluminum frames with a solid bottom plate and a folding lid on the top face. The sidewalls and lid were tightly covered with grey window-screening mesh made of PVC, with a mesh width of 1.4 mm. The enclosures were set up randomly in four blocks along a 50 m transect, parallel to the shore line, in a homogenous, shallow zone of *LB*. Each block consisted of three (one block) or four (three blocks) enclosures with an intermediate spacing of 1 m. The distance from shore was approximately 30 m, and there was no shading from trees. The enclosures were installed at depths of 0.80 to 0.87 m, to allow the stocked aquatic pulmonate gastropods to access the water surface for breathing.

Each enclosure was provided with 30 l of sediment and 10 l of coarse gravel (particle size 3.5 to 7.0 cm), as substrate for *Dreissena polymorpha*. The sediment consisted of fine sand, which was taken from the littoral zone of LR. The sand had been previously sampled for macroinvertebrates following the same scheme as described above, and it was found to contain only very few macroinvertebrates (mostly Oligochaeta). The gravel was taken from a nearby gravel pit and placed in the southeastern corner of each enclosure. In addition, each enclosure was provided with 5 g of conditioned *Salix* sp. leaves, 20 g of common oak leaves (*Quercus robur*) and 20 g of Norway maple leaves (*Acer platanoides*), as a source of detritus. The leaves had been collected in March, air dried and then exposed in LR in a gauze bag (mesh width 1 mm) at a depth of 2 m for six weeks, to allow colonization by microorganisms prior to the enclosure experiment (cf. Suren and Lake, 1989; Nyström, 2002). Each enclosure was also supplied with four pipes, made of dark-grey PVC, as shelter for the crayfish.

Each enclosure was then stocked with the three macrophyte species that were most commonly found in the study lakes: 60.0 g (± 0.6) of *Elodea nuttallii*, 60.3 g (± 0.8) of *Myriophyllum spicatum* and 300.0 g (± 2.1) of *Chara* sp.. The stocked biomass of the macrophyte species did not differ among the treatments (*E. nuttallii*: one-way ANOVA, $F_{2,12} = 0.731$, $P > 0.05$; *M. spicatum* and *Chara*: Kruskal-Wallis one-way ANOVA on ranks, $H_2 = 1.516$ and $H_2 = 4.534$, $P > 0.05$, respectively) nor did the number of *E. nuttallii* shoots per enclosure (18.7 ± 3.6 ; one-way ANOVA, $F_{2,12} = 0.805$, $P > 0.05$). The macrophytes had been previously collected from nearby gravel pit lakes and carefully washed several times with tap water in the laboratory to remove adherent macroinvertebrates. All of the macrophytes were stocked into the enclosures following the same scheme (based on a top view from the southern direction): *Elodea nuttallii* was placed in the upper left quarter, *Chara* in the upper right quarter, and *M. spicatum* in the lower right quarter. The *M. spicatum* and *E. nuttallii* shoots were carefully anchored in the sediment with long forceps, whereas *Chara* was loosely put on the sediment. The stocking with macrophytes was accomplished on August 4th and 5th, and one week later, it was checked that all of the macrophytes were firmly anchored in the sediment.

Three snail species (*Radix ovata*, *Lymnaea stagnalis* and *Planorbis corneus*) and *D. polymorpha* were added as macroinvertebrates to each enclosure. The snails were collected from a pond at the university campus, and *D. polymorpha* was taken from a nearby gravel pit lake. Each enclosure was stocked with four *L. stagnalis* (mean fresh weight of fifteen individuals: 3.5 g), four *Pl. corneus* (mean fresh weight of fifteen individuals: 1.6 g) and 49 *R. ovata* (mean fresh weight of fifteen individuals: 0.5 g), which corresponded to the relative abundance of these species observed in the pond. *Dreissena polymorpha* were grouped into three size classes and added to the enclosures according to the size distribution of the donor population, with 50 individuals with 7-14 mm shell length, 15 individuals with 15-21 mm shell length and one individual larger than 21 mm shell length per enclosure. Each macroinvertebrate species was added to all of the enclosures within one day, and on August 12th, the stocking was completed.

The crayfish for the crayfish treatments had been captured from LR three days before the start of the experiment and were held in solitary units at the laboratory. Only intact form I males with a *CL* of 50.8 to 57.3 mm were used (mean *CL* 53.8 mm ± 1.8). The crayfish were

larger than the specimens used by Gherardi and Acquistapace (2007) in their *in situ* enclosure experiment in a Mediterranean lake, to account for the larger size of *P. clarkii* at colder climates (Chucholl 2011a). There were no significant differences in *CL* or weight between the crayfish used for the low-density and high-density treatments (t-test, $P > 0.05$). To be able to compare the crayfish size and weight before and after the experiment, all of the crayfish were marked individually per enclosure by making a small cut in the distal margin of one uropod. On August 18th, the crayfish were added to the enclosures, which marked the start of the experiment.

Monitoring during the experiment

During the five-week experiment, the enclosure sidewalls and lids were carefully cleaned at least once per week with a brush to prevent clogging and biofouling of the mesh. On these occasions, it was also checked that all of the crayfish were alive and whether any individuals had undergone ecdysis. On September 24th, one crayfish in an enclosure of the *HT* was found dead after a molt and was replaced by a new form I male captured from *LR*.

Uprooting and consumption of macrophytes

Fragments of *E. nuttallii* and *M. spicatum* floating in the enclosures were collected once a week and weighed at the laboratory to distinguish between the macrophyte loss resulting from consumption by crayfish and the loss resulting from clipping of basal plant parts or uprooting by crayfish (*cf.* Lodge *et al.* 1994). The clipping of basal plant parts and uprooting by crayfish are together referred to as “uprooting” in this paper because both mechanisms result in large proportions of shoots floating on the water surface. The macrophyte fragments larger than 0.1 m (*E. nuttallii*) or 0.2 m (*M. spicatum*) were attached to small sinkers and re-anchored in their corresponding enclosures within the same day. The consumption of macrophytes (*C*) was calculated using the following equation:

$$C = \left(WT_0 \frac{WC_F}{WC_0} \right) - WT_F,$$

where *WT* is the macrophyte biomass in the crayfish treatments before (WT_0) and after (WT_F) the experiment, and *WC* is the mean biomass in the control treatment before (WC_0) and after (WC_F) the experiment (Cronin 1998). Prior to the calculation, WT_F was corrected by adding the weight of the floating plant fragments that were not re-anchored in the enclosures due to their small size.

Sampling at the end of the experiment

The experiment was ceased on September 29th, and the enclosures were destructively sampled. The crayfish were captured, and the PVC pipes that provided shelter were removed and carefully searched for attached *D. polymorpha* or snails. The remaining snails, *D. polymorpha*, macrophytes, and conditioned leaves were contemporaneously collected, and each species was packed into separate plastic bags, which were stored in the cool lake water until transportation to the laboratory. The plastic bags used for the macrophytes were opaque black to prevent photosynthesis after sampling.

All of the samples were processed in the laboratory at the evening of the same day. The macrophytes and conditioned leaves were weighed after being thoroughly searched for attached macroinvertebrates. The snails and *D. polymorpha* were counted per enclosure. The crayfish were measured (*CL*) and weighed. Upon the completion of the measurements, all samples were frozen and stored at -18°C for further studies.

Statistical analyses

All statistical analyses were performed using SigmaPlot 10.0 (with SigmaStat 3.5 Integration), except for the two- and three-way ANOVAs (see below), which were run in the software package R 2.14.0 (R Development Core Team 2011). Apart from the above-cited analyses, the *RI* values of the prey items and food categories were compared among the crayfish size classes and between the two sample months using two-proportion *z*-tests with Yates correction, to assess seasonal or ontogenetic shifts in the diet of *P. clarkii*. In addition, Pearson correlations were used to test for significant relationships between the number of consumed prey individuals and the *CL*. Differences in the *RI* of the prey items and food categories within each crayfish size class were also assessed using two-proportion *z*-tests with Yates correction, followed by a Bonferroni adjustment of the *P* level. Chi-squared tests were used to test for significant differences between the *RI* of prey items and the r_i .

The fresh weight of the conditioned leaves and macrophytes and the macroinvertebrate abundance (number of individuals per enclosure) at the end of the experiment were used as response variables of the enclosure experiment (*cf.* Matthews *et al.* 1992). The weight of the macrophytes was corrected for the loss through uprooting by subtracting the uprooted biomass from the final weight. The differences in the response variables between the treatments were assessed using three-way ANOVAs, in which the relative position of the enclosures within blocks (orientation) and the block number were entered as co-factors to detect potential side effects resulting from the spatial arrangement of the enclosures. The effects of treatment and macrophyte species on macrophyte consumption and uprooting were assessed using two-way ANOVAs. Furthermore, paired *t*-tests were used to compare the macrophyte and crayfish weights before and after the experiment. Prior to the statistical testing for differences, all variables were tested for normality (using a Kolmogorov-Smirnov test with Lilliefors' correction), and non-parametric tests were used where appropriate. Standard deviations are given after all mean values in the text and tables.

Results

Stomach-content analysis

All size classes of *Procambarus clarkii* were found to feed on detritus and macrophytes (*Chara* sp. and *E. nuttallii*) as well as a variety of macroinvertebrates (Figure 1A-D). In June, crayfish with a *CL* ≥ 50 mm fed predominately on detritus, followed by macroinvertebrates, *E. nuttallii* and *Chara* sp., whereas crayfish with a *CL* < 50 mm mostly consumed macroinvertebrates, followed by *E. nuttallii*, detritus and *Chara* sp. (Figure 1C). In September, crayfish with a *CL* ≤ 58 mm mostly fed on detritus and *E. nuttallii*, followed by macroinvertebrates and *Chara* sp., whereas crayfish with a *CL* > 58 mm showed the same

dietary preferences as in June (Figure 1D). However, the *RI* of the prey items and food categories did not differ significantly among the size classes or sample months, or within the size classes (based on two-proportion z-tests with a Yates correction, followed by a Bonferroni adjustment of the *P* level).

The macroinvertebrate prey ranged from aquatic insect larvae to crustacean plankton and terrestrial insect imagos, but mostly consisted of Chironomidae and Ephemeroptera larvae and *D. polymorpha* (Figure 1A, B). Ostracoda and Cladocera were also important prey in June and September, respectively. Crayfish remnants were only found in crayfish stomachs in September and typically in larger crayfish (Figure 1B). Several interdependencies between the crayfish size class and prey *RI* could be observed (*cf.* Figure 1A, B). In June, there was a significant negative relationship between the crayfish size (*CL*) and the number of consumed Chironomidae larvae, Ephemeroptera larvae and Ostracoda (Pearson correlation: $N = 54$, $P = 0.002$, $P = 0.003$ and $P = 0.03$ for Chironomidae, Ephemeroptera, and Ostracoda, respectively; *cf.* Figure 1A). A significant negative correlation between the number of consumed Ephemeroptera larvae and *CL* was also found in September (Pearson correlation: $N = 49$, $P = 0.02$), whereas the number of consumed Cladocera and *D. polymorpha* tended to increase with *CL* (Pearson correlation: $N = 49$, $P = 0.06$ and $P = 0.09$ for Cladocera and *D. polymorpha*, respectively; *cf.* Figure 2B).

The trophic diversity of the crayfish diet (*D*) was generally higher in June than in September, and crayfish with a *CL* < 50 mm consistently had a more diverse diet than larger crayfish in both of the sampled months (Table 1). In June, crayfish with a *CL* of 50-58 mm attained the lowest *D* value, but this was not the case in September, when the *D* gradually declined with increasing crayfish size (Table 1). The high *D* value of the largest crayfish size class (III) in June resulted mostly from the rather high *RI* of Coleoptera, other Diptera and other insects in the diet of this size class (*cf.* Figure 1A). These prey items were primarily terrestrial insects, such as Hymenoptera and Scolytinae. The diet similarity (*PSI*) was generally higher between the smallest (I) and medium crayfish size class (II) than between the medium (II) and largest (III) or the smallest (I) and largest size class (III; Table 1).

Table 1. Trophic diversity (*D*) and similarity (*PSI*) of the diet of *P. clarkii* in LR per sample month and size class. The *PSI* indicates the diet similarity between consecutive size classes (# denotes the similarity between size class I and III). *N* is the sample size of the crayfish used for the stomach-content analysis. Asterisks indicate a significant difference in the *CL* compared to the same size class of the previous sample month (Mann-Whitney rank sum test: $U = 324.0$, $P = 0.02$).

	Crayfish size class	Mean CL [mm]	Sample month	<i>N</i>	<i>D</i>	<i>PSI</i>
I:	< 50 mm <i>CL</i>	40.6 (± 5.8)	Jun 07	20	13.3	0.85
II:	50-58 mm <i>CL</i>	54.4 (± 3.1)	Jun 07	16	10.4	0.65
III:	> 58 mm <i>CL</i>	64.0 (± 1.9)	Jun 07	17	12.1	0.68#
I:	< 50 mm <i>CL</i>	44.9 (± 2.5) *	Sep 07	20	11.6	0.76
II:	50-58 mm <i>CL</i>	54.7 (± 2.0)	Sep 07	20	6.7	0.67
III:	> 58 mm <i>CL</i>	61.2 (± 2.1)	Sep 07	9	6.3	0.47#

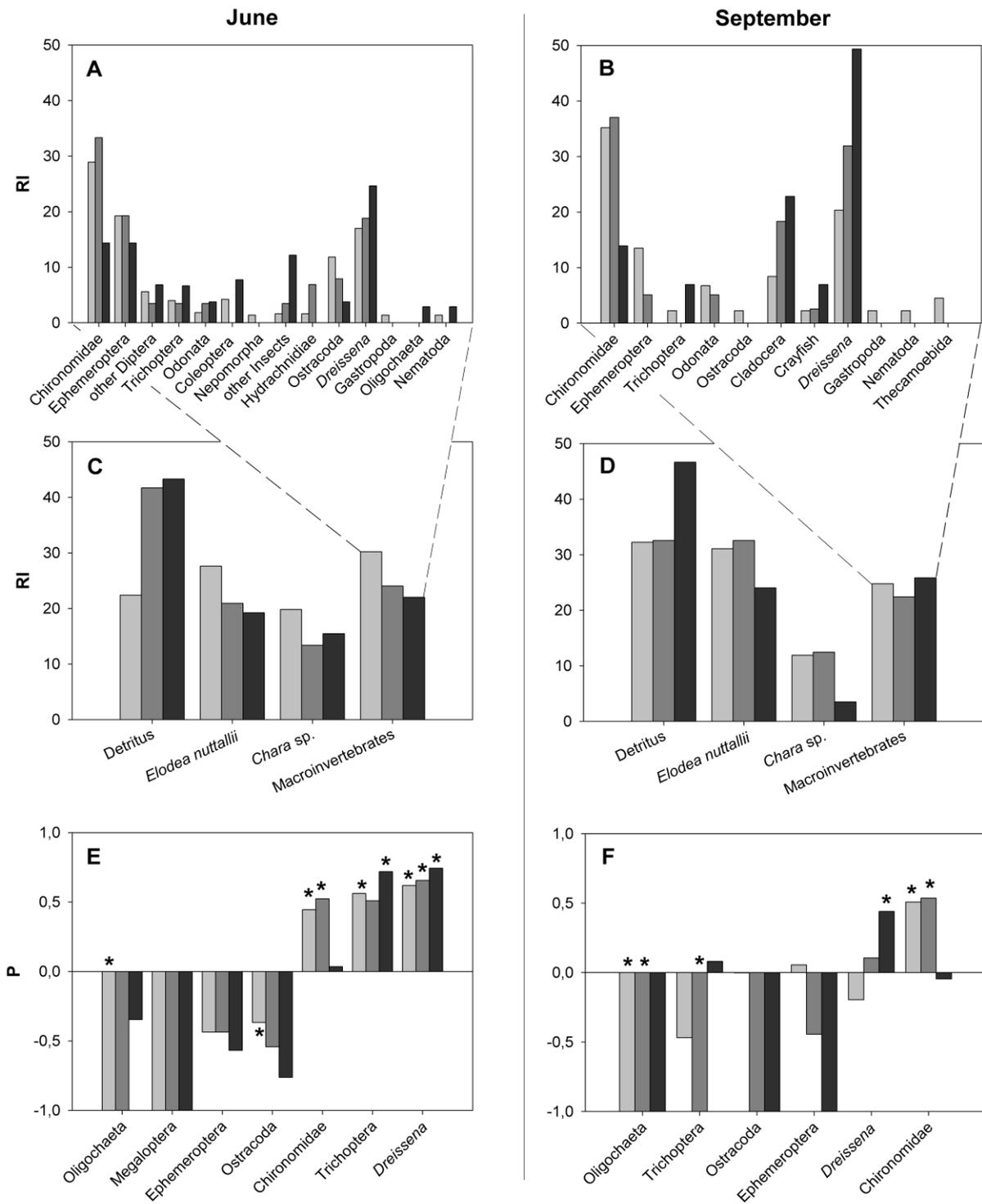


Figure 1. Relative importance of prey items (A, B) and food categories (C, D) in the diet and prey electivity (E, F) of *P. clarkii* per sample month (June 2007: left column; September 2007: right column) and size class (crayfish <math>< 50\text{ mm CL}</math>: light grey; crayfish with a $CL\text{ of }50\text{--}58\text{ mm}$: grey; crayfish $> 58\text{ mm CL}$: dark grey). The asterisks indicate significant differences between the prey *RI* and prey abundance in the environment, determined using a Chi-squared test ($P < 0.05$).

Table 2. Effects of treatment (0, 2.5, or 5 crayfish m⁻²), block, and enclosure orientation on the response variables of the enclosure experiment, determined using three-way ANOVAs. Significant effects are highlighted in bold ($P < 0.05$) and by symbols (: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$, and *** $P < 0.001$).

response variable	factor	d.f.	F	P
<i>Elodea nuttallii</i> ⁽¹⁾	treatment	2	6.376	0.057 .
	block	3	0.790	0.559
	orientation	3	1.063	0.490
	error	6		
<i>Myriophyllum spicatum</i> ⁽¹⁾	treatment	2	12.928	0.018 *
	block	3	2.377	0.211
	orientation	3	2.382	0.214
	error	6		
<i>Chara</i> sp. ⁽¹⁾	treatment	2	24.936	0.001 **
	block	3	1.402	0.331
	orientation	3	1.845	0.240
	error	6		
<i>Lymnaea stagnalis</i> ⁽²⁾	treatment	2	55.131	<0.001 ***
	block	3	0.577	0.651
	orientation	3	0.159	0.920
	error	6		
<i>Radix ovata</i> ⁽²⁾	treatment	2	9.460	0.014 *
	block	3	0.921	0.485
	orientation	3	0.215	0.882
	error	6		
<i>Planorbis corneus</i> ⁽²⁾	treatment	2	20.680	0.002 **
	block	3	0.199	0.893
	orientation	3	0.189	0.900
	error	6		
<i>Dreissena polymorpha</i> ⁽²⁾ > 21 mm	treatment	2	3.745	0.088 .
	block	3	2.645	0.143
	orientation	3	0.628	0.623
	error	6		
conditioned <i>Acer</i> leaves ⁽¹⁾	treatment	2	10.385	0.011 *
	block	3	1.199	0.387
	orientation	3	0.031	0.992
	error	6		
conditioned <i>Quercus</i> leaves ⁽¹⁾	treatment	2	31.403	<0.001 ***
	block	3	4.247	0.063 .
	orientation	3	1.785	0.250
	error	6		
conditioned <i>Salix</i> leaves ⁽¹⁾	treatment	2	7.981	0.020 *
	block	3	1.380	0.336
	orientation	3	0.158	0.921
	error	6		

⁽¹⁾: [g] enclosure⁻¹

⁽²⁾: indiv. enclosure⁻¹

Prey electivity

Chironomidae larvae and *D. polymorpha* were positively selected by *P. clarkii* in both of the sampled months, whereas Trichoptera were positively selected only in June (Figure 1E, F). Sediment-dwelling taxa, Ephemeroptera, and Ostracoda tended to be avoided as prey. The prey electivity varied also among the crayfish size classes; for instance, Chironomidae larvae were consistently preferred only by crayfish with a $CL \leq 58$ mm, whereas *D. polymorpha* tended to be more preferred by larger crayfish (Figure 1E, F). The prey RI and r_i significantly differed in Oligochaeta (size class I), Ostracoda (size class I), Chironomidae (size class I and II), Trichoptera (size class I and III) and *D. polymorpha* (all size classes) in June (chi-squared tests, $P < 0.05$), and in Oligochaeta (size class I and II), Trichoptera (size class II), *D. polymorpha* (size class III) and Chironomidae (size class I and II) in September (Chi-squared tests, $P < 0.05$; Figure 1E, F).

Enclosure experiment

The crayfish used in the crayfish treatments gained a significant amount of weight during the five-week enclosure experiment (Wilcoxon signed-rank test: $W = 404.0$, $T_+ = 405.0$, $T_- = -1.0$, $P < 0.001$), whereas the weight increase did not differ between the *LT* and *HT* (Mann-Whitney rank-sum test: $U = 161.0$, $P = 0.63$). Four crayfish (two each of the *LT* and *HT*) molted during the last two weeks of the experiment, resulting in a mean increase of the CL of 6.3 mm (12 %) and a mean increase of weight of 7.2 g (23 %).

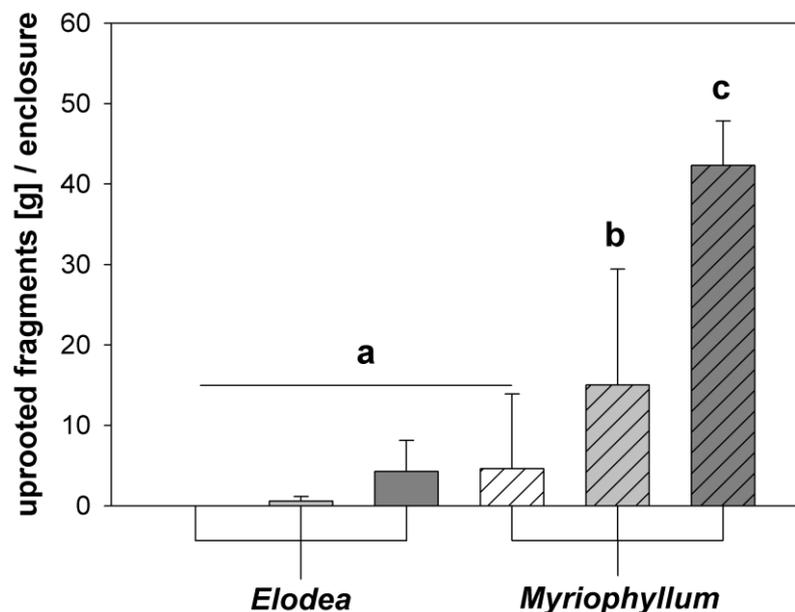


Figure 2. Uprooted plant fragments during the five-week enclosure experiment (pooled per enclosure) in relation to macrophyte species and treatment (white: control treatment; grey: low-density treatment; dark grey: high-density treatment). Different letters indicate significant differences, determined using a two-way ANOVA with a Holm-Sidak post-hoc test (treatment and macrophyte species as factors, $P < 0.05$). The whiskers show the standard deviation. The high-density treatment corresponds to 5 crayfish m^{-2} , the low-density treatment corresponds to 2.5 crayfish m^{-2} , and the control treatment included no crayfish. Each treatment was replicated five times.

The macrophyte uprooting differed significantly among the treatments and the macrophyte species (two-way ANOVA: $F_{1,24} = 47.96$, $P < 0.001$, and $F_{2,24} = 20.91$, $P < 0.001$, for species and treatment, respectively; Figure 2). Both of the factors showed a significant interaction (two-way ANOVA: $F_{2,24} = 13.02$, $P < 0.001$), indicating that the effect of the treatment depended on the macrophyte species. *Elodea nuttallii* uprooting was highest in the *HT*, followed by the *LT* and *CT*, but the differences among the treatments were not significant (two-way ANOVA with a Holm-Sidak post-hoc test: $P > 0.017$). The same trend could be observed in *M. spicatum*, where the differences among the treatments were significant (two-way ANOVA with a Holm-Sidak post-hoc test: $P < 0.04$). *Myriophyllum spicatum* uprooting was significantly higher than *E. nuttallii* uprooting in the *HT* and *LT* (two-way ANOVA with a Holm-Sidak post-hoc test: $P < 0.04$) but not in the *CT* (two-way ANOVA with a Holm-Sidak post-hoc test: $P > 0.04$).

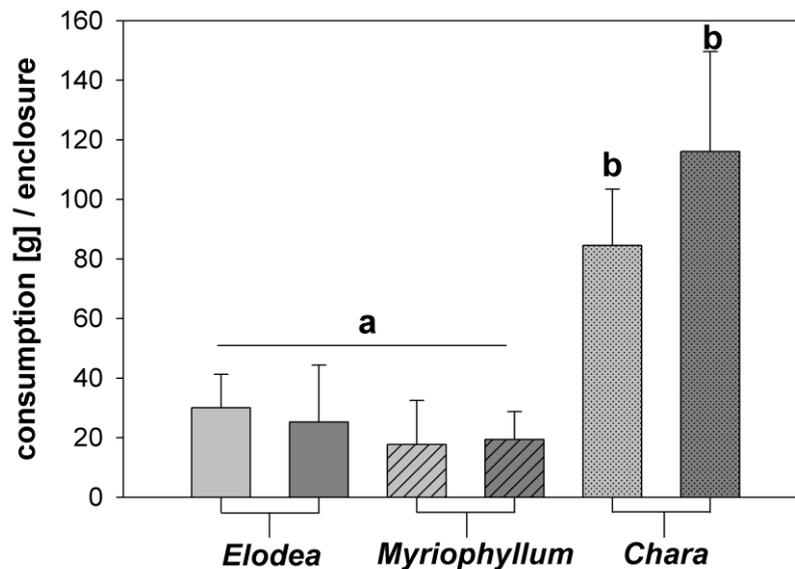


Figure 3. Macrophyte consumption in relation to macrophyte species and treatment (grey: low-density treatment; dark grey: high-density treatment). Different letters indicate significant differences, determined using a two-way ANOVA with a Holm-Sidak post-hoc test (treatment and macrophyte species as factors, $P < 0.05$). The whiskers show the standard deviation. The high-density treatment corresponds to 5 crayfish m^{-2} , and the low-density treatment corresponds to 2.5 crayfish m^{-2} . Each treatment was replicated five times.

The macrophyte consumption differed significantly among the macrophyte species but not among the treatments (two-way ANOVA: $F_{2,24} = 52.82$, $P < 0.001$ and $F_{1,24} = 1.77$, $P = 0.196$ for species and treatment, respectively; Figure 3). However, the *Chara* consumption tended to increase with crayfish density (*cf.* Figure 3). The *Chara* consumption was significantly higher than the *E. nuttallii* and *M. spicatum* consumption (two-way ANOVA with a Holm-Sidak post-hoc test: $P < 0.001$), whereas the consumption of the latter two species did not differ significantly (two-way ANOVA with a Holm-Sidak post-hoc test: $P = 0.307$).

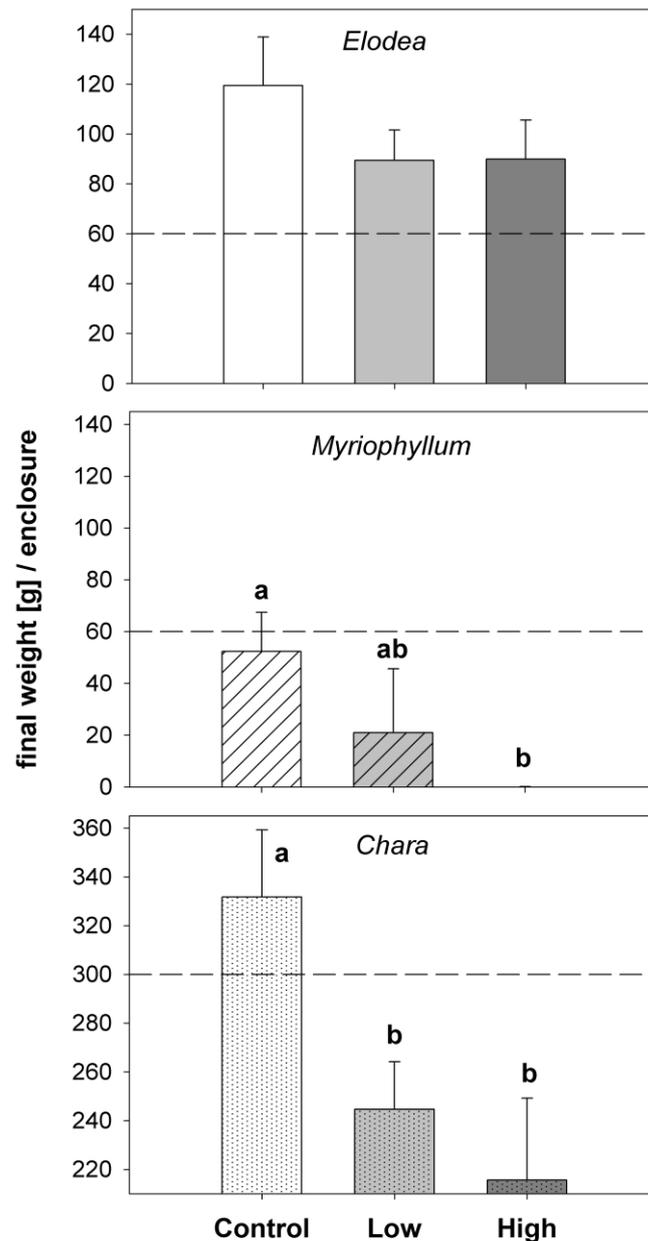


Figure 4. Net biomass of macrophytes at the end of the five-week enclosure experiment (accounting for loss through uprooting and consumption). Different letters indicate significant differences between the treatments, determined using a three-way ANOVA with a Holm-Sidak post-hoc test (block number and orientation as co-factors: $P = 0.057$, $P = 0.018$ and $P = 0.001$, for *E. nuttallii*, *M. spicatum* and *Chara* sp., respectively). The dashed lines denote the biomass at the start of the experiment. The whiskers show the standard deviation. High denotes the high-density treatment (5 crayfish m^{-2}), and low denotes the low-density treatment (2.5 crayfish m^{-2}); the control treatment included no crayfish. Each treatment was replicated five times.

The treatment had a significant effect on all of the response variables, except for *E. nuttallii* biomass and *D. polymorpha* abundance, whereas the block and enclosure orientation had no effect (three-way ANOVA; summarized in Table 2). For *E. nuttallii*, there was an obvious trend to a lower final biomass in the *LT* and *HT*; however, the differences from the *CT* were not significant (Table 2, Figure 4). *Myriophyllum spicatum* was completely eradicated from four of the five enclosures of the *HT*, when accounting for the loss through

uprooting and consumption. The final weight of this species in the *HT* was therefore significantly lower than its final weight in the *CT* (three-way ANOVA with a Holm-Sidak post-hoc test: $P = 0.007$; Figure 4). *Myriophyllum spicatum* was also eradicated from two enclosures of the *LT*, but its final weight in the *LT* did not differ significantly from its final weight in the *HT* or *CT* (three-way ANOVA with a Holm-Sidak post-hoc test: $P > 0.05$). Treatment also had a highly significant effect on the final *Chara* weight: in the crayfish treatments, the final *Chara* biomass was significantly lower than in the *CT* (three-way ANOVA with a Holm-Sidak post-hoc test: $P < 0.001$; Figure 4).

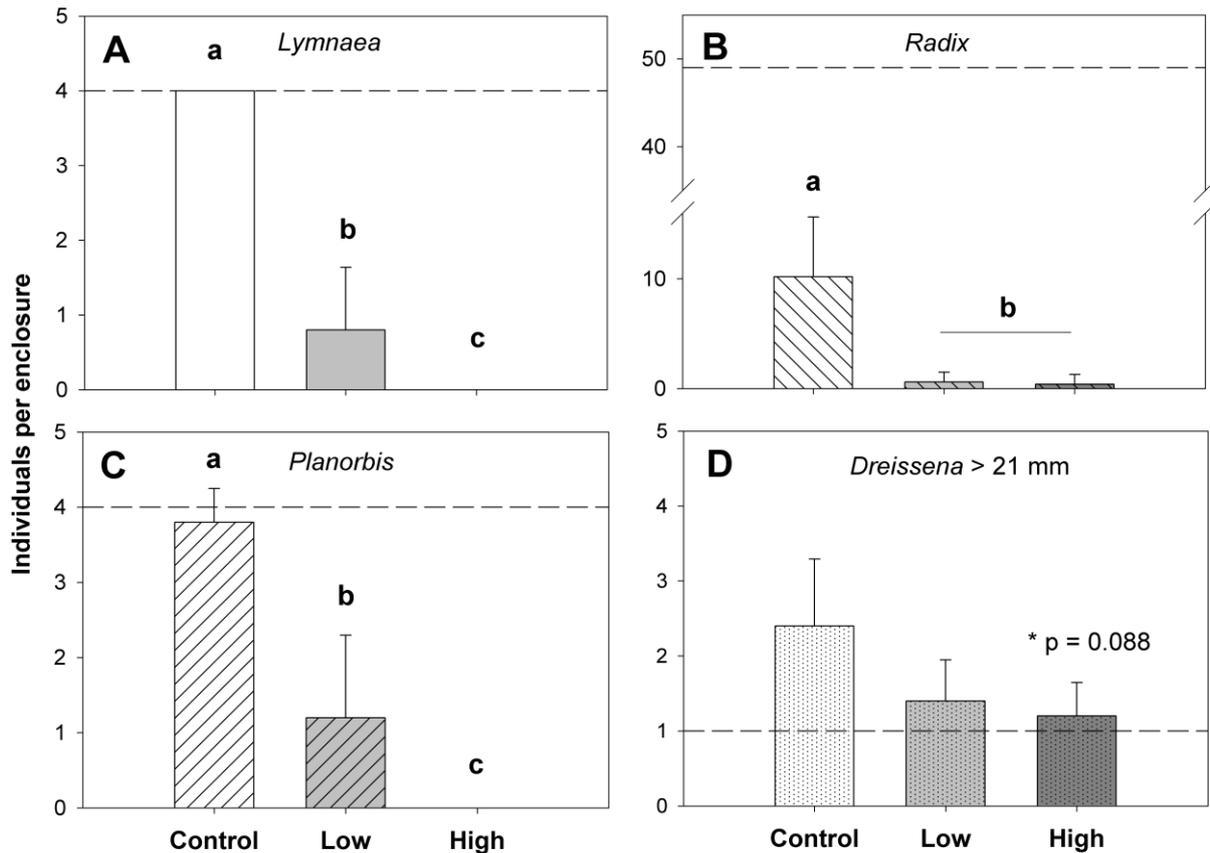


Figure 5. Final abundance of macroinvertebrates at the end of the five-week enclosure experiment (A: *Lymnaea stagnalis*, B: *Radix ovata*, C: *Planorbis corneus*, D: *Dreissena polymorpha* > 21 mm shell length). Different letters indicate significant differences between the treatments, determined using a three-way ANOVA with a Holm-Sidak post-hoc test (block number and orientation as co-factors, $P < 0.05$). The dashed lines denote the initial density at the start of the experiment. The whiskers indicate the standard deviation. High denotes the high-density treatment (5 crayfish m^{-2}), and low indicates the low-density treatment (2.5 crayfish m^{-2}); the control treatment included no crayfish. Each treatment was replicated five times.

The abundance of all three of the aquatic snail species was significantly reduced in the presence of crayfish (Table 2, Figure 5A-C). *Lymnaea stagnalis* and *Pl. corneus* were effectively eradicated in the *HT* but survived in low numbers in the *LT*. The differences among the treatments were significant for each species (three-way ANOVA with a Holm-Sidak post-hoc test: $P < 0.03$; Figure 5A,C). The final *R. ovata* abundance did not differ between the *LT* and *HT*; however, both treatments featured a significantly lower final abundance than the *CT* (three-way ANOVA with a Holm-Sidak post-hoc test: $P = 0.001$; Figure 5B). Treatment had no significant effect on any of the size classes of *D. polymorpha* (three-way ANOVA, $P > 0.05$), and only in the largest size class (> 21 mm shell length) was a trend to depletion in the crayfish treatments (three-way ANOVA, $P = 0.088$; Table 2, Figure 5D). The final weight of the conditioned leaves was significantly lower in the crayfish treatments than in the *CT* (Table 2, Figure 6). For *Acer* and *Quercus* leaves, there was a trend to a higher weight in the *LT* than in the *HT*; however, the differences were not significant (three-way ANOVA with a Holm-Sidak post-hoc test: $P = 0.062$ and $P = 0.284$ for *Acer* and *Quercus* respectively).

The final weight of the macrophytes in the crayfish treatments significantly differed from the initial weight at the start of the experiment (*cf.* Figure 4). The biomass of *E. nuttallii* increased (paired t-test: $t_4 = -5.410$, $P = 0.006$, and $t_4 = -4.226$, $P = 0.013$, for *LR* and *HT*, respectively), whereas *M. spicatum* and *Chara* sp. lost weight (paired t-test, *M. spicatum*: $t_4 = 3.528$, $P = 0.024$, and $t_4 = 179.521$, $P < 0.001$, and *Chara* sp.: $t_4 = 6.366$, $P = 0.003$, and $t_4 = 5.668$, $P = 0.005$, for *LR* and *HT*, respectively). The *E. nuttallii* biomass also increased significantly in the *CT* (paired t-test: $t_4 = -6.986$, $P = 0.002$). The same trend could be observed for *Chara* sp.; however, the weight increase was not significant (paired t-test: $t_4 = -2.540$, $P = 0.064$). The biomass of *M. spicatum* in the *CT* did not change significantly during the five-week experiment (paired t-test: $t_4 = 1.168$, $P = 0.308$).

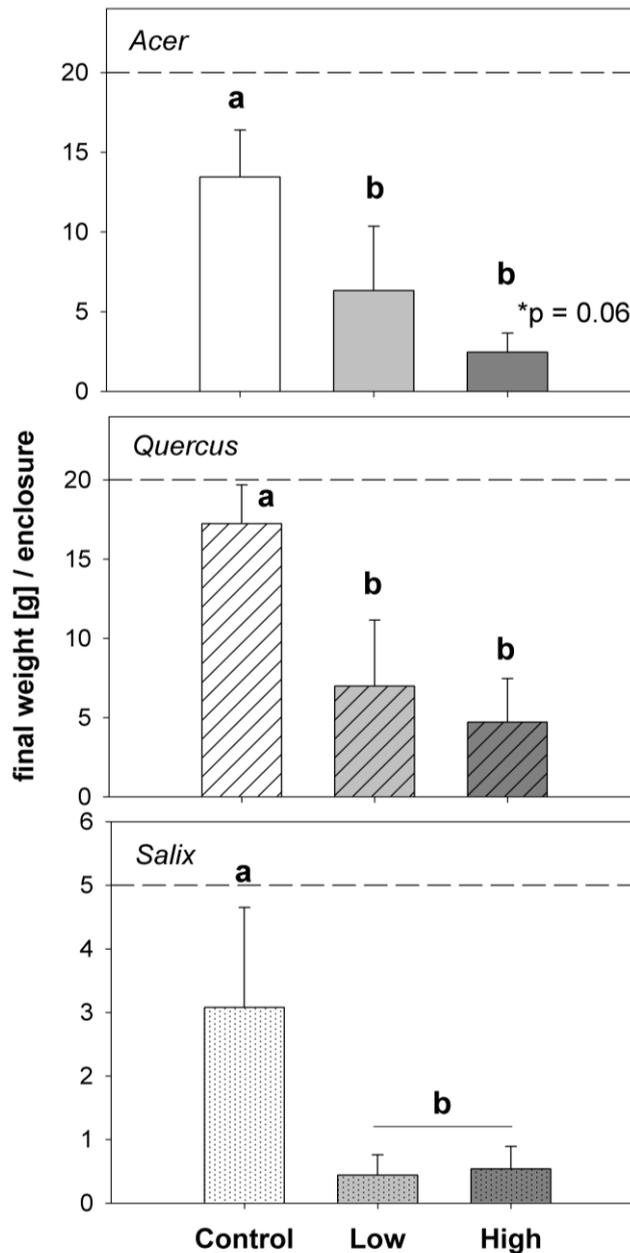


Figure 6. Final biomass of conditioned leaves per enclosure at the end of the five-week experiment. Different letters indicate significant differences between the treatments, determined using a three-way ANOVA with a Holm-Sidak post-hoc test (block number and orientation as co-factors; $P < 0.05$). The dashed lines denote the biomass at the start of the experiment. The whiskers indicate the standard deviation. High denotes the high-density treatment (5 crayfish m^{-2}), and low indicates the low-density treatment (2.5 crayfish m^{-2}); the control treatment included no crayfish. Each treatment was replicated five times.

Discussion

The present study attempted to investigate the feeding ecology and ecological impact of *P. clarkii*, a recent omnivorous invader in temperate lakes, by linking stomach-content analysis with an *in situ* enclosure experiment. While stomach-content data are crucial to elucidate the diet and trophic position of an invader, *in situ* enclosure experiments provide realistic data regarding how the recipient community is affected by the invader, including non-consumptive effects (*cf.* Lodge *et al.* 1994; Nyström *et al.* 1999; Gherardi and Acquistapace 2007).

Feeding ecology

Freshwater crayfish are generally considered as omnivorous (reviewed by Momot 1995 and Nyström 2002), and *P. clarkii* is no exception in that it was found to feed on macrophytes, detritus, and macroinvertebrates. Macrophytes (*E. nuttallii* and *Chara* sp.) and detritus were found to be the most important food categories; however, macroinvertebrates also formed an important part of the diet of *P. clarkii*, up to 100 % based on occurrence. Although macrophytes and detritus are low in energy, and laboratory studies frequently showed that crayfish prefer energy-rich macroinvertebrate prey when given a choice, macrophytes and detritus often occur in the stomach contents of crayfish in nature (Momot 1995; Nyström 2002, and citations therein). For instance, a high importance of macrophytes in the diet of *P. clarkii* living in lentic habitats has been previously shown by Alcorlo *et al.* (2004). To explain this apparent paradox, it was suggested that crayfish might rely on the protein-rich microbial fauna on decaying detritus, and macrophytes might provide minerals and nutrients essential for optimal survival and growth (reviewed by Nyström 2002). In addition, the diet of crayfish in nature may represent a trade-off between abundant low-quality foods, such as detritus and macrophytes, and less-abundant high-quality foods, such as energy-rich macroinvertebrate prey (Lodge and Hill 1994).

In the present study, *P. clarkii* was found to consume a wide spectrum of macroinvertebrates, ranging from benthic primary consumers (*e.g.*, *Caenis* sp. larvae) to predatory macroinvertebrates, such as Odonata and Coleoptera larvae, and planktivores, such as *D. polymorpha* and Cladocera. However, the main prey taxa were Chironomidae larvae and *D. polymorpha*, which were also significantly preferred as prey, followed by Ephemeroptera larvae in June and Cladocera in September. A similarly high dietary importance of Chironomidae larvae was recently reported for the invasive crayfish *Orconectes immunis* (Chucholl 2012) and is also known for other *Orconectes* species (Whitledge and Rabeni 1997), suggesting that Chironomidae larvae are generally a preferred prey of crayfish. Sediment-dwellers, such as Oligochaeta and Megaloptera larvae, in contrast, were underrepresented in *P. clarkii* stomachs, representing a common phenomenon in crayfish (*cf.* Alcorlo *et al.* 2004; Nyström 2002; Chucholl 2012). Generally, *P. clarkii* positively selected for energy-rich, less-vagile or sessile prey that can be easily captured, such as *D. polymorpha* and Chironomidae larvae (*cf.* Nyström 2002).

Interestingly, these two prey taxa showed a diametrically opposed relationship to crayfish size; the *RI* of *D. polymorpha* in the diet increased with crayfish size, whereas the number of consumed Chironomidae larvae negatively correlated with crayfish size. A probable explanation for this negative correlation is the growing size difference between the prey, *i.e.*, Chironomidae larvae, and crayfish, making it harder for larger crayfish to capture the relatively smaller prey (Lodge and Hill 1994; Alcorlo *et al.* 2004). Consequently, the handling time of small prey becomes inefficient for large crayfish. The same explanation is likely true for Ephemeroptera larvae and Ostracoda, where the number of consumed individuals was also negatively correlated with crayfish size. The increase of the *RI* of *D. polymorpha* with crayfish size, in contrast, may be a result of the stronger mandibles of larger crayfish, making it easier for them to break the shell of *D. polymorpha*. The handling time of *D. polymorpha* therefore presumably decreases with increasing crayfish size, resulting in *D.*

polymorpha being more attractive as prey for large crayfish than for small crayfish. The influence of crayfish size on the number of consumed prey, as well as the different *RI* of the food categories across the three crayfish size classes, concordantly suggest an ontogenetic shift in the diet of *P. clarkii*. An ontogenetic shift in diet is a common phenomenon in crayfish (Lodge and Hill 1994; Momot 1995; France 1996; Nyström 2002) and was also evidenced for *P. clarkii* populations in southern Europe (Alcorlo *et al.* 2004). The possible underlying mechanisms are discussed in detail by Nyström (2002).

The trophic diversity of *P. clarkii*'s diet, as measured using the diversity index of Herrera (*D*), was generally higher in smaller crayfish and in June. The variation with crayfish size can most likely be attributed to the ontogenetic shift in diet, with small crayfish being able to prey on a wider spectrum of prey species than large crayfish, which typically avoided small prey species (*cf.* Lodge and Hill 1994). For instance, Ostracoda and Ephemeroptera larvae (mostly small and agile *Caenis* sp. larvae) were predominately found in the stomachs of smaller crayfish. A remarkable exception was the occurrence of terrestrial insects, such as Hymenoptera and Scolytinae, in the stomachs of the largest crayfish size class in June, which were most likely gleaned from the water surface or from nearby terrestrial habitats. This suggestion is backed up by random observations of large crayfish accessing the water surface or migrating over land. The temporal variation in the trophic diversity of the diet is most likely because many larger aquatic insect larvae had emerged from the water by September. For instance, Diptera larvae, other than Ephemeroptera and Chironomidae, and Coleoptera larvae were present in crayfish stomachs in June but not in September. In addition, terrestrial insects were also consumed in June only (see above).

Crayfish remnants were mostly found in large crayfish, suggesting predation by large crayfish on smaller individuals, as was shown to be the case in the similar-sized signal crayfish (*Pacifastacus leniusculus*; Blake *et al.* 1994). However, cannibalism was apparently rare, and some of the crayfish remnants found in *P. clarkii* stomachs might have been consumed crayfish exuviae rather than consumed conspecifics (*cf.* Stenroth and Nyström 2003). This low frequency of cannibalism is surprising given the high population density in *LR* (Chucholl 2011a) and the high frequency of cannibalism found in studies on *P. clarkii*'s diet in southern Europe (*e.g.*, Alcorlo *et al.* 2004; Pérez-Bote 2005). Cannibalism is known to decrease when the small and vulnerable crayfish find abundant shelters to escape predation by larger individuals (Blake *et al.* 1994). In *LR*, the abundant *E. nuttallii* and *Chara* sp. stands probably provided adequate shelter for small crayfish.

In summary, the results of the stomach-content analysis clearly demonstrate that *P. clarkii* is a polytrophic omnivore, a finding that is in accordance with previous studies of the diet of *P. clarkii* (Gutiérrez-Yurrita *et al.* 1998; Alcorlo *et al.* 2004; PérezBote 2005). The high occurrence of macroinvertebrates in the diet of *P. clarkii* highlights the importance of crayfish as functional predators, as suggested by Momot (1995). By selectively preying upon preferred prey taxa, *P. clarkii* may eliminate sensitive macroinvertebrates (*e.g.*, aquatic snails) from invaded communities (*cf.* Alcorlo *et al.* 2004). Larger crayfish also frequently consumed terrestrial insects, a process that, in combination with the consumption of terrestrial detritus (*e.g.*, leaves), enhanced land-water ecotonal coupling (*cf.* France 1996). By consuming detritus, primary producers, primary and secondary benthic consumers as well as

planktivores, *P. clarkii* greatly enhanced the connectance (fraction of all possible links that are realized) of the food web of *LR* and accessed all energy pools and trophic pathways. The ability to feed on virtually all organic matter enables *P. clarkii* to build up and maintain the high population densities typical for this species (*cf.* Nyström 2002, and citations therein).

Enclosure experiment

The ecological impact mechanisms of crayfish fall generally into one of three categories, namely, direct trophic interactions (*e.g.*, grazing and predation), non-consumptive effects (*e.g.*, uprooting of macrophytes or competition), and indirect cascading effects through the food web (*e.g.*, increases of periphyton biomass due a depletion of benthic grazers by crayfish; *cf.* Momot 1995; Nyström 1999, 2002 and citations therein). The *in situ* enclosure experiment quantified both the direct trophic interactions and non-consumptive effects of *P. clarkii* on the basal levels of a typical littoral food web of temperate lakes, that is, detritus (conditioned leaves), primary producers (macrophytes), primary benthic consumers (aquatic snails) and benthic planktivores (*D. polymorpha*). The littoral species that were used in the *in situ* enclosure experiment included several indigenous species as well as the two common IAS, *E. nuttallii* and *D. polymorpha*. The tested crayfish densities were consistent with the densities observed in *LR* and the crayfish densities used in previous experimental studies of the ecological impact of crayfish (summarized in Table 3), thus providing a realistic and comparable setting. All of the *P. clarkii* used in the experiment increased their weight, except for one individual of the *HT* that died upon molting, indicating that sufficient resources for growth were provided, and the general conditions were good.

The breakdown of the conditioned leaves was significantly higher in the presence of *P. clarkii* than in the *CT* without crayfish, highlighting the important functional role of crayfish as shredders and producers of particulate organic matter (POM; Usio and Townsend 2001). A higher rate of leaf decomposition in the crayfish treatments was expected, based on related studies (Usio and Townsend 2001) and the obtained stomach content data, which showed that detritus, including decaying leaves, forms an important part of *P. clarkii*'s diet. The crayfish indiscriminately consumed the conditioned leaves of all three of the tree species, suggesting that the detritus consumption may have been largely driven by the protein-rich microbial fauna on the detritus (which was most likely the same for all three of the tree species) rather than by the initial nutritional composition and size/shape of the detritus. The species-specific anti-nutritional compounds of the leaves were most likely lost prior to the experiment, when the leaves were exposed to lake water for decomposition (Suren and Lake 1989; Nyström 2002).

Crayfish also had a significant effect on the macrophyte biomass, resulting from both consumption and uprooting; however, the impact mechanisms and outcomes differed markedly among the macrophyte species. Uprooting, measured as the biomass of floating plant fragments, was significantly higher in the crayfish treatments than in the *CT* and higher for *M. spicatum* than for *E. nuttallii*. The non-consumptive destruction of macrophytes by invasive crayfish is well known (*e.g.*, Lodge and Lorman 1987; Lodge *et al.* 1994; Nyström and Strand 1996) and results from crayfish clipping the basal parts of stems or uprooting the stems and then losing grasp of the fragments (Lodge *et al.* 1994; Nyström 1999, and citations

therein). The higher uprooting of *M. spicatum* was most likely due to the different growth form of this species than that of *E. nuttallii*. Single stems of *M. spicatum* are larger, and therefore also heavier, than single stems/shoots of *E. nuttallii*, resulting in a higher biomass loss per uprooted stem/shoot. A similar phenomenon was reported by Lodge and Lorman (1987), who found large, single-stemmed macrophytes to be especially prone to uprooting by crayfish. In fact, *M. spicatum* loss caused by uprooting was higher than *M. spicatum* loss resulting from consumption, which was not the case for *E. nuttallii* and *Chara* sp., highlighting the strong effect of uprooting by crayfish on large, single-stemmed macrophytes (cf. Lodge and Lorman 1987; Gherardi and Acquistapace 2007; cf. Table 3). The macrophyte consumption was significantly higher for *Chara* sp. than for the other two macrophyte species, coinciding with the preference of *P. clarkii* for *Chara* sp. reported by Cronin (1998). A preference for *Chara* sp. is also known from other crayfish species, including indigenous and alien species, e.g., *Austropotamobius pallipes* and *Pa. leniusculus* (Matthews *et al.* 1992; Nyström *et al.* 1999; Table 3), leading Nyström (1999) to suggest that *Chara* species are generally preferred by crayfish. Easy handling due to the highly branched, filamentous growth form and the high content of calcium of *Chara* species were proposed as explanations for this general preference (Cronin 1998; Nyström 1999, and citations therein).

Overall, *P. clarkii* led to a depletion of the indigenous *M. spicatum* and *Chara* sp. but had no significant effect on the alien *E. nuttallii* and, thus, induced a marked shift in the relative abundance of these macrophyte species. This finding coincides with the results of Nyström *et al.* (1999); in their experimental study, both *Astacus astacus* and *Pa. leniusculus* reduced *Chara* sp., while *Elodea canadensis* was not affected (Table 3). However, caution should be applied when generalizing the ecological impacts of crayfish, as Lodge *et al.* (1994) demonstrated a clearly negative effect of the alien *Orconectes rusticus* on *E. canadensis* abundance in a more diverse macrophyte community (Table 3). The species identity of the invader and the species composition and richness of the recipient community are most likely important determinants of the impact outcome. In the present study, *E. nuttallii* was apparently able to compensate for the grazing and uprooting by *P. clarkii*, most likely due to its high growth rate and high potential for regeneration (Barrat-Segretain *et al.* 2002; Thiébaud 2007, and citations therein). Moreover, *E. nuttallii* may have experienced lower competition for sunlight in the *HT* due to the depletion of the larger *M. spicatum* by crayfish. The ability of *E. nuttallii* to tolerate grazing and uprooting by *P. clarkii* most likely explains the fact that both species have co-existed in *LR* at high densities since the early 2000s. In comparable cases in southern Europe, an invasion of *P. clarkii* into macrophyte-dominated habitats has led to a collapse of macrophytes in short time (Rodríguez *et al.* 2005).

Dreissena polymorpha constituted a preferred prey of *P. clarkii* in *LR*; yet, in the enclosure experiment, *P. clarkii* had no significant effect on this species. Only in the largest *D. polymorpha* size class was a trend to depletion in the crayfish treatments. Interestingly, in all of the treatments, the total *D. polymorpha* abundance at the end of the experiment was considerably higher than the initially stocked abundance, suggesting additional colonization of the enclosures by planktonic veliger larvae during the experiment. This interpretation is supported by the occurrence of *D. polymorpha* shorter than 7 mm shell length in all of the enclosures. The final abundance of *D. polymorpha* > 14 mm also exceeded the initially

stocked abundance in all of the treatments, indicating that smaller individuals grew into the two biggest size classes. These findings exemplify the high recruitment potential and growth rate of this invasive species (Füreder and Pöckl 2007, and citations therein) and may explain why *P. clarkii* had no overall effect on *D. polymorpha*. Moreover, the crayfish used were rather large and therefore presumably preyed on larger *D. polymorpha*, leaving the smaller, most abundant size classes largely unaffected.

In contrast to the weak effect on *D. polymorpha* abundance, *P. clarkii* had a dramatic density-dependent effect on the final abundance of the three pulmonate snail species. In the *HT*, snails were effectively eradicated from the enclosures, while in the *LT*, only low numbers survived. This finding is consistent with the strong trophic link between crayfish and snails found in related studies (Lodge *et al.* 1994; Nyström and Pérez 1998; Gherardi and Acquistapace 2007; Table 3). For instance, Gherardi and Acquistapace (2007) demonstrated a strong negative effect of *P. clarkii* on the Mediterranean *Haitia acuta*, and in Kenya, *P. clarkii* was deliberately introduced as biological control agent for aquatic snails that are intermediary vectors of human parasites, such as *Schistosoma haematobium* (Lodge *et al.* 2005). However, it is interesting to note that, in the present study, *P. clarkii* impacted the three snail species unequally: while all species were effectively eradicated from the enclosures of the *HT*, a few *L. stagnalis* and *P. corneus* survived in the *LT*, as opposed to *R. ovata*, which was also eradicated in the *LT*. Apparently, *P. clarkii* preferentially preyed on *R. ovata*, probably due to its rather thin shell and small size, resulting in a low handling time (Alexander and Covich 1991; Nyström and Pérez 1998; Nyström 1999). *Lymnaea stagnalis* individuals were considerably larger than *R. ovata* individuals and because shell-thickness and handling time increase with snail size (Nyström and Pérez 1998), *L. stagnalis* was probably a less attractive prey for crayfish than *R. ovata*. The same explanation probably holds also true for *P. corneus*, which has the thickest shell of the three tested snail species (*cf.* Alexander and Covich 1991). Moreover, the shell of *P. corneus* is highly rounded and features only a few angles where the mandibles of the crayfish can effectively attach. This suggestion is supported by scars and small dismantled fragments around the opening of the shell of surviving *P. corneus* individuals of the *LT*.

Conclusion

The impact of *P. clarkii* on its food resources, assessed using the *in situ* enclosure experiment, differed from the expectations based on the stomach content data, signifying that caution should be applied when forecasting the ecological consequences of an omnivorous invader based on its diet. Specifically, both *E. nuttallii* and *D. polymorpha* formed an important part of *P. clarkii*'s diet in *LR*, yet both species were not affected by top-down pressure in the *in situ* enclosure experiment. In fact, *E. nuttallii* even increased in biomass in the presence of *P. clarkii*. Given that *P. clarkii* reduced at the same time the biomass of indigenous *M. spicatum* and *Chara* sp., one may argue that *P. clarkii* indirectly facilitated a dominance of the alien *E. nuttallii* by reducing its indigenous competitors. In addition, *E. nuttallii* fragments resulting from clipping by crayfish may aid the dispersal of *E. nuttallii* because stem fragmentation is considered to be the main dispersal mode for *E. nuttallii* in Europe (Barrat-Segretain *et al.* 2002; Thiébaud 2007). *Procambarus clarkii*, in contrast, may also benefit from *E. nuttallii* in

that *E. nuttallii* constitutes an important food resource and most likely provides abundant shelter for smaller crayfish, suggesting an indirect positive mutualism between both of the species, consistent with an invasional meltdown scenario (Simberloff and Von Holle 1999; Von Holle 2011). Based on the results of the *in situ* enclosure experiment, high densities of *P. clarkii* are likely to result in an *Elodea*-dominated littoral community deprived of large, single-stemmed macrophytes and larger aquatic snail species. Interestingly, this was the situation found at LR, where *E. nuttallii* was distinctly the dominant macrophyte species, and the aquatic snail community almost exclusively consisted of small (< 2 mm) *Gyraulus* species that are most likely too small to be a preferred prey for crayfish.

In summary, the stomach-content analysis and the *in situ* enclosure experiment concordantly suggest that *P. clarkii* is a keystone species that, despite being a ‘warm-water’ species in a cold habitat, can profoundly alter recipient communities *via* direct trophic links and non-consumptive destruction. Other IAS, such as *D. polymorpha* and *E. nuttallii*, were not affected though and may even be indirectly facilitated by *P. clarkii*. The arising implication for species management is that *P. clarkii* should be subjected to population control, especially in habitats of high conservation value, such as nature reserves and special areas of conservation (SAC). Finally, the presented findings raise the question of whether other ‘New’ NICS in Central Europe, such as *Orconectes* sp. and the Marmorcrebs (*Procambarus fallax* f. *virginalis*; Holdich *et al.* 2009; Chucholl 2012), have similar pronounced effects on the recipient communities. Conservation concerns associated with NICS in Central Europe are often focused on the displacement of indigenous crayfish species (*cf.* Holdich *et al.* 2009); the ecological role and impacts of NICS have received much less attention and should be targeted in future research.

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References

- Alcorlo, P., Geiger, W., Otero, M., 2004. Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. *Crustaceana* 77, 435–453.
- Alexander, J.E., Covich, A.P., 1991. Predation risk and avoidance behavior in two freshwater snails. *Biol. Bull.* 180, 387–393.

- Barrat-Segretain, M.H., Elger, A., Sagnes, P., Puijalon, S., 2002. Comparative life-history traits of two invasive macrophyte species, *Elodea canadensis* Michaux and *Elodea nuttallii* (Planchon) H. St John. *Aquat. Bot.* 74, 299–313.
- Bernauer, D., Jansen, W., 2006. Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. *Aquat. Inv.*, 1, 55–71.
- Blake, M., Nyström, P., Hart, P., 1994. The effect of weed cover on juvenile signal crayfish (*Pacifastacus leniusculus* Dana) exposed to adult crayfish and non-predatory fish. *Ann. Zool. Fenn.* 31, 297–306.
- Chang, H.H., Lin, Y.S., Chuaung, L.C., 1999. Effects of dams on fish assemblages of the Tachia river, Taiwan. *Acta Zool. Taiwanica* 10, 77–90.
- Chucholl, C., 2011a. Population ecology of an alien “warm water” crayfish (*Procambarus clarkii*) in a new cold habitat. *Knowl. Managt. Aquatic Ecosyst.* DOI: 10.1051/kmae/2011053.
- Chucholl, C., 2011b. Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquat. Inv.* 6, DOI: 10.3391/ai.2011.6.1.
- Chucholl, C., 2012. Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae). *Knowl. Managt. Aquatic Ecosyst.* DOI: 10.1051/kmae/2011082.
- Chucholl, C., Dehus, P., 2011. Flusskrebse in Baden-Württemberg, Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen.
- Clavero, M., García-Berthou, E., 2005. Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.* 20, 110.
- Cronin, G., 1998. Influence of macrophyte structure, nutritive value, and chemistry on the feeding choices of a generalist crayfish. In: Jeppesen, E., Sondergaard, M., Sondergaard, M., Christofferson, K. (Eds.). *The structuring role of submerged macrophytes in lakes*. Springer Press, New York, pp. 307–317.
- Cruz, M.J., Pascola, S., Tejedo, M., Rebelo, R., 2006. Predation by an exotic crayfish, *Procambarus clarkii*, on Natterjack Toad, *Bufo calamita*, embryos: its role on the exclusion of this Amphibian from its breeding ponds. *Copeia* 2, 274–280.

- DAISIE European Invasive Alien Species Gateway, 2010. One hundred of the worst, available from: <http://www.europe-aliens.org/speciesTheWorst.do> [accessed 1st August 2010].
- Dümpelmann, C., Bonacker, F., Häckl, M., 2009. Erstnachweis des Rotem Amerikanischen Sumpfkrebsses *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia* 67, 39–47.
- France, R., 1996. Ontogenetic shift of crayfish $\delta^{13}\text{C}$ as a measure of land-water ecotonal coupling. *Oecologia* 107, 239–242.
- Frutiger A., Borner S., Büsser T., Eggen R., Müller R., Müller S. and Wasmer H.R., 1999. How to control unwanted populations of *Procambarus clarkii* in central Europe? *Freshw. Crayfish* 12, 714–726.
- Füreder, L., Pöckl, M., 2007. Ecological traits of aquatic NIS invading Austrian fresh waters. In: Gherardi, F. (Ed.), Biological invaders in inland waters: profiles, distribution and threats, Springer, Dordrecht, pp. 233–257.
- Gherardi, F., 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. *Mar. Freshw. Behav. Phy.* 39, 175–191.
- Gherardi, F. (Ed.), 2007. Biological invaders in inland waters: profiles, distribution and threats, Springer, Dordrecht.
- Gherardi, F., Baldaccini, G.N., Ercolini, P., Barbaresi, S., De Luise, G., Mazzoni, D., M. Mori, 1999. Alien crayfish: the situation of Italy. *Crustacean Issues* 11, 107–128.
- Gherardi, F., Acquistapace, P., 2007. Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biol.* 52, 1249–1259.
- Gutiérrez-Yurrita, P.J., Sancho, G., Bravo, M.A., Baltanás, A., Montes, C., 1998. Diet of the red swamp crayfish *Procambarus clarkii* in natural ecosystems of the Dónana National Park temporary fresh-water marsh (Spain). *J. Crust. Biol.* 18, 120–127.
- Henttonen, P., Huner, J.V., 1999. The Introduction of alien species of crayfish in Europe: A historical introduction. *Crustacean Issues* 11, 13–22.
- Herrera, C.M., 1976. A trophic diversity index for presence-absence food data. *Oecologia* 25, 187–191.

- Hobbs, H.H., Jass, J.P., Huner J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56, 299–316.
- Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Managt. Aquatic Ecosyst.* 394-395, 11.
- Huner, J.V., 2002. *Procambarus*. In: Holdich, D.M. (Ed.), *Biology of Freshwater Crayfish*, Blackwell Scientific Press, Oxford, pp. 541–574.
- Jacobs, J., 1974. Quantitative measurement of food selection. *Oecologia* 14, 413–417.
- Lodge, D.M., Lorman, J.G., 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. *Can. J. Fish. Aquat. Sci.* 44, 591–597.
- Lodge, D.M., Hill, A.M., 1994. Factors governing species composition, population size, and productivity of cool-water crayfishes. *Nordic J. Freshw. Res.* 69, 111–13.
- Lodge, D.M., Kershner M.W. Aloi, J.E., Covich, A.P., 1994. Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology* 75, 1265–1281.
- Lodge, D.M., Rosenthal, S.K., Mavuti, K.M., Muohi, W., Ochieng, P., Stevens, S.S., Mungai, B.N., Mkoji, G.M., 2005. Louisiana crayfish (*Procambarus clarkii*) (Crustacea: Cambaridae) in Kenyan ponds: non-target effects of a potential biological control agent for schistosomiasis. *Afr. J. Aquat. Sci.* 30, 119–124.
- Matthews, W.J., R. Cashner, R.C., Gelwick, F.P., 1988. Stability and persistence of fish fauna and assemblage in three midwestern streams. *Copeia* 1988, 945–955.
- Matthews, M.A., Reynolds, J.D., Keatinge, M.J., 1992. Macrophyte reduction and benthic community alteration by the crayfish *Austropotamobius pallipes* (Lereboullet). *Feshw. Crayfish* 9, 289–299.
- McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J., Hoffmann, M., 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Divers. Distrib.* 16, 95–108.
- Momot, W.T., 1995. Redefining the role of crayfish in aquatic ecosystems. *Rev. Fish. Sci.* 3, 33–63.

- Nyström, P., 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. *Crustacean Issues* 11, 63–85.
- Nyström, P., 2002. Ecology. In: Holdich, D.M. (Ed.), *Biology of Freshwater Crayfish*, Blackwell Scientific Press, Oxford, pp. 192–224.
- Nyström, P., Strand, J.A., 1996. Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biol.* 36, 673–682.
- Nyström, P., Pérez, J.R., 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia* 368, 201–208.
- Nyström, P., Brönmark, C., Granéli, W., 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* 85, 545–553.
- Pérez-Bote, J.L., 2005. Feeding ecology of the exotic Red Swamp Crayfish, *Procambarus clarkii* Girard, 1852, in the Guadiana River (SW Iberian Peninsula). *Crustaceana* 77, 1375–1387.
- R Development Core Team, 2011. R 2.14, available from: <http://www.r-project.org/> (accessed November 12, 2011).
- Reynolds, J.D., 2002. Growth and Reproduction. In: Holdich, D.M. (Ed.), *Biology of Freshwater Crayfish*, Blackwell Scientific Press, Oxford, pp. 152–191.
- Rodríguez, C.F., Bécares, E., Fernández-Aláez, M., Fernández-Aláez C., 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biol. Invasions* 7, 75–85.
- Rodríguez, C.F., Bécares, E., Fuertes, B., 2006. Comparison of the impact of the freshwater decapod species *Austropotamobius pallipes* (indigenous) and *Procambarus clarkii* (non-indigenous), on the submerged vegetation of two Mediterranean wetlands. *Freshw. Crayfish* 15, 166–175.
- Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sannwald, E., Huenneke, L., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, B.H., Walker, B.H., Walker, M., Wall, D.H., 2000. Biodiversity scenario for the year 2100. *Science* 287, 1770–1774.
- Simberloff, D., Von Holle, B., 1999. Positive interactions of non-indigenous species: Invasional meltdown? *Biol. Inv.* 1, 21–32.

- Soes, M., van Eekelen, R., 2006. Rivierkrefeten, een oprukkend probleem? *De Levede Natur* 107, 56–59.
- Soes, M., Koese, B., 2010. Invasive crayfish in the Netherlands: a preliminary risk analysis. Interim report, Bureau Waardenburg bv, Stichting EIS-Nederland, Invasive Alien Species Team, Waardenburg.
- Souty-Grosset, C., Holdich, D.M., Noel, P.Y., Reynolds, J.D., Haffner, P. (Eds.), 2006. Atlas of Crayfish in Europe, Museum national d'Histoire naturelle, Paris.
- Stenroth, P., Nyström, P., 2003. Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshwater Biol.* 48, 466–475.
- Suren, A.M., Lake, P.S., 1989. Edibility of fresh and decomposing macrophytes in three species of freshwater invertebrate herbivores. *Hydrobiologia* 178, 165–178.
- Thiébaud, G., 2007. Non-indigenous plants in inland waters of France. In: Gherardi, F. (Ed.), Biological invaders in inland waters: profiles, distribution and threats, Springer, Dordrecht, pp. 209–229.
- Usio, N., Townsend, C.R., 2001. The significance of the crayfish *Paranephrops zealandicus* as shredders in a New Zealand headwater stream. *J. Crust. Biol.* 21, 354–359.
- Von Holle, B., 2011. Invasional Meltdown. In: Simberloff, D., Rejmánek, M., (Eds.), Encyclopedia of Biological Invasions. University of California Press, Los Angeles, pp. 360-364.
- Whitledge, G.W., Rabeni, C.F., 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analyses. *Can. J. Fish. Aquat. Sci.* 54, 2555–2563.
- Windell, J.T., 1971. Food analysis and rate of digestion. In: Ricker, W.E. (Ed.), Methods for assessment of fish production in freshwaters, Blackwell Scientific Press, Oxford, pp. 197–203.

Table 3. Overview of experimental studies on the ecological impact of crayfish in lentic habitats. Outcomes are shown by arrows: →: no effect, ↓: reduction, ↓↓: strong reduction/depletion, ↑: increase.

Reference	Species	Species status	Crayfish density		Method	Impact on		
			indiv. m ⁻²	g m ⁻²		Macrophytes	Macroinvertebrates	Other
present study	<i>Procambarus clarkii</i>	alien	2.5	91	<i>in situ</i> enclosures	<i>Chara</i> sp. (↓↓), <i>Myriophyllum spicatum</i> (↓), <i>Elodea nuttallii</i> (→)	<i>Radix ovata</i> (↓↓), <i>Planorbis corneus</i> (↓), <i>Lymnaea stagnalis</i> (↓), <i>Dreissena polymorpha</i> (→)	conditioned leaves
	<i>Procambarus clarkii</i>	alien	5	182	<i>in situ</i> enclosures	<i>Chara</i> sp. (↓↓), <i>Myriophyllum spicatum</i> (↓↓), <i>Elodea nuttallii</i> (→)	<i>Radix ovata</i> (↓↓), <i>Planorbis corneus</i> (↓↓), <i>Lymnaea stagnalis</i> (↓↓), <i>Dreissena polymorpha</i> (→)	conditioned leaves
Gherardi & Acquistapace 2007	<i>Procambarus clarkii</i>	alien	4	88	<i>in situ</i> enclosures and laboratory study	<i>Nymphoides peltata</i> (↓), <i>Potamogeton</i> spp. (↓↓), <i>Utricularia australis</i> (→)	<i>Haitia acuta</i> (↓↓)	<i>Gambusia affinis</i> (→)
	<i>Procambarus clarkii</i>	alien	8	176	<i>in situ</i> enclosures and laboratory study	<i>Nymphoides peltata</i> (↓↓), <i>Potamogeton</i> spp. (↓↓), <i>Utricularia australis</i> (→)	<i>Haitia acuta</i> (↓↓)	<i>Gambusia affinis</i> (→)
Matthews <i>et al.</i> 1992	<i>Austropotamobius pallipes</i>	indigenous	2	110	<i>in situ</i> enclosures	<i>Chara</i> (↓)	Chironomidae (↓), <i>Gammarus</i> sp. (↓), Ephemeroptera (→), <i>Asellus</i> (→), Gastropoda (→)	
	<i>Austropotamobius pallipes</i>	indigenous	10	594	<i>in situ</i> enclosures	<i>Chara</i> (↓)	Chironomidae (↓), <i>Gammarus</i> sp. (↓), Ephemeroptera (→), <i>Asellus</i> (→), Gastropoda (→)	
Nyström <i>et al.</i> 1999	<i>Pacifastacus leniusculus</i>	alien	6	x	artificial ponds	<i>Chara hispida</i> (↓), <i>Elodea canadensis</i> (→)	predatory invertebrates (→), Gastropoda (↓↓), Ephemeroptera (→)	Periphyton (↑)
	<i>Astacus astacus</i>	indigenous	6	x	artificial ponds	<i>Chara hispida</i> (↓), <i>Elodea canadensis</i> (→)	predatory invertebrates (→), Gastropoda (↓), Ephemeroptera (↑)	Periphyton (↑)
Lodge <i>et al.</i> 1994	<i>Orconectes rusticus</i>	alien	8	x	<i>in situ</i> enclosures	12 species, including <i>Elodea canadensis</i> (↓↓)	Gastropoda (↓↓), non-snail taxa (→)	Periphyton (↑)

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Curriculum vitae

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Education

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June 1999	Secondary school (Luitpold-Gym.), Wasserburg a. Inn, Germany	“Allgemeine Hochschulreife” (equivalent to A level exams)
2000-2002	University of Ulm, Germany	Life sciences “Vordiplom” (equivalent to B.Sc.)
2003	University of Salzburg, Austria	Semester abroad, biology
2003-2006	University of Ulm, Germany	Ecology, tropical biology, system. zoology, computer sciences “Diplom” (equivalent to M.Sc.). Thesis topic: “Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: <i>Orconectes immunis</i> vs. <i>O. limosus</i> ” (Prof. Dr. G. Maier)
2006-present	University of Ulm, Germany	Ph.D. candidate in invasion ecology (Institute of Experimental Ecology)

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Professional experience

Date	Institution/Activity
<i>1998-1999</i>	Secondary school project (term paper) and follow-up study on Stone crayfish conservation, Isen, Germany. Participant at regional and national youth research competitions (“Jugend Forscht” and “Bundesumweltwettbewerb”)
<i>1999-2000</i>	Community service at “Bund Naturschutz e.V.” Mühldorf, Germany (nature conservation NGO). Research assistant on terrestrial arthropod sampling
<i>2003</i>	Practical training in bird conservation at “Landesbund für Vogelschutz in Bayern e.V.” (nature conservation NGO), Hilpoltstein, Germany
<i>2003</i>	Research assistance, Institute of Neurobiology, University of Ulm
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Memberships

- Deutsche Gesellschaft für Limnologie e.V. (DGL)
- International Association of Astacology (IAA)
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List of publications

Peer-reviewed publications

- Chucholl, C. (submitted). Feeding ecology and ecological impact of an alien “warm-water” omnivore in temperate lakes.
- Chucholl, C. (in press). Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. *Biol. Inv.*
- Chucholl, C. (2012). Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae). *Knowl. Managt. Aquatic Ecosyst.* 404: art. no. 04.
- Chucholl, C. (2011b). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquat. Invas.* 6: 109–113.
- Chucholl, C. (2011a). Population ecology of an exotic ‘warm water’ crayfish (*Procambarus clarkii*) in a new cold habitat. *Knowl. Managt. Aquatic Ecosyst.* 401: art. no. 29.
- Chucholl, C. and Pfeiffer, M. (2010). First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquat. Invas.* 5: 405–412.
- Chucholl, C. and Daudey, T. (2008). First record of *Orconectes juvenilis* from eastern France. Update to the species identity of a recently introduced Orconectid crayfish (Crustacea: Astacida). *Aquat. Invas.* 3: 105–107.
- Chucholl, C., Morawetz, K., Groß, H. (in press). The clones are coming – strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. *Aquat. Invas.*
- Chucholl, C., Stich, H.B., Maier, G. (2008). Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. *Fund. App. Lim./Arch. Hydrobiol.* 172: 27–36.

Filipová, L., Grandjean, F., Chucholl, C., Soes, D.M., A., Petrusek (2011). Identification of exotic North American crayfish in Europe by DNA barcoding. *Knowl. Managt. Aquatic Ecosyst.* 401: art. no. 11.

Conference contributions

Chucholl, C. (in preparation). Marmorkrebs gaining ground in Europe: the role of the pet trade as invasion pathway. Talk at the 19th Symposium of the International Association of Astacology, Innsbruck, Austria.

Chucholl, C. (2012). Notorisch gefährdet: Probleme, Strategien und Perspektiven im Artenschutz für Flusskrebse. Talk at the 23th SVK-Fischereitagung, Fulda, Germany.

Chucholl, C. (2011). Situation der Flusskrebse in Baden-Württemberg. Talk at the 5th International Crayfishmeeting, Schaffhausen, Schweiz.

Kutschera, V., Haug, C., Haug, J.T., Maas, A., Chucholl, C., Waloszek, D. (2010). Stereoscopic 3D documentation of crayfish with a simple flatbed scanner. Poster presentation at the 21st International Senckenberg-Conference: Biology of Freshwater Decapods, held in Frankfurt, Germany.

Chucholl, C. (2010). Invaders for sale: Does the ornamental freshwater crayfish trade constitute an actual and overlooked risk? Poster presentation at the European Crayfish Food, Flagship & Ecosystem Services conference held in Poitiers, France.

Chucholl, C. (2009). Cooled down: Populationsökologie des Roten Sumpfkrebs in Süddeutschland. Talk at the 4th International Crayfishmeeting, Gersfeld/ Rhön, Germany.

Chucholl, C. (2009). Ökologischer Impact von *Procambarus clarkii* in Süddeutschland. Talk at the 4th International Crayfishmeeting, Gersfeld/ Rhön, Germany.

Chucholl C., Stich, H.B., Maier, G. (2008). Konkurrenz zwischen zwei Neozoen: Verdrängungs-Mechanismen zwischen Kamberkreb (*Orconectes limosus*) und Kalikokrebs (*O. immunis*). Talk at the annual DGL meeting, Konstanz, Germany.

Other publications and public outreach

Chucholl, C. and Dehus, P. (2011). Flusskrebse in Baden-Württemberg. Fischereiforschungsstelle Baden-Württemberg (FFS), Langenargen, 92 p.

Chucholl, C. (2011). Der Handel mit exotischen Flusskrebsen. *Forum Flusskrebse* 15: 33–39.

Chucholl, C. (2009). The ‘newcomer’ *Orconectes immunis* keeps spreading in the Upper Rhine plain. *Crayfish News* 31: 4–5.

Chucholl, C. (2009). Lange unbeachtet, lange unterschätzt: Der Rote Sumpfkrebs bei Ulm. *Forum Flusskrebse* 12: 23–27.

Chucholl, C. (2009). Flusskrebse bei Ulm. Radio interview provided for SWR 4.

Chucholl, C. (2007). Der Kalikokrebs (*Orconectes immunis*). *Forum Flusskrebse* 8: 3–23.

Reviewing activities

- *Aquatic Invasions*
- *Behaviour*
- *Knowledge and Management of Aquatic Ecosystems*
- *Marine and Freshwater Research*
- *Zoology*

Erklärung

Ich versichere hiermit, dass ich die vorliegende Dissertationsarbeit selbständig angefertigt habe und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, sowie die wörtlich oder inhaltlich übernommenen Stellen als solche kenntlich gemacht habe.

Kümbach, den 4. Juni 2012

APPENDIX

- Chucholl, C., Daudey, T. (2008). First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida). *Aquat. Invas.* 3: 105–107.

- Chucholl, C., Pfeiffer, M. (2010). First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquat. Invas.* 5: 405–412.

- Chucholl, C. (2011b). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquat. Invas.* 6: 109–113.

- Figures



First record of *Orconectes juvenilis* (Hagen, 1870) in eastern France: update to the species identity of a recently introduced orconectid crayfish (Crustacea: Astacida)

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Abstract

In 2005 a new, non-indigenous crayfish species was discovered in the Dessoubre River (eastern France). The species was initially considered as *Orconectes rusticus* (Girard, 1852). However, an examination of form I males caught in 2007 revealed that the species is in fact *Orconectes juvenilis* (Hagen, 1870), a closely related congener of *O. rusticus*. According to our knowledge, this is the first record of *O. juvenilis* outside the North American continent. The ecological implications as well as the possible impact on the native European crayfish are discussed briefly.

Key words: non native crayfish, *Orconectes*, France, pond introduction

In November 2005 a previously unknown *Orconectes* species was discovered in the Dessoubre River, a tributary of the Doubs (C. Rossignon pers. com. 2005). The origins of introduction were two ponds (UTM: 32T, 324734, 523051; Rosureux), adjacent to a restaurant which advertises crayfish as a delicacy. Since the ponds are in close proximity to the Dessoubre River, it is likely that some crayfish escaped upon stocking.

Initially, the introduced species was identified erroneously as rusty crayfish [*Orconectes (Procericambarus) rusticus* (Girard, 1852)], a common and largely translocated species in North America (cf. Souty-Grosset et al. 2006). In August 2007 we collected several specimens from the restaurant ponds, including seven form

I males. The form I males were subsequently compared to preserved *O. rusticus* specimens and identified as *Orconectes (Procericambarus) juvenilis* (Hagen, 1870; Figure 1), a species closely related to *O. rusticus* (Taylor 2000). *Orconectes juvenilis* resembles the habitus of *O. rusticus* and only differs from rusty crayfish in form I gonopod characteristics: In *O. juvenilis* the terminal elements of form I gonopods are more elongated and the ratio of central projection length to total gonopod length is greater (Taylor 2000, see Taylor and Schuster 2004 for gonopod terminology). *Orconectes juvenilis* has been only recently elevated to species rank (Taylor 2000), thus justifying the misidentification as *O. rusticus* of the former collected specimens.

In 2006 only a few adult specimens were found in the Dessoubre River along a 700m stretch, while the restaurant ponds showed high densities of *O. juvenilis* of all size-classes (Daudey 2006). In August 2007 the ponds were dominated by young of the year crayfish, indicating successful reproduction and recruitment. From September 5 to October 12,

2007 an attempt was made to eradicate the pond population by intense trapping (80 trap nights per pond; one 'trap night' equals one trap set for one night). The trapping effort yielded 716 crayfish, ranging from 5 to 17 g wet weight. Crayfish with a wet weight of more than 10 g accounted for 54 % of the total catch (T. Perrine, N. Guibert and C. Rossignon pers. com. 2007).



Figure 1. *Orconectes juvenilis* form I male. Photo by Chris Lukhaup

However, these numbers are presumably biased towards larger size classes, since juveniles do not readily enter traps (France et al. 1991; Frutiger et al. 1999). As Holdich et al. (1999) pointed out, it is probably impossible to eradicate a crayfish population solely by trapping (cf. Hein et al. 2006).

Little is known about the ecology of *O. juvenilis* (e.g. Dickey and McCarthy 2007). Within its native range, *O. juvenilis* inhabits a variety of lotic habitats (Taylor and Schuster 2004) and lakes (Rorer and Simmons 1975). Form I males can be found year-round and egg laying takes place in spring (Taylor and Schuster 2004). Since *O. juvenilis* was long considered a synonym of *O. rusticus* (cf. Taylor and Schuster 2004), we assume that their ecological traits might be in a comparable order of magnitude: *O. rusticus* is considered a keystone species in littoral habitats (Lodge et al. 1994; Momot 1995; Dorn and Wojdak 2004). Its presence can inflict

perturbing effects upon multiple trophic levels of native freshwater communities (e.g. Charlebois and Lamberti 1996; Dorn and Mittelbach 1999; Dorn and Wojdak 2004; McCarty et al. 2006).

The introduced population of *O. juvenilis* also poses a serious threat to the native European crayfish fauna since it might carry the crayfish plague [*Aphanomyces astaci* (Schikora 1906)], a disease that causes dramatic mortality in the susceptible European crayfish species (Souty-Grosset et al. 2006). *Orconectes juvenilis* may also outcompete the native European crayfish due to its presumably faster life cycle, high fecundity and higher pollution-tolerance (cf. Rorer and Simmons 1975; Vigneux 1997). This may be especially true for the imperiled white-clawed crayfish [*Austropotamobius pallipes* (Lereboullet, 1858)], which is still present in some headwaters of the Dessoubre river (Daudey 2006). Since the *O. juvenilis* population is still spatially restricted, we argue that it may be

advisable to consider eradication of the population by different means than solely trapping. We stress, that any range expansion of *O. juvenilis*, especially in the Dessoubre River, should be followed carefully.

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References

- Charlebois CM and Lamberti GA (1996) Invading crayfish in a Michigan stream: direct and indirect effects on periphyton and macroinvertebrates. *Journal of the North American Benthological Society* 15: 551-563
- Daudey T (2006): Diagnostic des peuplements astacicoles de la vallée du Dessoubre. Rapport de Master QTEBV, Université de Franche-Comté, Fédération de Pêche du Doubs. 65 p + Annexes
- Dickey BF and McCarthy TM (2007) Predator-prey interactions between crayfish (*Orconectes juvenilis*) and snails (*Physa gyrina*) are affected by spatial scale and chemical cues. *Invertebrate Biology* 126: 57-66
- Dorn NJ and Mittelbach GG (1999) More than Predator and Prey: A Review of Interactions between Fish and Crayfish. *Vie et Milieu* 49: 229 – 237
- Dorn NJ and Wodjak JM (2004) The role of omnivorous crayfish in littoral communities. *Oecologia* 140: 150-159
- France R, Holmes J and Lynch A (1991) Use of Size-Frequency Data to Estimate the Age Composition of crayfish Populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 2324-2332
- Frutiger A, Borner S, Busser T, Eggen R, Muller R, Muller S and Wasmer H (1999) How to control unwanted populations of *Procambarus clarkii* in central Europe. *Freshwater Crayfish* 12: 714-726
- Hein CL, Roth BM, Ives AR and Vander Zanden MJ (2006) Fish predation and trapping for rusty crayfish (*Orconectes rusticus*) control: a whole-lake experiment. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 383-393
- Holdich DM, Gydemo R and Rogers WD (1999) A review of possible methods for controlling nuisance populations of alien crayfish. *Crustacean Issues* 11: 245-270
- Lodge DM, Kersher MW and Aloï JE (1994) Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology* 75: 1265-1281
- McCarty JM, Hein CL, Olden JD and Vander Zanden MJ (2006) Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology* 51: 224-235
- Momot WT (1995) Redefining the Role of Crayfish in Aquatic Ecosystems. *Reviews in Fisheries Science* 3: 33-63
- Rorer B and Simmons GM (1975) Competitive Exclusion of *Cambarus bartoni* by *Orconectes juvenilis* in Mountain Lake, Virginia [Abstract]. *ASB Bulletin* 22:76
- Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD and Haffner P (eds) (2006) Atlas of Crayfish in Europe. Museum national d'Histoire naturelle, Paris: 187 pp (Patrimoines naturels, 64)
- Taylor CA (2000) Systematic studies of the *Orconectes juvenilis* complex (Decapoda: Cambaridae), with descriptions of two new species. *Journal of Crustacean Biology* 20 (1): 132-152
- Taylor CA and Schuster GA (2004) Crayfishes of Kentucky. Illinois Natural History Survey, Special Publication 28
- Vigneux E (1997) Les introductions de crustacés décapodes d'eau douce en France. Peut-on parler de gestion ? *Bulletin Français de la Pêche et Pisciculture* 344-345: 357-370

Research article

First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817)

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Abstract

Marmorkrebs are one of 12 currently known non-indigenous crayfish species (NICS) to be found in Central European waters. It is unique in the manner that there exist only females which reproduce parthenogenetically, i.e. eggs develop unfertilized and all offspring are genetically identical. Marmorkrebs have been first discovered in the German aquarium trade in the mid 1990s and became a very common pet species since then. Here, we present first evidence for a well established Marmorkrebs population in a small lake in the Upper Rhine catchment near Freiburg (Germany). The population occurs syntopically with *Orconectes limosus*, another NICS which invaded the Rhine system about 50 years ago. Morphometric and ovary weight measurements were taken from 12 Marmorkrebs specimens that were captured on July 3, 2010. The rostrum spination was pronounced and resembled the one found on a free-living individual captured in Saxony (Germany). Ovary development stages (Gonadosomatic Index) were heterogeneous and single berried females were found from early June to late July, which might indicate an asynchronous breeding habit. The relative abundance and distribution of both crayfish species were assessed by visual counts at nighttime at two occasions. Both species attained a comparable, moderate density throughout the lake margin. The Marmorkrebs was the prevalent species on shallow, swampy habitat patches, which are presumably similar to its natural prime habitats. The successful establishment of Marmorkrebs despite a pre-existing *O. limosus* population, stresses the competitive ability of Marmorkrebs. In addition to the recently suggested hypothesis that Marmorkrebs might be temperature limited in most parts of Europe, we feel that it is also necessary to consider its probable natural prime habitats and life cycle: Marmorkrebs are presumably able to colonize summer-warm, lentic habitats in most parts of Central Europe.

Key words: Marmorkrebs, marbled crayfish, non-indigenous species, invasion success, parthenogenesis, *Procambarus fallax*

Introduction

Crayfish (Astacida) are the largest mobile freshwater invertebrates and are considered as key members of littoral communities. They attain frequently a high biomass and interact with multiple trophic levels due to their omnivorous feeding habits and non-trophic activities, e.g. burrowing and bioturbation (Momot 1995; Nyström 2002). The three indigenous crayfish species (ICS) of Central Europe are largely outnumbered by 12 introduced, non-indigenous crayfish species (NICS), of which at least eight are established (Holdich et al. 2009). All ICS species are endangered throughout their Central European range and the presence of NICS is among the biggest threats to the remaining

stocks (Souty-Grosset et al. 2006; Holdich et al. 2009). NICS may also have strong detrimental effects on the integrity of the receiving community, including a reduction in α -diversity and species abundance (Nyström 2002; Rodriguez et al. 2005).

The Marmorkrebs ('marbled crayfish') is a medium sized crayfish species of unknown origin and enigmatic phylogenetic position. It was first discovered in the German pet trade in the mid 1990s and became a very popular pet species since then. It is unique in the manner that it is the only known decapod crustacean that reproduces by parthenogenesis (Scholtz et al. 2003; Martin et al. 2007; Vogt 2008): there exist only females, which lay unfertilized eggs and all offspring are genetically identical. The phylogenetic position and status of the Marmorkrebs

was long unresolved, although it has been shown to belong to the American genus *Procambarus* Ortmann, 1905 (Scholtz et al. 2003; Braband et al. 2006). Most recently, Martin et al. (2010a) suggested that the Marmorkrebs is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870) and proposed the tentative scientific name *Procambarus fallax* f. *virginalis*. *Procambarus fallax* occurs in southern Georgia and Florida and it is therefore reasonable to assume that the Marmorkrebs originates also from the southeastern United States, although an indigenous population has never been reported.

Marmorkrebs have been introduced into natural ecosystems in Europe (Germany, The Netherlands and Italy; Holdich and Pöckl 2007; Marzano et al. 2009; Martin et al. 2010b) and Africa (Madagascar; Jones et al. 2008) and there is concern that its wide spread in the North American pet trade will inevitably also result in releases from captivity there (Faulkes 2010). Parthenogenesis permits a high reproductive potential and there have been persistent concerns that the Marmorkrebs will become an invasive pest species (Vogt et al. 2004; Jones et al. 2008). Since the females do not need to mate in order to reproduce, one single specimen is sufficient to create a new population. Jones et al. (2008) went so far as to label the Marmorkrebs as “the perfect invader”, which is probably no understatement with regard to the situation in Madagascar. However, most records of free-living Marmorkrebs from Central Europe are based on the collection of single specimens, which led Martin et al. (2010b) to suggest that “in spite of all previous expectations, [the Marmorkrebs] does not have the potential for wide expansion within Europe”.

Here, we present the first evidence for an established Marmorkrebs population in a small lake in the Upper Rhine catchment near Freiburg (Germany). The Marmorkrebs population was found to occur syntopically with *Orconectes limosus* (Rafinesque, 1817), another NICS which invaded the Rhine system about 50 years ago. We assessed the relative abundance and distribution of both crayfish species along the shore line and examined the morphology and reproductive traits of Marmorkrebs in order to gain preliminary insights into the ecology of wild Marmorkrebs populations in temperate zones. We also discuss our findings with regard to the recent suggestion that Marmorkrebs might be temperature limited within most parts of Europe (Martin et al. 2010b).

Materials and methods

Study site and sampling

The presence of the Marmorkrebs in Lake Moosweiher (48°01'53.13"N, 7°48'18.10"E) was first confirmed by one of us (M.P.) on July 6, 2009, when an adult female of 95 mm total length was captured by snorkeling. The initial clue to the presence of Marmorkrebs in Lake Moosweiher was provided by a local pet shop owner, who told M.P. that he had repeatedly heard of Marmorkrebs in the lake. Lake Moosweiher is a small (7.6 ha; 8 m max. depth), mesotrophic lake, situated near Freiburg and is a popular swimming lake. Surface water temperature ranged between 25.7°C on July 5, 2010 to 26.2°C on July 20, 2010. The lake is fed from ground-water and features lower temperatures near the ground-water influx (22.7°C on July 20, 2010). Conductivity was low and ranged between 337 and 299 $\mu\text{S cm}^{-1}$.

In order to assess the relative crayfish abundance and distribution, the lake was visited during night time at two occasions (July 5 and July 20, 2010). At each sampling occasion, twelve sample plots, distributed along the northeastern lake margin (Figure 1), were searched for crayfish by hand lamps for one hour, beginning at 22.00 h. Plots were chosen for easy accessibility and moderate depth - deep water was avoided because it was difficult to spot crayfish in deeper areas. Plot area was estimated and varied between plots but not between sample occasions and ranged between 5 and 50 m². Each observed crayfish was identified by distinct morphological and color peculiarities (e.g. marbled colour pattern, chelae shape, spination) and noted.

On July 3, 2010, twelve Marmorkrebs were captured by hand nets and transported to the laboratory to take morphological and weight measurements. Carapace length and width as well as chelae length (*prodopus*) were measured to the nearest 0.1 mm with an electronic slide caliper. The captured crayfish were then frozen to -18°C and later dissected to calculate the gonadosomatic index (GSI = gonad weight/ fresh weight \times 100). Only females with intact chelae were used for GSI calculation to avoid bias from the lower fresh weight of specimen with regenerated or missing chelae. Prior to weight measurement, specimens were put on blotting paper and subsequently gently shaken ten times to remove adherent water drops. Dissected

gonads were also gently shaken ten times prior to weighting (Kern balance, type 822-67).

Statistical analyses

All statistical analyses were performed with SigmaPlot 10.0 (with SigmaStat 3.5 Integration). All data were tested for normality prior to analysis. Relationships between chelae length, fresh weight, carapace width and carapace length were assessed by Pearson Correlations. Weight and carapace length data were used to describe the relationship between carapace length and fresh weight by means of the power equation $y = a \times x^b$, with x = carapace length. Parameters a and b were estimated by employing a linear regression after log transformation of the data.

Results

Distribution and relative abundance

In total, 56 Marmorkrebs and 48 *Orconectes limosus* were recorded from Lake Moosweiher. Both species occurred syntopically at nine sample plots, whereas two plots had only Marmorkrebs (Figure 1). The latter two plots (one and six) were adjacent to each other and featured a shallow, soft-bottomed backwater area, with an extensive detritus layer and dead wood. At one plot (eleven) we found no crayfish. Total crayfish density was estimated at 0.07 to 1.50 individuals m^{-2} and was highest on plots seven (1.50) and five (1.33). On both plots, Marmorkrebs was more abundant than *O. limosus* (0.83 vs. 0.67 and 1.00 vs. 0.33 individuals m^{-2} , respectively). Mean total crayfish abundance was estimated at 0.27 individuals m^{-2} , with both species being equally abundant.

Morphology and reproductive traits

Chelae length, carapace width and fresh weight correlated significantly positive with carapace length (Pearson Correlation: correlation coefficient=0.92, $p < 0.001$, correlation coefficient = 0.98, $p < 0.001$ and correlation coefficient = 0.96, $p < 0.001$, respectively; Figure 2). The linear regression coefficients are summarized in Table 1. The rostrum spination of all twelve examined Marmorkrebs resembled the one found on a free-living individual captured in Saxony, i.e. the tip of the acumen, the lateral spines on the rostrum and the anterior angle of the postorbital ridges were pronounced and acute (Martin et al. 2010b; Figure 3).

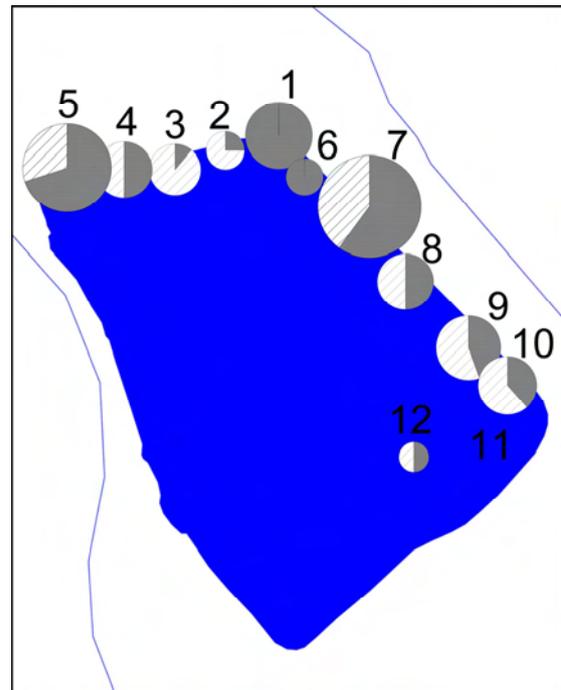


Figure 1. Distribution and relative abundance of Marmorkrebs (grey) and *Orconectes limosus* (hatched) in Lake Moosweiher. Symbol size gives estimated crayfish abundance (not to scale), numbers indicate the corresponding plot number. Marmorkrebs was the prevalent species on plots 1, 5 and 6, which were dominated by shallow, swampy areas (without stones). Plots 2, 3 and 10 featured stony substratum and were predominated by *O. limosus*.

The GSI values of the examined females ranged between 0.24 and 4.9 (mean = 0.9 +/- 1.4 SD, N = 11) and did not correlate with carapace length (Pearson Correlation: $p > 0.05$; Figure 4). Egg bearing females were captured on June 10, July 9 and July 21, 2010 (Figure 3). Fecundity was determined from three females and was seven, 160 and 724 eggs.

Discussion

The considerable number of specimens observed, the lake-wide distribution and the presence of different size classes, as well as reproducing individuals, strongly suggest that Marmorkrebs has established a stable population in Lake Moosweiher. This conclusion is further supported by a Marmorkrebs record made in summer 2009 and reported observations that date back even further. The presented findings are the first evidence for an established, free-living

Marmorkrebs population in Germany and possibly even Europe. There exist some uncertainties about the status of Marmorkrebs populations in the Netherlands, where the species was reported from the wild in Dordrecht in 2004 (Soes and van Eekelen 2006) and was still present there in 2008 (according to www.marmorkrebs.org). With the possible Dutch exception, all previously published records of Marmorkrebs in Europe were based on single specimens (summarized in Martin et al. 2010b). Martin et al. (2010b) attributed the apparent lack of records of established wild Marmorkrebs populations from Europe to the fact that Marmorkrebs might fail to establish sustaining populations. The species is very common in the pet trade and the records of single Marmorkrebs specimens across Europe stress the inability of the pet trade to keep animals captive (unpubl. data; Duggan 2010). The first stage of the invasion process, i.e. the introduction into the wild is certainly accomplished; however, subsequent establishment success seems to be poor.

In order to understand and predict the invasive success of an introduced species, the authors feel that it is important to consider its natural life history and habitat preferences. Since there are no known natural Marmorkrebs populations it is reasonable to consider the life history and habitat requirements of its closest relative *Procambarus fallax* (Scholtz et al. 2003; Vogt 2008; Martin et al. 2010a): Although *P. fallax* may occur in streams and rivers, it seems to prefer lentic or slow flowing habitats and is typically found in marshes, wet prairies and sloughs (Hendrix and Loftus 2000). This preference is also reflected by the common name of *P. fallax*: slough crayfish (Dorn et al. 2005). *Procambarus fallax* inhabits also temporary wetlands, which feature seasonal dry-downs during which crayfish retreat into refugia or simple burrows (Hendrix and Loftus 2000; Martin et al. 2010a). Although crayfish might reproduce within the refugia or burrows,

growth is most likely inhibited or slowed down. In this respect, they are adapted to a boom and bust scenario, i.e. growth and recruitment during inundation and reduced activity during dry periods. Granted that Marmorkrebs has a similar natural life history, this boom and bust strategy might facilitate its success at moderate European temperatures: while growth and recruitment is most likely inhibited during winter months, Marmorkrebs might be able to thrive during the

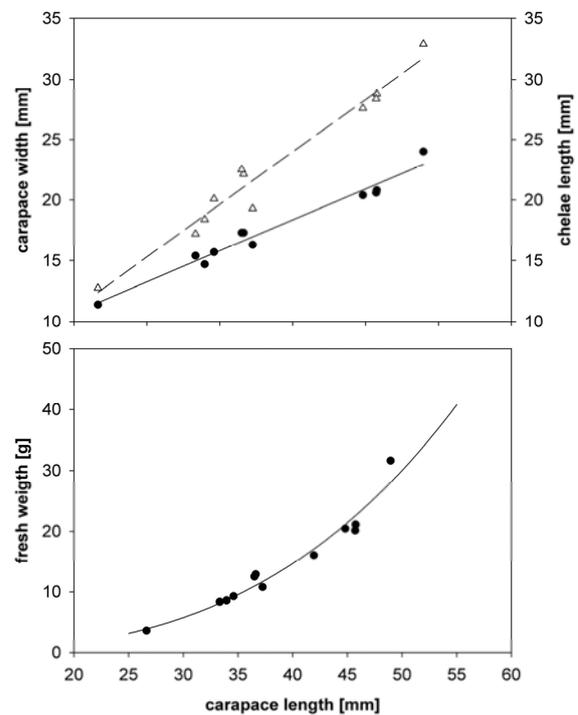


Figure 2. Carapace width (top, circles), chelae length (top, triangles) and fresh weight (bottom) of free-living Marmorkrebs in relation to carapace length. See text and Table 1 for correlation and linear regression statistics.

Table 1. Linear regression statistics. Regression equations are $y = a \times x + b$ for carapace width and chelae length and $y = 10b \times x^a$ for fresh weight, respectively. $x =$ carapace length [mm].

	a	b	R	p	N
carapace width [mm]	0.510	-2 034	0.98	< 0.001	11
chelae length [mm]	0.868	-10 742	0.98	< 0.001	11
fresh weight [g]	3 211	-3 978	0.99	< 0.001	12



Figure 3. Left: Rostrum spination of Marmorkrebs from Lake Moosweiher. Arrows indicate acute spines that are usually less pronounced or absent in laboratory specimens (Martin et al. 2010b; pers. obs.). Right: Egg bearing Marmorkrebs, captured from Lake Moosweiher on July 9, 2010. The female carried 724 eggs. Photographs by CC (left) and MP (right).

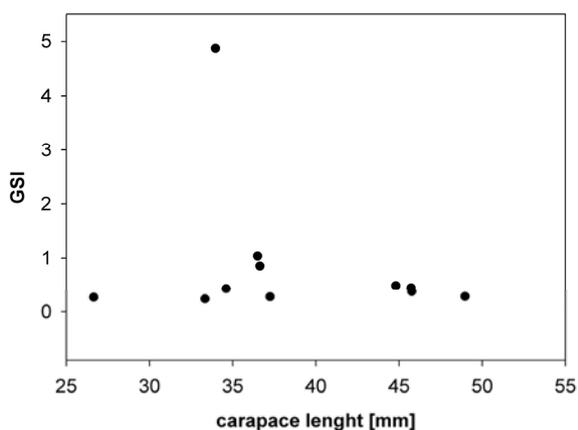


Figure 4. Gonadosomatic Index (GSI) of Marmorkrebs captured from Lake Moosweiher on July 3, 2010.

warm summer months. It was shown that Marmorkrebs can survive ice cover (Pfeiffer 2005) and reproduces at temperatures above 15°C (Seitz et al. 2005). The inactivity forced by low temperatures during winter might not be that different from its natural life history as one would expect, because Marmorkrebs might also exhibit periods of inactivity within its natural habitat. Similarly, the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), was often considered as 'warm-water' species (Henttonn and Huner 1999), but proved recently to be able to thrive also at cooler climates at higher latitudes and altitudes (Frutiger et al. 1999; Mueller 2007; Ellis and England 2008; Dümpelmann et al. 2009; unpubl. data). The red swamp crayfish has a comparable natural prime habitat to *P. fallax* and shows considerable life cycle plasticity, including reduced activity during winter in Central Europe (Frutiger et al. 1999; unpubl. data). The case of *P. clarkii*

illustrates well that *Procambarus* species can cope with seasonally low temperatures and may exceed expectations of their invasive potential that are based on assumed temperature constraints (cf. Bohl et al. 1989). Although this is speculative with regard to Marmorkrebs, it gives rise to the idea that temperature constraints are not the single factor that determines the invasive success (cf. Martin et al. 2010b).

Based on the habitat preferences of its closest relatives, it is reasonable to expect that introduced Marmorkrebs will be especially successful in lentic habitats, such as ponds, shallow lakes, backwaters and wetlands. This assumption is supported by reports from Madagascar, where Marmorkrebs thrives in brick pits, drainage ditches, rice fields and fish ponds (Jones et al. 2008). Martin et al. (2010b) on the other hand, found a single specimen in a lowland brook in Saxony. They were unsuccessful in detecting further specimens, which indicates that the Marmorkrebs was not able to propagate. They concluded that the temperatures were probably too low. However, the failure of the Saxony specimen to establish a population might also be related to the unsuitable habitat. Marmorkrebs need temperatures between 20 and 25°C for optimal development and reproduction (Seitz et al. 2005) and Martin et al. (2010b) argue that these temperatures are significantly higher than that found in most European waters. While this holds certainly true for many rivers and streams, small or shallow lentic habitats may offer more favorable temperatures for the Marmorkrebs, because those habitats heat up faster and reach higher summer temperatures than most lotic habitats. Interestingly, shallow lentic habitats are presumably also close to the natural prime habitat of the Marmorkrebs as pointed out above. In the present study, surface water temperatures clearly exceeded 20°C, thus permitting optimal temperature conditions for growth and recruitment. We found both freshly moulted and breeding individuals. Lake Moosweiher features also shallow, swampy areas (plots one and six), at which Marmorkrebs occurred exclusively.

Given that the habitat type has probably an influence on the invasion success, it is not surprising that there exists a lack of records of established wild Marmorkrebs populations from Europe: Small, lentic systems are often not monitored by routine sampling (e.g. within the scope of the EU Habitats Directive and Water Framework Directive). In fact, all recent

Marmorkrebs records from Europe were made coincidentally (Marzano et al. 2009; Martin et al. 2010b). This is also well illustrated by the present study, where the initial clue to the presence of Marmorkrebs was obtained by mere chance. The estimated number of unreported Marmorkrebs populations is therefore presumably high, because Marmorkrebs may establish stable populations especially in under-sampled habitat types. The currently published records represent most likely only the 'tip of the iceberg'.

We also obtained preliminary morphological and reproductive data. The length-weight relationship of the individuals examined shows that growth is not isometric, which coincides with results of laboratory studies on Marmorkrebs (Seitz et al. 2005). The length-weight regression coefficients are similar to values reported for female *Procambarus clarkii* (Huner and Barr 1991; unpubl. data). Interestingly, the rostrum spination of Marmorkrebs from Lake Moosweiher resembles that of one found on a free-living individual captured in Saxony (Figure 3), which might support the tentative interpretation that the spines are a sign of predator-induced plasticity (Martin et al. 2010b). However, this needs further investigation. Ovary development stages (i.e. GSI values) were heterogeneous and single berried females were found from early June to late July (cf. Figure 4), which might indicate an asynchronous breeding habit. In Madagascar, approximately 12 % of Marmorkrebs sampled were breeding, both in summer and winter (Jones et al. 2008). Both findings suggest that individuals breed independently of each other whenever conditions are favorable (e.g. at water temperatures above 15°C). However, it is not clear whether individuals are able to propagate more than once per year in Lake Moosweiher. Marmorkrebs reproduce approximately every three months at temperatures between 20 and 25°C (Seitz et al. 2005). These temperatures can be attained in Lake Moosweiher for three months and individuals breeding in early June might be able to reproduce again by late summer.

The presented findings demonstrate that Marmorkrebs are able to establish wild populations in Central Europe. Its invasion success is presumably affected by both temperature and habitat type: summer-warm, lentic habitats may be colonized readily. Based on a comparison of climate data of its probable native range and its established range (assessed

in CLIMATCH v.1.0; Invasive Animals CRC, Bureau of Rural Sciences 2008), we expect that Marmorkrebs are also able to develop reproductively active populations on the Iberian and the Balkan Peninsula, in Italy, France and possibly in parts of England and Eastern Europe. The pre-existence of another crayfish species does not seem to mitigate the potential of Marmorkrebs to establish a stable population (cf. Jimenez and Faulkes 2010). The Marmorkrebs population in Lake Moosweiher poses a latent risk to a population of the highly endangered white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet, 1858), which is situated within 3.6 km linear distance. Future research should therefore address the potential of Marmorkrebs to spread via smaller brooks or over land. Moreover, its population ecology and interactions with other crayfish species (here *Orconectes limosus*) are only poorly understood and need more attention (Jimenez and Faulkes 2010). Unlike other NICS, Marmorkrebs don't need to maintain a minimum viable population size, which makes eradication difficult, or even impossible. We stress that it is therefore urgent to minimize the risk of further Marmorkrebs introductions.

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References

- Bohl E, Strohmeier W, Leuner E, Negele RD, Pscheidl M, Heise J (1989) Untersuchungen an Flusskrebsbeständen. Bayrische Landesanstalt für Wasserforschung. Wielenbach, Bayern, 237 pp
- Braband A, Kawai T, Scholtz G (2006) The phylogenetic position of the East Asian freshwater crayfish Cambaroides within the Northern Hemisphere Astacoidea (Crustacea, Decapoda, Astacida) based on molecular data. *Journal of Zoological Systematics and Evolutionary Research* 44: 17–24, doi:10.1111/j.1439-0469.2005.00338.x
- Dorn NJ, Urgelles R, Texler JC (2005) Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *Journal of the North American Benthological Society* 24: 346–356, doi:10.1899/04-037.1
- Duggan I (2010) The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biological Invasions* 12: 3757–3770, doi: 10.1007/s10530-010-9768-x
- Dümpelmann C, Bonacker F, Häckl M (2009) Erstnachweis des Rotem Amerikanischen Sumpfkrebse *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia* 67: 39–47
- Ellis A, England J (2008) Red swamp crayfish on the move in the UK. *Crayfish News: IAA Newsletter* 30: 4
- Faulkes Z (2010) The spread of the parthenogenetic marbled crayfish, Marmorkrebs (*Procambarus* sp.) in the North American pet trade. *Aquatic Invasions* 5: 447–450, doi: 10.3391/ai.2010.5.4.16
- Frutiger A, Borner S, Büsser T, Eggen R, Müller R, Müller S, Wasmer HR (1999) How to control unwanted populations of *Procambarus clarkii* in Central Europe? *Freshwater Crayfish* 12: 714–726
- Hendrix AN, Loftus WF (2000) Distribution and relative abundance of the crayfishes *Procambarus alleni* (Faxon) and *P. fallax* (Hagen) in southern Florida. *Wetlands* 20: 194–199, doi:10.1672/0277-5212(2000)020[0194:DARAOJ]2.0.CO;2
- Henttonen P, Huner JV (1999) The Introduction of alien species of crayfish in Europe: A historical introduction. *Crustacean Issues* 11: 13–22
- Holdich DM, Pöckl M (2007) Invasive crustaceans in European inland waters. In: Gherardi F (ed) *Biological invaders in inland waters: profiles. Distribution and threats*. Springer, Dordrecht, pp 29–75, doi:10.1007/978-1-4020-6029-8_2
- Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ (2009) A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 394–395, 11, doi: 10.1051/kmae/2009025
- Huner JV, Barr LE (1991) *Red Swamp Crawfish: Biology, Culture, and Exploitation*. Louisiana State University Sea Grant College System, Louisiana State University, Baton Rouge, Louisiana, 128 pp
- Jimenez SA, Faulkes Z (2010) Can the parthenogenetic marbled crayfish Marmorkrebs compete with other crayfish species in fights? *Journal of Ethology*, doi: 10.1007/s10164-010-0232-2
- Jones JPG, Rasamy JR, Harvey A, Toon A, Oidtmann B, Randrianarison MH, Raminosoa N, Ravoahangimalala OR (2008) The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions* 11: 1475–1482, doi:10.1007/s10530-008-9334-y
- Martin P, Kohlmann K, Scholtz G (2007) The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. *Naturwissenschaften* 94: 843–846, doi:10.1007/s00114-007-0260-0
- Martin P, Dorn NJ, Kawai T, van der Heiden C, Scholtz G (2010a) The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contributions to Zoology* 79: 107–118
- Martin P, Shen H, Füllner G, Scholtz G (2010b) The first record of the parthenogenetic Marmorkrebs (Decapoda, Astacida, Cambaridae) in the wild in Saxony (Germany) raises the question of its actual threat to European freshwater ecosystems. *Aquatic Invasions* 5: 397–403, doi: 10.3391/ai.2010.5.4.09
- Marzano FN, Scalici M, Chiesa S, Gherardi F, Piccinini A, Gibertini G (2009) The first record of the marbled crayfish adds further threats to fresh waters in Italy. *Aquatic Invasions* 4: 401–404, doi:10.3391/ai.2009.4.2.19
- Mueller KW (2007) Reproductive Habits of Non-native Red Swamp Crayfish (*Procambarus clarkii*) at Pine Lake, Sammamish, Washington. *Northwest Science* 81: 246–250, doi:10.3955/0029-344X-81.3.246
- Momot WT (1995) Redefining the Role of Crayfish in Aquatic Ecosystems. *Reviews in Fisheries Science* 3: 33–63, doi:10.1080/10641269509388566
- Nyström P (2002) Ecology. In: Holdich DM (ed) *Biology of Freshwater Crayfish*. Blackwell Scientific Press, Oxford, pp 192–224

- Pfeiffer M (2005) Marmorkrebse überleben im Eis. *Fischer and Teichwirt* 6: 204
- Rodríguez CF, Bécares E, Fernández-Aláez M, Fernández-Aláez C (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions* 7: 75–85, doi:10.1007/s10530-004-9636-7
- Scholtz G, Braband A, Tolley L, Reiman A, Mittmann B, Lukhaup C, Steuerwald F, Vogt G (2003) Parthenogenesis in an outsider crayfish. *Nature* 421: 806, doi:10.1038/421806a
- Seitz R, Vilpoux K, Hopp U, Harzsch S, Maier G (2005) Ontogeny of the Marmorkrebs (Marbled Crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. *Journal of Experimental Zoology* 303A: 393–405, doi:10.1002/jez.a.143
- Soes M, van Eekelen R (2006) Rivierkrefeten, een oprukkend probleem? *De Levede Natuur* 107: 56–59
- Souty-Grosset C, Holdich DM, Noel PY, Reynolds JD, Haffner P (eds) (2006) Atlas of Crayfish in Europe. Museum national d'Histoire naturelle (Patrimoines naturels, 64), Paris, 187 pp
- Vogt G (2008) The marbled crayfish: a new model organism for research on development, epigenetics and evolutionary biology. *Journal of Zoology* 276: 1–13, doi:10.1111/j.1469-7998.2008.00473.x
- Vogt G, Tolley L, Scholtz G (2004) Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. *Journal of Morphology* 261: 286–311, doi:10.1002/jmor.10250

Short communication

Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany

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Abstract

Procambarus clarkii is among the worst invasive species that have invaded Europe. It originates from the central south of the USA and northeastern Mexico and is a hardy, r-selected generalist. There exists a marked gradient in population numbers from southwestern to northeastern Europe: The Iberian Peninsula, Italy and France have the bulk of the populations, while northeastern populations are mainly scattered and isolated from each other. In the present study I report on the distribution of *P. clarkii* in an artificial lake system along the River Danube in Southwestern Germany, which is within its northeastern range limit. The presence of *P. clarkii* was confirmed in nine lakes and one canal with stagnant water. *Procambarus clarkii* was absent from lotic situations, which may indicate that *P. clarkii* thrives chiefly in stagnant or slow flowing waters. A probable explanation is that those habitats heat up faster and reach higher summer temperatures than most lotic habitats. Since *P. clarkii* is considered as a 'warm water' species, lentic habitats probably offer more favorable temperatures. The populations form two disjunct distribution centres, isolated from each other by around seven km linear distance. It is not known for sure when the species was initially introduced, but it may have been present in the western distribution centre since the mid 1970s. The emerging disjunct distribution pattern is a synergistic result of introductions into two lakes and subsequent active spread to surrounding habitats, including migration overland. *Procambarus clarkii* numbers are often high and the species has become a nuisance to recreational fishery, since it tends to grab on to exposed fish bait. Two noble crayfish (*Astacus astacus*) populations were wiped out rapidly by *P. clarkii*, presumably due to crayfish plague transmission. Although eradication may prove impossible, population management, e.g. intense trapping and stocking of predatory fish, is strongly suggested. Early eradication, if necessary relying on extreme control methods (e.g. chemical agents), should be attempted when *P. clarkii* invades further lakes.

Key words: non-indigenous crayfish, active spread, red swamp crayfish, migration overland

Introduction

Freshwater crayfish (Astacida) are considered as keystone species in littoral communities. They are the largest mobile freshwater invertebrates, are long-lived, act as polytrophic omnivores and alter their habitats (Momot 1995; Nyström 1999, 2002). The three indigenous crayfish species (ICS) of Central Europe face the competition of at least eight established non-indigenous crayfish species (NICS; Holdich et al. 2009). All ICS species are endangered throughout their Central European range and the ongoing spread of NICS is among the biggest threats to the remaining stocks (Souty-Grosset et al. 2006; Holdich et al. 2009). NICS can also cause ecological havoc and may eliminate plant or animal species from ecosystems (Rodríguez et al. 2005; Nyström 1999).

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is one of the most important freshwater decapods farmed for consumption (Huner 2002). It shows considerably ecological plasticity and is listed among the "100 of the worst" invasive species by the 'Delivering Alien Invasive Species In Europe' project (DAISIE 2010). It is a known carrier of the parasitic oomycete *Aphanomyces astaci* Schikora 1906, the causative agent of the crayfish plague (Souty-Grosset et al. 2006). The crayfish plague is fatal for all ICS and has inflicted pan-European mass mortalities since its initial introduction in 1859 (Holdich et al. 2009).

Procambarus clarkii originates from the central south of the USA and northeastern Mexico, but has been translocated widely. It is now established in every continent except Australia and Antarctica (Huner 2002). The red swamp crayfish was first introduced into Europe

in 1973, when Spain intentionally imported the species for commercial crayfish production. In the subsequent decades, *P. clarkii* expanded its introduced range rapidly, supported by illegal introductions, and is nowadays widespread and abundant in whole southwestern Europe and northern Italy. Wild populations were also reported from most Central European countries, although there exists a marked gradient in population numbers from southwestern to northeastern Europe: Northeastern populations are mainly scattered and isolated from each other, while Spain, Italy and France have the bulk of the populations (Huner 2002; Souty-Grosset et al. 2006; Holdich et al. 2009).

In the present study, I report on the distribution of *P. clarkii* in an artificial lake system along the River Danube in Southwestern Germany, which is within its northeastern range limit. The presence of an 'American crayfish' in some of these lakes has long been known to local fishermen but only recently drew scientific attention when a strong noble crayfish, *Astacus astacus* (Linnaeus, 1758), population was rapidly wiped out by *P. clarkii*, presumably due to crayfish plague transmission (cf. Keller 1996). Up to date no attempt has been made to evaluate the distribution of *P. clarkii* in the lake system.

Crayfish survey

Most habitats in the study area are artificial gravel pit lakes along the River Danube, in total numbering approx. 100. In order to assess the current distribution of *P. clarkii*, I interrogated local fishing associations and authorities. Specific localities (N = 19) were selected on the basis of the gathered information and each locality was visited at least once from July to September 2008. Two of the localities were at small streams, one at a shallow backwater of the Danube River and 16 at gravel pit lakes. Localities were visited at night, beginning two hours after sunset, and the shallow water at the lake or stream margins was monitored for 30 to 45 minutes with flashlights. Apart from living crayfish, I searched after burrowing activities (Figure 1) and shed exuviae. Living crayfish and exuviae were identified by examining distinct morphological peculiarities of *P. clarkii*, e.g. a closed areola (space between the cardiobranchic grooves), a distinct median spine on carpus of the large chelipeds and prominent tubercles on the median margin of the chelae palm (propodus; see Figure 1).

Results

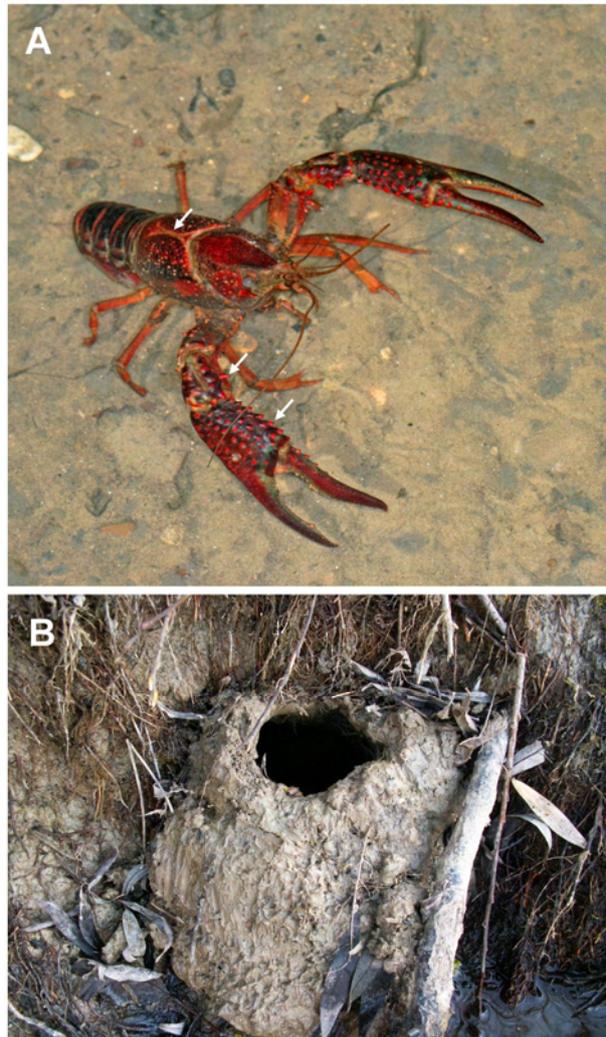
The presence of *P. clarkii* was confirmed in nine lakes and one canal with stagnant water (Figure 2, Table 1). Two disjunct distribution centres were found to exist: one south of the Danube River in the vicinity of Burlafingen and one north of the Danube River in the proximity of Lake Riedheim. Both distribution centres are isolated from each other by around 7 km linear distance; no crayfish were found either in-between or in the Danube River.

Discussion

The presence of *P. clarkii* was confirmed at ten localities, but due to the large number of nearby lakes in the area and the difficulties in detecting crayfish at low population densities, it is possible that further lakes are colonized by this crayfish. Nevertheless, the distribution of *P. clarkii* in the lake system was assessed for the first time and the present data may serve as a basis for future studies (primary geo-referenced data are available in Table 1).

In the study area, *P. clarkii* seems to be confined to artificial lentic habitats, chiefly gravel pit lakes, although lotic systems (e.g. the River Danube, canals and smaller streams) are found nearby (Figure 2). The absence of *P. clarkii* from lotic situations is in accordance with its prime habitats in its original home range, which are temporary lentic systems (Huner and Romaine 1978; Huner 2002). Introduced populations in the Netherlands, Switzerland, England and Germany were also predominately reported from lentic habitats, although some records exist from smaller streams and rivers (Frutiger et al. 1999; Henttonen and Huner 1999; Soes and van Eekelen 2006; Holdich and Sibley 2009; Dümpelmann et al. 2009; unpublished data). The prevalence of records from lentic habitats in Central Europe may indicate that *P. clarkii* thrives chiefly in stagnant or slow flowing waters. A probable explanation is that those habitats heat up faster and reach higher summer temperatures than do most lotic habitats. Since *P. clarkii* is considered as 'warm water' species and prefers temperatures between 21 and 27°C (Huner and Barr 1991), lentic habitats probably offer more favorable temperatures. This may especially hold true at higher latitudes/altitudes, where low winter temperatures probably restrict breeding season and growth (cf. Frutiger et al. 1999; unpublished data).

Figure 1. A: *Procambarus clarkii* form 1 male (reproductive form). Arrows indicate distinct morphological peculiarities (from left to right): closed areola (no space between the cardiobranctic grooves), distinct median spine on carpus of the large chelipeds and prominent tubercles on the median angle of the chelae palm (propodus). **B:** typical red swamp crayfish burrow along the shoreline of Lake Riedheim.



It is not known for sure when the species was initially introduced, but it may have been present in the vicinity of Burlafingen (western distribution centre) since 1975/6. According to local fishermen, less than 50 specimens of an 'exotic crayfish species' were introduced into a gravel pit lake (Hechtsee) at this time, which today features a dense *P. clarkii* population (see Figure 2). The commercial success of *P. clarkii* in Spain led to a series of illegal introductions in Spain, Italy and France in the 1970s and 1980s (Souty-Grosset et al. 2006) and it is conceivable that *P. clarkii* was stocked into Lake Hechtsee for the same motive. This scenario is also in accordance with the invasion history of the surrounding lakes: the most southern lakes in the western distribution centre were colonized only

within the last decade and one small pond still holds a noble crayfish population. The populations in the eastern distribution centre are reportedly younger than the initially stocked population in the western distribution centre and occurred later in the mid 1990s. The absence of *P. clarkii* from the lakes in-between and the Danube River suggests human mediated translocation to Lake Riedheim, the first lake in the eastern distribution centre that was colonized. After the loss of a noble crayfish population in Lake Riedheim, fishermen were aware of the risks of transplanting NICS and the subsequent invasion of the adjacent lakes was therefore most likely the result of natural active spread. The active colonisation of these lakes stresses the high potential of *P. clarkii* to migrate

Figure 2. Distribution of *Procambarus clarkii* and indigenous noble crayfish (*Astacus astacus*) in the study area. 'H' denotes Lake Hechtsee (site of first *P. clarkii* introduction) and 'R' Lake Riedheim (second introduction). The surrounding lakes were most likely colonized by migration overland.

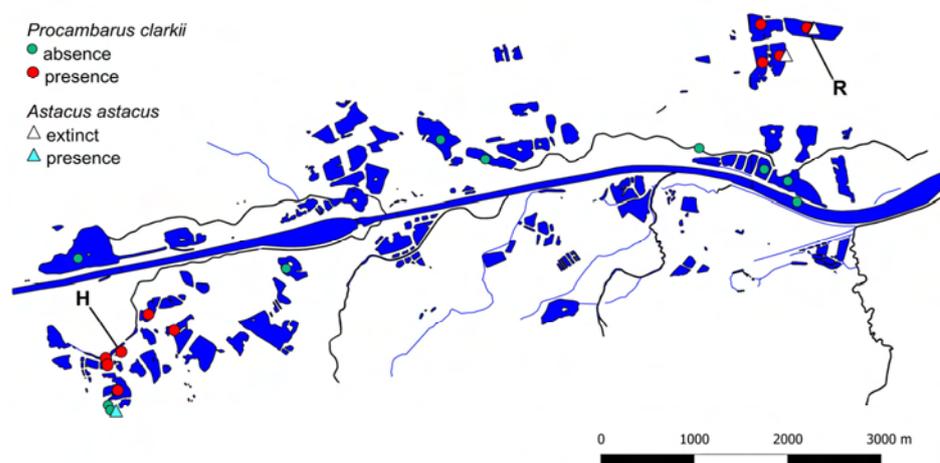


Table 1. Localities with confirmed presence of *Procambarus clarkii*. NN means no name.

Locality	Surface area [ha]	Latitude N	Longitude E	Comment
NN	0.9	48°25'57.56"	10°04'21.47"	
Burlafinger Vereins See	5.1	48°25'44.63"	10°04'29.00"	
Hechtsee	0.4	48°25'59.55"	10°04'30.30"	probable site of initial introduction
NN	2.9	48°26'12.67"	10°04'43.80"	
NN	5.0	48°26'04.87"	10°04'58.51"	
NN	6.8	48°27'50.68"	10°10'08.16"	
Riedheimer See	9.3	48°27'49.53"	10°10'32.35"	former <i>A. astacus</i> population
NN	5.2	48°27'40.08"	10°10'15.92"	former <i>A. astacus</i> population
NN	5.9	48°27'38.49"	10°10'06.51"	
NN	1.0	48°25'59.52"	10°04'27.80"	

considerable distances overland as there are no connecting surface water bodies (cf. Huner and Barr 1991). Crayfish migrating overland were indeed encountered during the study period and crayfish roadkills were frequently reported from a nearby freeway. Overall, the emerging, disjunct distribution pattern is a synergistic result of initial translocation by man (Lake Hechtsee and Lake Riedheim) and subsequent active spread to surrounding habitats.

The introduction and spread of *P. clarkii* has led to adverse economic, and probably also ecological effects: The invasion of Lake Riedheim by *P. clarkii* was followed by a rapid disappearance of resident noble crayfish (cf. Keller 1996). A second noble crayfish population in the lake south of Lake Riedheim was wiped out soon after. The noble crayfish populations have been valuable to the local fishing

associations, because noble crayfish were sold for stocking purposes and consumption. Although *P. clarkii* has reached high population densities in some lakes (e.g. an estimated adult population of approx. 13,400 crayfish in Lake Riedheim; unpubl. data) it was never harvested intensively. Currently, recreational fishermen harvest only one *P. clarkii* population extensively, chiefly for personal consumption. Red swamp crayfish numbers are often high and dense populations may structure benthic communities (unpublished data). *Procambarus clarkii* also constructs deep burrows along the shoreline of Lake Riedheim and the openings are occasionally covered by typical 'crayfish chimneys' (excavated mud; Figure 1). The species has become a nuisance to recreational fishery since it tends to grab on to exposed fish bait.

In Central Europe, *Procambarus clarkii* might not spread along rivers to the same substantial extent as other NICS, e.g. signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)) and *Orconectes* spp. (Dehus et al. 1999; Souty-Grosset et al. 2006; Chucholl 2009). However, it has proved to migrate considerable distances overland and the existing populations are latent 'bridgeheads' for a further active spread. Although eradication of the existing populations may prove impossible (Aquiloni et al. 2009; Freeman et al. 2010), population management, e.g. intense trapping and stocking of predatory fish, is strongly suggested (Frutiger and Müller 2002; Hein et al. 2006; Freeman et al. 2010). Early eradication, if necessary relying on extreme control methods (e.g. chemical agents, Sandodden and Johnsen 2010), should be attempted when *P. clarkii* invades further lakes.

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References

- Aquiloni L, Beccioloni A, Berti R, Porciani S, Trunfio C, Gherardi F (2009) Managing invasive crayfish: use of X-ray sterilisation of males. *Freshwater Biology* 54: 1510–1519, doi:10.1111/j.1365-2427.2009.02169.x
- Chucholl C (2009) The 'newcomer' *Orconectes immunis* keeps spreading in the Upper Rhine Plain. *Crayfish News* 31: 4–5
- DAISIE European Invasive Alien Species Gateway (2010) One hundred of the worst. Available from: <http://www.europe-aliens.org/speciesTheWorst.do> (Accessed 1st August 2010)
- Dehus P, Phillipson S, Bohl E, Oidtmann B, Keller M, Lechleiter S (1999) German conservation strategies for native crayfish species with regard to alien species. *Crustacean Issues* 11: 149–159
- Dümpelmann C, Bonacker F, Häckl M (2009) Erstnachweis des Rotem Amerikanischen Sumpfkrebsses *Procambarus clarkii* (Decapoda: Cambaridae) in Hessen. *Lauterbornia* 67: 39–47
- Freeman MA, Turnbull JF, Yeomans WE, Bean CW (2010) Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 211–223, doi:10.1002/aqc.1065
- Frutiger A, Borner S, Büsser T, Eggen R, Müller R, Müller S, Wasmer HR (1999) How to Control unwanted Populations of *Procambarus clarkii* in Central Europe? *Freshwater Crayfish* 12: 714–726
- Frutiger A, Müller R (2002) Der Rote Sumpfkrebs im Schübelweiher (Gemeinde Küsnacht ZH, Schweiz). Auswertung der Maßnahmen 1998–2001 und Erkenntnisse. EAWAG, Dübendorf, 26 p
- Hein CL, Roth BM, Ives AR, Vander Zanden MJ (2006) Fish predation and trapping for rusty crayfish (*Orconectes rusticus*) control: a whole-lake experiment. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 383–393, doi:10.1139/f05-229
- Henttonen P, Huner JV (1999) The Introduction of alien species of crayfish in Europe: A historical introduction. *Crustacean Issues* 11: 13–22
- Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ (2009) A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 394–395, 11, doi:10.1051/kmae/2009025
- Holdich DM, Sibley PJ (2009) ICS and NICS in Britain in the 2000s. In: Crayfish Conservation in the British Isles. Brickland J, Holdich DM, Imhoff EM (eds). Proceedings of a conference held on 25th March 2009 in Leeds, UK, pp 13–33
- Huner JV (2002) *Procambarus*. In: Holdich DM (ed) Biology of Freshwater Crayfish. Blackwell Scientific Press, Oxford, pp 541–574
- Huner JV, Barr LE (1991) Red Swamp Crawfish: Biology, Culture, and Exploitation. Louisiana State University Sea Grant College System, Louisiana State University, Baton Rouge, Louisiana, 128 pp
- Huner JV, Romaine RP (1978) Size at maturity as a means of comparing populations of *Procambarus clarkii* (Girard) (Crustacea, Decapoda) from different habitats. *Freshwater Crayfish* 4: 53–64
- Keller M (1996) Crayfish situation in Germany, Switzerland and Austria. *Crayfish News* 18: 4
- Momot WT (1995) Redefining the Role of Crayfish in Aquatic Ecosystems. *Reviews in Fisheries Science* 3: 33–63, doi:10.1080/10641269509388566
- Nyström P (1999) Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. *Crustacean Issues* 11: 63–85
- Nyström P (2002) Ecology. In: Holdich DM (ed) Biology of Freshwater Crayfish. Blackwell Scientific Press, Oxford, pp 192–224
- Rodríguez CF, Bécares E, Fernández-Aláez M, Fernández-Aláez C (2005) Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions* 7: 75–85, doi:10.1007/s10530-004-9636-7
- Sandodden R, Johnsen SI (2010) Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET. *Aquatic Invasions* 5: 75–81, doi:10.3391/ai.2010.5.1.9
- Soes M, van Eekelen R (2006) Rivierkrefeten, een oprukkend probleem? *De Levede Natuur* 107: 56–59
- Souty-Grosset C, Holdich DM, Noel PY, Reynolds JD, Haffner P (eds) (2006) Atlas of Crayfish in Europe. Museum national d'Histoire naturelle (Patrimoines naturels 64), Paris, 187 pp

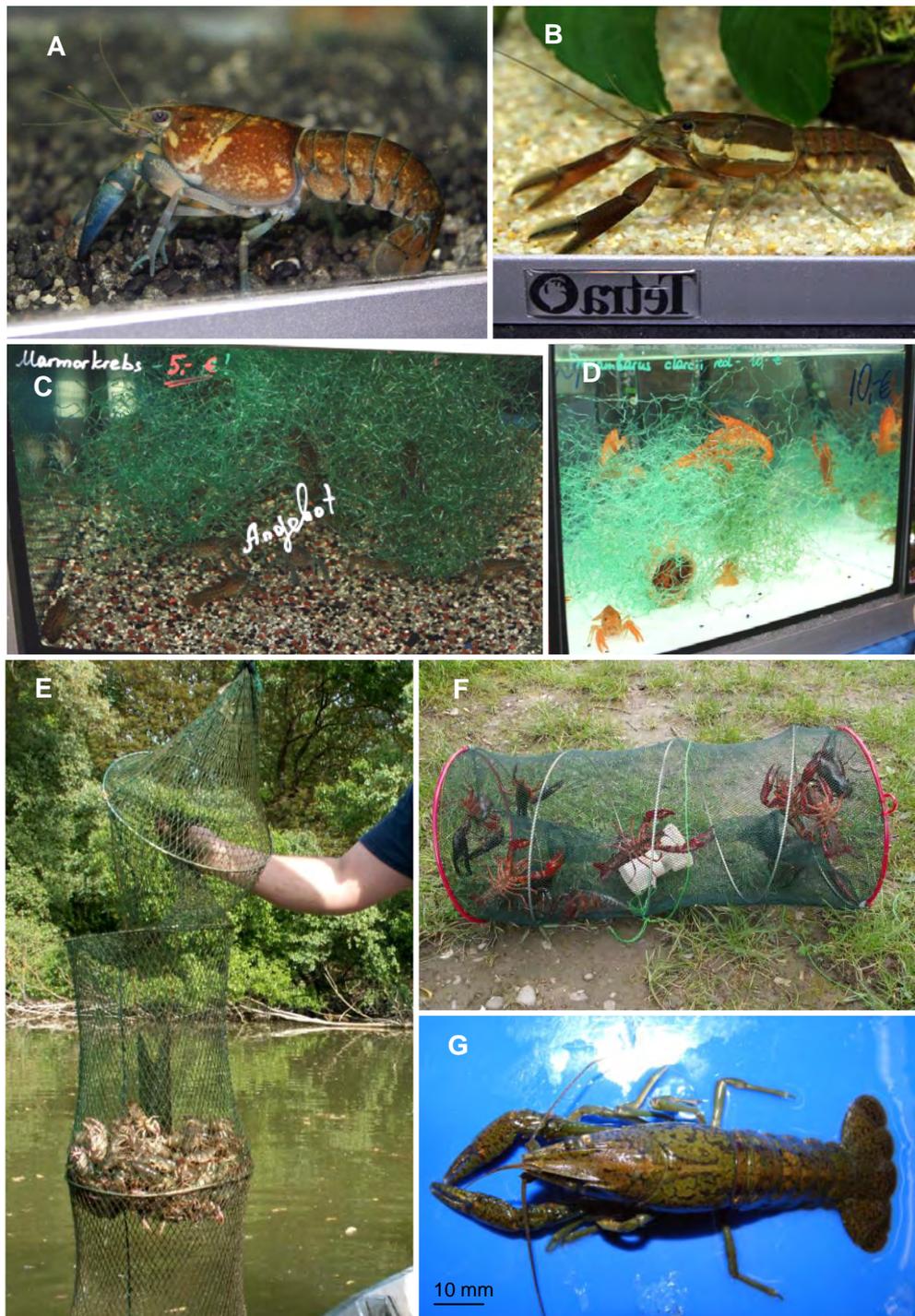


Figure 1. A-D: selection of NICS offered at an aquarium trade show in southern Germany in spring 2012. A) *Cherax* cf. *monticola*, a newly imported species from Irian Jaya (probably a low-risk species), B) *Procambarus llamasii* (medium-risk species of Central American origin; photo courtesy of T. Lang), C) Marmorcrebs (high-risk species; “Angebot” means on sale), D) *Procambarus clarkii*, orange color strain (high-risk species). The risk ratings are based on the FI-ISK scores from Chucholl (in press).

E-F: crayfish sampling gear. E) Unbaited funnel traps used to capture *O. immunis* (Chucholl 2012), F) baited minnow traps used to sample *P. clarkii* (Chucholl 2011a).

G) Marmorcrebs captured by way of nocturnal manual search (Chucholl and Pfeiffer 2010).



Figure 2. A-B: typical *O. immunis* habitats in the Upper Rhine Plain. A) Backwater at Au am Rhein, B) canal near the location where the species was first recorded. C-D: *Procambarus clarkii* habitats. C) Typical gravel pit lake along the River Danube, D) burrows at Lake Riedheim (arrows point towards the burrow openings) (Chucholl 2011a). E-F: enclosure cages used in the *in situ* enclosure experiment. E) Prior to the experiment in front of Lake Burlafingen, F) top view during the experiment (lid opened) (Chucholl submitted). G) *Procambarus clarkii* female with developed eggs, displaying the high fecundity typical for this species (photo courtesy of Dr. H. Bellmann) (Chucholl 2011a). H) *Orconectes immunis* male (right) performing a successful strike, *i.e.*, an unilateral attack, on a larger *O. limosus* male (both individuals were in form I) (Chucholl *et al.* 2008).