ECOLOGY AND EVOLUTION IN NOVEL COMMUNITIES: THE MARBLED CRAYFISH AND ITS INTERACTION PARTNERS

Inaugural-Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy

of Freie Universität Berlin

by

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2019

The scientific work presented in this thesis was conducted from 24.11.2014 until 13.06.2019 at the Institute of Biology (FU) and the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB). The work was supervised by Prof. Dr. Jonathan M. Jeschke and Dr. Sabine Wollrab.

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Disputation am	02.10.2019

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my first supervisor, Jonathan Jeschke, for his extensive and enduring support throughout each phase of this thesis. He gave me the freedom to pursue my own research interests and guided me towards a coherent thesis. Also, I want to thank my second supervisor, Sabine Wollrab, for her support and ideas, especially during the early stages of my thesis.

Next, I want to thank Larissa Goebel, who was an indispensable help for me during her time as my master student and student assistant. She really dedicated herself to researching crayfish and exceeded my expectations by far.

Furthermore, I want to thank all the people that shared their knowledge with me and helped me to advance as a scientist. Thank you to all the collaborators, especially Marcin Penk for his enriching stay in our research group and the opportunity to contribute to Chapter 1; Kate Laskowski for our exchanges on behavioral ecology as well as study design and for having an ear for my scientific and logistical problems; David Bierbach for his remarks on the behavior of clonal organisms and his help during the setup of my laboratory; and Gregor Kalinkat for his ideas and help on a multitude of questions and constant forwarding of new and important publications in my research field. I especially want to thank Chris Chucholl for passing on some of his profound knowledge on crayfish and for helping me during sampling.

Also, I want to express my gratitude towards the people that provided more hands-on support. I want to thank David Lewis for his technical support in the lab and for the occasional chat. Thanks also to Jorrit Lucas, Sofia Schütt and Alban Sagouis for their helping hands during field work for staying up late to catch some “crawdads”.

I want to thank my colleagues, who helped me to find solutions to the problems I encountered in this thesis. I especially want to thank Florian Ruland as well as Maud Bernard-Verdier for their statistical advice and Camille Musseau for deepening my knowledge on stable isotope ecology and scientific writing. Also, I want to thank Martin Enders for the delightful exchanges in our office.

Finally, I want to thank my wife Julia, who backed me up throughout my time as a doctoral candidate, especially after the birth of our son Valentin.

„Jedes Naturgesetz, das sich dem Beobachter offenbart,
läßt auf ein höheres, noch unerkanntes schließen.“

Alexander Freiherr von Humboldt (1769 – 1859)

SUMMARY

Ecosystems worldwide are undergoing drastic changes caused by the intentional and unintentional transfer of species between them. This anthropogenic process has accelerated within the last centuries, creating novel ecosystems which harbor species assemblages devoid of a shared evolutionary history. When novel organisms interact with their new environment and the members of the invaded community, they often exhibit different or even new traits compared to established species. These interactions can thus be difficult to predict, and they can have far-reaching consequences on biotic and abiotic components of the ecosystem. Especially differences in trophic interactions and behaviors can cause the most severe repercussions of species invasions. Therefore, a deeper understanding of the mechanisms underlying the invasion process informs general ideas of community assembly and can help predict potential invasions and the risks associated with it. To gain an understanding of the interactions within novel communities, this dissertation combines empirical and theoretical approaches from the fields of community, invasion and behavioral ecology.

The first chapter of the thesis presents a framework for risk assessments of novel organisms based on trophic interactions. A fundamental ecological principle, the functional response (i.e. the per-capita consumption rate as a function of resource density) is used to identify and quantify trophic traits of novel organisms linked to invasion success. The new approach presented in this chapter prioritizes and selects subsets of trophic links within the system in question and demonstrates the application of functional responses while including multiple potential interaction partners in the invaded system. The invasion of marbled crayfish *Procambarus virginalis* into Germany and a resident non-native congener (spiny-cheek crayfish *Faxonius limosus*) are used to illustrate the framework. However, the framework is applicable to a variety of novel organisms and invasion scenarios.

The second chapter implements the framework from Chapter 1 by executing the aforementioned example within a laboratory study and the parameterization of a mechanistic functional response model. Predator-prey interactions between the crayfish species and individuals feeding on a key aquatic primary consumer (*Dreissena* spp.) are examined in great detail to mechanistically explain trophic-trait differences. Data from video-recordings of foraging and feeding events are used to model and predict functional responses from independently derived predation parameters. In addition, modeled and empirically observed functional responses are linked to individual behavioral traits. Furthermore, this chapter demonstrates and discusses the explanatory power of the predation parameters on functional responses.

The third chapter assesses and compares behavioral traits that are important for the invasion success of crayfish species. Individual traits related to interspecific interactions – such as agonistic behavior between two crayfish and the response to predators, but also activity, which is related to foraging – are tested therein. In addition, correlations between behaviors, or so-called behavioral syndromes, are evaluated. This chapter also compares behavioral differences between naïve aquarium and naturalized individuals of marbled crayfish, and discusses the overall importance of the observed behavioral traits for novel communities.

In the fourth and final chapter, the prey-choice of marbled crayfish compared to that of established spiny cheek crayfish from field sites were investigated in the laboratory and contrasted with diet data from invaded lakes. Also, the trophic position and trophic niche size are determined to assess the ecological function of each species. To understand what resources the species use and which prey items or resources are mostly impacted, preferences and consumption rates were measured in predator-free environments and computed from stable isotopes of lake ecosystems. This part of the dissertation delivers insights into the in situ impacts of marbled crayfish in invaded food webs by highlighting particularly important interactions in an ecosystem context.

My thesis provides a novel interaction framework applicable for risk assessments of novel organisms (Chapter 1). It advances fundamental principles of ecology and invasion biology by providing a detailed, mechanistic examination and modeling of predator-prey interactions (Chapter 2), including the behavioral aspects (Chapter 3) and the food web effects (Chapter 4) of the novel, invasive marbled crayfish and a functionally similar comparator species, the spiny-cheek crayfish.

Keywords

Behavior; biological invasions; crayfish; functional response; predator-prey interactions; trophic position

ZUSAMMENFASSUNG

Durch den bewussten und unbewussten Austausch von Arten werden weltweit drastische Veränderungen von Ökosystemen verzeichnet. In den vergangenen Jahrhunderten beschleunigte sich dieser menschengemachte Prozess zusehends und brachte Artengemeinschaften ohne eine gemeinsame Evolutionsgeschichte hervor. Wenn neuartige Organismen mit der fremden Umwelt und den Mitgliedern der Organismengemeinschaft interagieren, zeigen diese oft unterschiedliche oder gänzlich neue Eigenschaften im Vergleich zu den etablierten Arten. Diese Interaktionen sind schwer vorherzusagen, können aber weitreichende Konsequenzen auf biotische und abiotische Komponenten der Ökosysteme haben. Vor allem Unterschiede in den trophischen Wechselwirkungen und im Verhalten können zu schwerwiegenden Auswirkungen bei Invasionen führen. Ein tiefer gehendes Verständnis der Mechanismen, die dem Invasionsprozess zugrunde liegen, erklärt die Grundlagen der Entstehung von Artengemeinschaften und kann dabei helfen, potenzielle Invasionen vorherzusagen und mögliche Risiken aufzuzeigen. Arten mit besonders gravierenden Auswirkungen rechtzeitig zu identifizieren ist heutzutage eine wichtige Aufgabe für das Ökosystemmanagement, damit künftige Invasionen verhindert werden können. Um die Beziehungen in neuartigen Organismengemeinschaften besser zu verstehen, vereint diese Dissertation empirische und theoretische Ansätze aus der Gemeinschafts-, Invasions- und Verhaltensökologie.

Im ersten Kapitel der Dissertation wird ein Schema zur Risikobewertung neuartiger Organismen basierend auf trophischen Interaktionen vorgestellt. Trophische Eigenschaften neuartiger Organismen, die auf eine erfolgreiche Invasion hindeuten, werden mithilfe eines fundamentalen ökologischen Konzepts, der funktionellen Reaktion (das ist die Pro-Kopf-Konsumptionsrate als Funktion der Ressourcendichte) identifiziert und quantifiziert. Dieser neue Ansatz priorisiert und selektiert eine Auswahl der im System vorhandenen trophischen Verbindungen und zeigt, wie funktionelle Reaktionen dazu genutzt werden können, wobei mehrere potenzielle Interaktionspartner berücksichtigt werden. Beispielhaft wird die Invasion von Marmorkrebsen (*Procambarus virginalis*) in Deutschland in Systeme mit einer etablierten Vergleichsart (dem Kamberkrebs *Faxonius limosus*) vorgestellt. Das Schema ist auf ganz unterschiedliche neuartige Organismen und Invasionsszenarien anwendbar.

Im zweiten Kapitel wird das Schema aus Kapitel 1 mithilfe einer Laborstudie auf das erwähnte Beispiel angewandt und ein mechanistisches Modell zu funktionellen Reaktionen parametrisiert. Die Räuber-Beute-Beziehung zwischen den Krebsarten sowie der Individuen und einem wichtigen aquatischen Primärproduzenten (*Dreissena* spp.) werden ausführlich untersucht, um Unterschiede in trophischen Eigenschaften zu erklären. Daten aus Videoaufzeichnungen von Krebsen bei der Nahrungssuche und -aufnahme werden genutzt, um phänomenologisch gemessene funktionelle Reaktionen durch unabhängig gemessene Prädationsparameter zu modellieren und vorherzusagen. Zusätzlich werden die empirisch gemessenen und modellierten funktionellen Reaktionen mit individuellen Verhaltensweisen in Beziehung gesetzt. Außerdem wird in diesem Kapitel die Aussagekraft der Prädationsparameter in Bezug auf funktionelle Reaktionen gezeigt und erörtert.

Im dritten Kapitel werden ausgewählte Verhaltensweisen, die für den Invasionserfolg von Bedeutung sein können, evaluiert und verglichen. Individuelle Eigenschaften, welche die zwischenartliche Beziehung betreffen – wie z.B. agonistisches Verhalten zwischen zwei Krebsen und deren Reaktion auf Räuber – werden getestet, aber auch die Aktivität, welche in Bezug zur Nahrungssuche steht werden hier untersucht. Zusätzlich werden Korrelationen zwischen Verhaltensweisen untersucht, so genannte Verhaltenssyndrome. In diesem Abschnitt werden außerdem die Verhaltensunterschiede zwischen naiven Individuen aus Aquarien und in Seen gefangenen Tieren verglichen und die Bedeutung der beobachteten Verhaltensweisen für neuartige Organismengemeinschaften diskutiert.

Schließlich werden im vierten Kapitel die Nahrungswahl von Marmorkrebsen und frei lebenden Kamberkrebsen im Labor mit im Freiland erhobenen Daten zur Ernährungsweise verglichen. Die trophische Position und Nahrungsnischengröße werden erhoben, um die ökologische Funktion der Arten einzuordnen. Um zu verstehen, welche Ressourcen die beiden Arten nutzen und welche Beute oder Ressource am meisten beeinträchtigt wird, wurden die Präferenzen und Konsumption in räuberfreier Umgebung gemessen und durch stabile Isotopendaten von Seen errechnet. Dieser Abschnitt zeigt den Einfluss von Marmorkrebsen auf Nahrungsnetze, in denen sie bereits vorkommen und hebt besonders bedeutende Interaktionen in einem Ökosystemzusammenhang hervor.

Meine Dissertation präsentiert ein neuartiges Interaktionsschema für die Anwendung in der Risikobewertung (Kapitel 1), detaillierte und mechanistische Ansätze in der Bestimmung von Räuber-Beute-Beziehungen (Kapitel 2) sowie Verhaltensweisen (Kapitel 3) und Nahrungsnetzauswirkungen (Kapitel 4) neuartiger Marmorkrebse und einer funktional ähnlichen Vergleichsart, dem Kamberkrebs. Dadurch trägt diese Arbeit zum grundlegenden Verständnis von Ökologie und Invasionsbiologie sowie angewandtem Ökosystemmanagement mit einem Fokus auf Krebsen bei.

Schlagwörter:

Biologische Invasionen; Flusskrebse; Funktionelle Reaktion; Trophische Position; Räuber-Beute Beziehung; Verhalten

THESIS OUTLINE

The dissertation consists of a general introduction, four separate chapters and a general discussion. The general introduction describes the background and purpose of the study and defines the research objective. Each of the following chapters represents an independent manuscript and, except Chapter 1, follows the conventional structure for research papers, with subsections for the introduction, methods, results and discussion. All manuscripts have either been published (Chapter 1 and 3) or have been submitted (Chapter 2 and 4) to a peer-reviewed scientific journal. In the last section, the thesis' findings are synthesized, evaluated and discussed with respect to prior research on the topics, and recommendations for future research are proposed.

LIST OF PUBLICATIONS WITH AUTHOR CONTRIBUTIONS

Author of this dissertation: Stefan Markus Linzmaier (SML)

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The following publications derived from this thesis:

Penk, M., W.-C. Saul, J. T. A. Dick, I. Donohue, M. E. Alexander, **S. Linzmaier**, and J. M. Jeschke. 2017. A trophic interaction framework for identifying the invasive capacity of novel organisms. Methods in Ecology and Evolution **8**:1786–1794. https://doi.org/10.1111/2041-210X.12817

JMJ and MP jointly conceived the idea of the study, developed the framework and discussed it with the other authors. SML developed the case study. MP drafted the manuscript. All authors reviewed and revised the manuscript.

Linzmaier, S. M. and J. M. Jeschke: *Towards a mechanistic understanding of individual-level functional responses: invasive crayfish as model organisms*.

SML and JMJ jointly conceived the idea of the study and designed the experiments. SML performed the experiments and analyzed the data. SML drafted the manuscript and both authors contributed to revisions. The study has been submitted to the journal Freshwater Biology.

Linzmaier, S. M., L. S. Goebel, F. Ruland, and J. M. Jeschke. 2018. Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. Ecosphere **9**:e02385. https://doi.org/10.1002/ecs2.2385

SML and JMJ conceived the idea of the study and designed the experiments. SML and LSG performed the experiments. SML gathered the data. FR developed and performed the statistical models. SML drafted the manuscript. All authors reviewed and revised the manuscript.

Linzmaier, S. M., C. Musseau, S. Matern and J. M. Jeschke: *Trophic ecology of invasive marbled and spiny-cheek crayfish populations.*

SML and JMJ conceived the idea of the study. SML designed and performed the laboratory experiments and field sampling and processed the samples. SML and CM jointly analyzed the data and developed the structure of the manuscript. SM contributed additional data from field sites. SML drafted the manuscript. All authors reviewed and revised the manuscript. The study has been submitted to the journal Biological Invasions.

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[1] The figure and table numbers of published articles have been adjusted to meet the format of the dissertation.

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THESIS INTRODUCTION

Communities and ecosystems around the globe change at an accelerating pace due to human-driven changes, such as pollution, the exchange of species or climate change (Alexander et al. 2015, Burdon et al. 2016, Seebens et al. 2017). *Homo sapiens* colonized most of the planet and manages to thrive under conditions that are vastly different from where it had evolved (Levin 2015). Since humans started colonizing the lands beyond their African origin, they never traveled alone. At the beginning of this exodus, it might have been mostly viruses, bacteria and other parasites, associated with the human body, but soon humans deliberately took other species along on their travels (Zeder 2015). Thus, humans helped to spread a relatively small number of species across vast areas that were formerly inaccessible for these species. Species were exchanged between human populations back and forth if they seemed to be useful and other less conspicuous species joined them as contaminants or stowaways. These processes have been going on for millennia, but deliberate relocation and the increasing globalization of trade within the last couple of hundred years enabled ever more species to escape their somehow defined habitats and invade foreign territory, which is also very different from their origins. Globalization and economic development have led to an unpredictable legacy of introduced, non-native species described as invasion debt, which will possibly lead to the establishment and spread of an unknown number of species in the future (Essl et al. 2011).

Species invasions have blurred human ideas of nature. The number of species that thrive far away from their origins steadily increases (Seebens et al. 2017). Simultaneously, the loss of species in their home range takes the form of a mass extinction (Ceballos et al. 2015). Landscapes and ecosystems cannot be defined as natural or unnatural, but are instead subject to different degrees of modification (Hobbs et al. 2014). Therefore, nature conservation and society as a whole will have to reevaluate nature and the species that are part of it (Kueffer and Kull 2017). Unsurprisingly, questions such as (1) “Can invasive species facilitate native species?” (Rodriguez 2006) or (2) when are novel species considered native (Carthey and Banks 2012), can be hotly debated.

New and novel organisms arise and the invaders that we observe today might be over-invaded in the future (Jeschke et al. 2013, Russell et al. 2014). Ecological novelty summarizes these fast new forms of anthropogenically-driven changes in ecosystems (Hobbs et al. 2009, Kueffer 2015). Novel organisms (such as invasive species, genetically modified organisms, emerging pathogens, artefactual organisms), novel species interactions and communities within novel ecosystems demand increasing attention as environmental change accelerates (Williams and Jackson 2007, Hobbs et al. 2009, Lurgi et al. 2012, Jeschke et al. 2013, Bezemer et al. 2014, Carthey and Banks 2014). The success or failure of these novel organisms depends on human activities and evolutionary processes (Mooney and Cleland 2001). For example, the numbers of introduced individuals of a species (i.e. propagule pressure) is a decisive factor for the establishment of populations (Lockwood et al. 2009). But successful novel species rapidly adapt to their environment (Sih et al. 2011) and have shown fast-paced adaptations to ongoing changes (Colautti and Barrett 2013, Ricciardi et al. 2013). In some cases, interactions of new and novel species with biotic and abiotic elements of the invaded systems resulted in species loss and the devastation of ecosystems (Clavero and García-Berthou 2005, Mollot et al. 2017). Some invasive species even provided new resources or functionally substituted extinct taxa (Hubbell 2005, Pejchar et al. 2009). Thus, species invasions do not only tell the history of human development, but they also provide ideal models for ecological and evolutionary theories.

Understanding the mechanisms behind the processes of emergence, introduction, establishment, spread and impact of novel species gives us the means to react appropriately to the negative or positive consequences in the wake of ecosystem changes (Jeschke et al. 2013). Since invasive species are thought to drive ecological change, the functional differences and commonalities of species from different origins are thought to be the main reason for their success or failure (Mitchell et al. 2006, Hufbauer and Torchin 2007, Sih et al. 2010). New and novel species assemblages harbor species that have traits unfamiliar to the species they interact with (Saul and Jeschke 2015). The interactions within such communities are often unknown and can alter ecosystem stability (Traveset et al. 2013). Species invasions therefore provide insightful research opportunities for community assembly and dynamics (Puth and Post 2005).

Finding traits that promote invasiveness has always been a goal of invasion ecologists, who compare the traits of invasive and native species or among invasive species. Especially plant traits and plasticity of potentially favorable traits have been studied intensively (e.g. van Kleunen et al. 2010, Davidson et al. 2011). In animals, life-history traits, associations with humans or differences in behavioral or personality traits have all been related to successful invaders (Keller et al. 2011, Chapple et al. 2012, Sih et al. 2012). Another approach to assess invasiveness of organisms, independently of taxonomy, is to look at their resource use in relation to resource density (i.e. functional responses) and abundance (i.e. numerical response; Dick et al. 2013, Dick et al. 2017). Combining the insights of several of these concepts is needed to better understand the invasion process and invasion success or failure of novel species (Jackson et al. 2017).

When novel species enter communities, they engage in multiple interactions. These interactions can take the form of direct links (e.g. predation) but also indirect links (competition, trophic cascades) and indirect non-trophic effects that affect, for example, the behavior of other species (Jackson et al. 2017). Novel species can be phylogenetically and functionally very different from resident species (Gallardo et al. 2016), or, in some instances, species can be functionally equivalent, interacting in similar ways as established members of the community (Hubbell 2005). Especially, interactions among multiple functionally similar novel species are rarely studied empirically (Russell et al. 2014). However, some exceptions include Hudina et al. (2011), Burlakova et al. (2014), Russell et al. (2015) and James et al. (2016). Overall, these interactions are part of greater networks or webs, such as plant-pollinator networks, host-parasite networks or food webs

(Lopezaraiza–Mikel et al. 2007, Lafferty et al. 2008, Kéfi et al. 2012, Pocock et al. 2012, Wollrab et al. 2012). While food webs are a classic research object in community ecology, it is an open question how they change due to the arrival of novel organisms. How invasive species can dominate or are succumbed by interacting species of the recipient community can be studied in predator-prey relationships and competitive interactions (Procheş et al. 2008, Thuiller et al. 2010, Carthey and Banks 2014). The exact quantification of stronger and weaker interaction strength among these predator-prey interactions is essential to gain insights on system stability with novel organisms (Wootton and Emmerson 2005).

All networks are rooted in the behaviors and traits of individuals, which are increasingly studied to mechanistically understand general ecological relationships, and invasion success (Ings et al. 2009, Chapple et al. 2012). The role of individual behavior in invasions gained more research interest in the last decade (Sih et al. 2010). But the link between individual behavioral traits and functional feeding reactions is still largely unexplored (Toscano et al. 2016). For example, changes in diet or foraging behavior may affect predator-prey interactions and ultimately influence ecosystem structure (Lima and Dill 1990, Peckarsky et al. 1997, Levin et al. 2000). The capability to adapt to new resources or diets is crucial for the success of novel organisms (Shea and Chesson 2002, Sih et al. 2011). Many animals, however, often do not seem to maximize net energy intake (optimal foraging) because prey encounter rate, prey capture success or prey behavior and abundance may skew prey preference away from predicted patterns (Einfalt and Wahl 1997, Naef-Daenzer et al. 2000, Estes et al. 2003). Additionally, risk of predation, competition or interactions from other foragers affects foraging behavior (Sih 1993, Bateson 2002, Höner et al. 2002, Guillemain et al. 2007). In particular, territorial, agonistic and cannibalistic animals affect individual survival and energy intake (Olsson and Nyström 2009, Tigreros et al. 2018). Therefore, it is important to include behavioral data if food-web effects of novel species should be quantified.

Crayfish as invaders and model organisms

As exceptionally successful invaders, freshwater decapod crayfish from North America have had major impacts on recipient ecosystems all over the world (Holdich et al. 2009, Lodge et al. 2012). They are so successful that nine out of ten crayfish species become established after translocation to areas outside their native range (Holdich 1999). Most notably, these crayfish carried the crayfish plague pathogen *Aphanomyces astaci* that is deadly to most non-American crayfish species and which has already eradicated large portions of indigenous crayfish (IC) populations in Europe (Svoboda et al. 2017). In addition, non-indigenous crayfish (NICs) threaten native crayfish through intraguild predation and competition for resources (Holdich et al. 2009). Thus, crayfish are of high concern for nature conservation and pose a good model organism for invasions in aquatic systems.

> *"Yes, the crayfish are the great worldly-wise men that know the philosopher's stone. On the bottom of the rivers and brooks, they search out the little grains of gold, and enjoy every day as delicious a dish as Antony only once tasted out of the hands of his beloved Cleopatra"* (Herbst et al. 1790, p. 28)[2].

With these words, Johann Friedrich Willhelm Herbst poetically described the frugality of crayfish regarding their diet in his pioneering work on decapod biology. The omnivorous diet and central trophic function of crayfish was already very much acknowledged in the 18th century, as Herbst et al. (1790, p. 29) continue:

> *"The most common and certain food of the crabs is meat. But I mean by this all animal bodies; it is now carrion of animals, or in the sea that of drowned men, or fish, frogs, insects, worms, shells, water-snails; even her own family is not spared by them* [...]. *In the meantime, the flesh of animals is not their only food, but they also find so much good taste in plants and fruits* [...]. *Yes, even the greasiness of the soil and some impurities serve as food for them."*[2]

But, at the time of this treatise, the changes that were to come with the first translocated crayfish were still unforeseeable.

Opportunistic, generalist predators, such as crayfish in aquatic systems or rats in terrestrial systems, are among the most impactful invaders that interact with abiotic and biotic parts of ecosystems (Snyder and Evans 2006, Clout and Russell 2008, Gallardo et al. 2016). In temperate freshwaters, crayfish are the largest invertebrates and can occur at high densities where they usually take up the role of intermediate consumers (Holdich and Crandall 2002, Roth et al. 2006). Crayfish can strongly influence community structure and alter food webs bottom-up and top-down, being important prey and predator alike (e.g. Nyström et al. 2001, Moore et al. 2012, Pulkkinen et al. 2013, van der Wal et al. 2013, Jackson et al. 2014). Moreover, ICs and NICs modify sediments (Usio and

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[2] Translated into English from the German original by Stefan Linzmaier.

Townsend 2004), the breakdown of organic matter (Dunoyer et al. 2014, Doherty-Bone et al. 2018), the macrophyte fauna (van der Wal et al. 2013) and the composition and abundance of zoobenthic organisms (McCarthy et al. 2006). Also, they provide an energy-rich food source for many (top) predators (Fischer et al. 2009, Aquiloni et al. 2010, Boyle et al. 2014). The magnitude of these effects can be modified by habitat characteristics (Weinländer and Füreder 2012, Ruokonen et al. 2014) and the ability of predators, competitors and prey (Nyström et al. 2001) to cope with the new species. Finally, intraspecific variability among a novel species can determine the magnitude of impact (Evangelista et al. 2019). Overall, there are multiple biotic and abiotic interactions among crayfish and their host ecosystems.

NICs typically replace ICs, which means that their behavior and resource use should not be completely novel to colonized ecosystems (Gherardi et al. 2011). In that case, invader effects or interaction strength may still be strong if they reach higher densities or larger sizes than the native species or a distinctive mode of resource exploitation (Strayer et al. 2006, Gherardi 2007, Gherardi et al. 2011, Hansen et al. 2013). Even at similar density and size, introduced species may have a more flexible diet, be more efficient predators or have higher resource intake rates, and can thus exert higher pressure on native communities than resident crayfish (Usio et al. 2006, Haddaway et al. 2012, Ercoli et al. 2014). For example, NICs have been proposed to be more opportunistic feeders and to switch faster to different prey items than ICs (invasion displacement dichotomy – Gherardi et al. (2001)). NICs have also been shown to consume more prey items than their native congeners (Olsen et al. 1991, Haddaway et al. 2012). And NICs are often different in their behavior, being more aggressive, active and bolder than ICs (Weis 2010). But how do formerly successful NICs and the invaded communities react to the arrival of another, similar NIC?

Some NICs, especially from the aquarium trade, are either so new in Europe or so obscure that their impact on natural water bodies is completely unknown (Chucholl and Wendler 2017). Over-invasions are increasingly observed in crustaceans, especially amphipods that successively altered the species composition of major European river systems like the Rhine (Bernauer and Jansen 2006, Leuven et al. 2009). Also, decapods increasingly co-occur with other invaders as they expand ranges and additional species are released into nature (Chucholl et al. 2008, Hudina et al. 2011, James et al. 2016). Since crayfish are omnivorous, some scientists assume functional equivalence among crayfish species (Ercoli et al. 2014). But other studies found species-specific effects on food webs (Dunoyer et al. 2014, Larson et al. 2017). Management efforts and resources to control the aquatic invaders are limited and have to be directed at critical organisms (Vander Zanden and Olden 2008). Thus, the question whether novel species cause fundamental changes and add new functions or replace existing nodes in the food web is crucial for managers.

Marbled crayfish as novel organisms

In 2003, a new Cambarid crayfish was found and described for the first time in a lake near Freiburg, Germany (Marten et al. 2004), the marbled crayfish (*Procambarus virginalis*), which was recognized as an independent species in 2017 (Lyko 2017; Figure 1). This species descends from the American slough crayfish (*Procambarus fallax*) and suddenly appeared in the German pet trade in 1995 (Lukhaup 2001, Seitz et al. 2005, Martin et al. 2010, Lyko 2017, Gutekunst et al. 2018). In other words, this truly novel species lacks a natural distribution as it apparently emerged by autopolyploidy in the tank of a German hobby aquarist (Vogt et al. 2015, Lyko 2017, Gutekunst et al. 2018).

Most likely originating from aquarium releases, marbled crayfish managed to establish populations in lotic and lentic environments of various trophic states, temperature regimes and even in polluted water bodies (Vogt 2017). They know occur in Germany (Chucholl et al. 2012), Italy (Marzano et al. 2009), Slovakia (Lipták et al. 2016), Hungary (Lőkkös et al. 2016), Croatia (Samardžić et al. 2014), Czech Republic (Patoka et al. 2016), The Netherlands (Souty-Grosset et al. 2006), Sweden (Bohman et al. 2013), Romania (Pârvulescu et al. 2017), Madagascar (Jones et al. 2009, Kawai et al. 2009), Ukraine (Novitsky and Son 2016), Estonia (Estonian Research Council 2018) and Japan (Kawai and Takahata 2010).

Due to their unique set of traits and fast life history, Jones et al. (2009) named marbled crayfish the *perfect invader*. Its most striking trait is the capacity to reproduce parthenogenetically, which is unique for decapod crustaceans and thus makes them a functionally novel species (Scholtz et al. 2003, Saul and Jeschke 2015). Notably, it was found that every marbled crayfish worldwide stems from a single clone or single individual (Vogt et al. 2015), which was confirmed by whole genome analysis (Gutekunst et al. 2018). Its novelty and parthenogenetic mode of reproduction spark the interest of biologists from different fields (Hossain et al. 2018 and references therein). Marbled crayfish have been enthusiastically embraced as model organisms for a variety of biological research fields like evolution, ecology and behavior as well as epigenetics and biogerontology (Vogt 2008, Luna et al. 2009, Vogt 2012).

Scientists and management authorities estimate that marbled crayfish will further spread and become a problematic invader throughout Europe (Chucholl et al. 2012, Chucholl and Wendler 2017). Hence, marbled crayfish and four other American crayfish have been classified as invasive alien species of European Union concern under the Regulation 1143/2014 to curb further spread. However, knowledge on the behavior of marbled crayfish is very limited and their ecology is mostly unknown (Chucholl et al. 2012, Vogt et al. 2018). If this species does not bring about the anticipated impacts, the scarce resources for prevention and management might be better spend on other invaders (Simberloff et al. 2013). More information is needed to estimate the risk of further spread and impact of marbled crayfish on colonized water bodies (Chucholl 2015). Usually, invasion biologists look at a species' native habitat and how it behaves in its native community to get an idea how a species might behave in the invaded range. However, this is not possible for marbled crayfish due to its obscure origin in aquaria ("Anökozoon"). Overall, the marbled crayfish are still obscure and their invasive potential has never been empirically assessed.

Figure 1 – Marbled crayfish (*Procambarus virginalis*).

Marbled crayfish that are released from their artificial habitats into European water bodies today encounter mostly NICs that have been introduced decades ago. These species share a phylogenetic history or eco-evolutionary experience, which can pose a disadvantage for the novel marbled crayfish (Saul and Jeschke 2015). Another species, which is also on the EU list, is an "old NIC", the spiny-cheek crayfish (*Faxonius limosus*; Figure 2). Spiny-cheek crayfish were introduced in the 1890s and are now the most common decapod in Central and Eastern Europe (Kouba et al. 2014). The spiny-cheek crayfish frequently co-occurs with marbled crayfish (Chucholl and Pfeiffer 2010, Chucholl et al. 2012, Lipták et al. 2016), but it is unclear if the syntropy of marbled crayfish and spiny-cheek crayfish can be stable in the long-term or whether one species will outcompete the other. NICs have been shown to outcompete ICs even without the devastating effects of crayfish plague (Westman et al. 2002). For example, exploitative or interference competition could lead to the replacement of established old NICs by new NICs. Marbled crayfish in Europe can be considered in the stage of initial dispersal, which is the most critical phase of the invasion process to enact management and provides the opportunity to trace community assembly and meta-community dynamics (Puth and Post 2005). Given their recent and early stages of dispersal and their interactions with established NICs, naturalized marbled crayfish can present insights into adaptation to interaction partners and the outcome of over-invasions.

Figure 2 – Male spiny-cheek crayfish (*Faxonius limosus*).

Thesis objectives

Invasion success or failure often depends on the trait- and density-mediated interactions related to predation and competition (Werner and Peacor 2003, Sih et al. 2010). Using the marbled crayfish as an example, I addressed the following questions: (1) How do ecological interactions change in communities due to the arrival of novel organisms, and (2) which species adaptations might result from these changes? And (3) what role does individual behavior play? To address these questions, I combined empirical and theoretical approaches from the fields of community, invasion and behavioral ecology (Jackson et al. 2017). Research in these fields is often phenomenological and rarely mechanistic (Simberloff 2004, McGill et al. 2006). Therefore, a main objective was to create a mechanistic understanding of the (changes in) interactions between competitors and between predators and prey, as novel organisms enter communities.

Chapter 1 exemplarily compares the focal novel organism (marbled crayfish) in a quantitative trait analysis with a species that is already present in the community and exhibits comparable traits and a similar ecology: the spiny-cheek crayfish. The idea is to derive expected community changes by comparing the autecological traits of two species. For example, Figure 3 depicts a food web that is invaded by a novel organism whose traits can be compared to a very similar native organism (in this case the planktivorous fish species no. 2). The framework behind this concept is explained in detail in Chapter 1. It would also be possible to compare the community where the introduced species is native to the community that it is invading. Yet, since the marbled crayfish is not native to any community, I looked at the organisms of a model focal community it would readily feed on to proceed with the first type of comparison. What type of food do they prefer? What sizes do they select for?

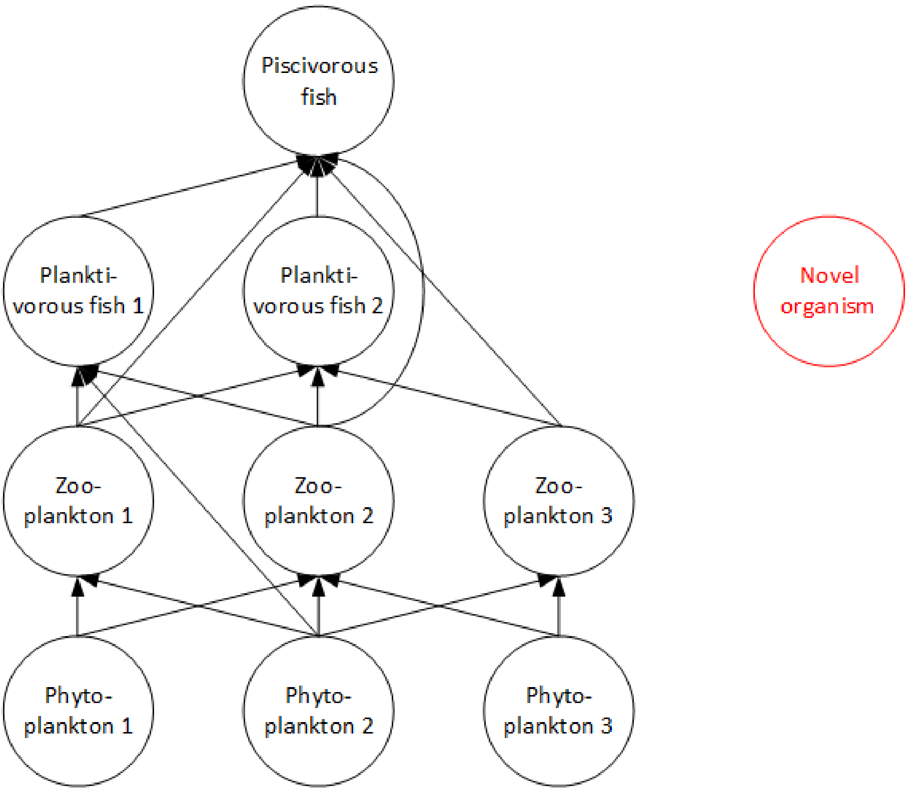

Figure 3 – A hypothetical simplified food web before the arrival of a novel organism.

To get a mechanistic understanding of food intake and therefore predator-prey interactions, Chapter 2 compares the steps of the predation cycle (search, encounter, detection, attack and consumption) and investigates where the two species differ and how individuals differ in these parameters. The link between parameters and the per-capita effects of predator functional response is verified as well, with appropriate functional response models. Functional response curves proceed in three basic types (I, II and III) that describe the food intake and its dependence on food density (Holling 1959, Jeschke et al. 2002). So far, only few studies have empirically tested the parameters of mechanistic functional response models (Jeschke et al. 2002, Jeschke and Hohberg 2008). In the context of novel species interactions, only theoretical predictions have been made thus far (Saul and Jeschke 2015), which are addressed empirically in this chapter. Other studies have shown that successful invasive species tend to have functional responses that proceed above their comparator species, i.e. they have higher consumption rates across a range of prey densities (Dick et al. 2013, Dick et al. 2014). Assuming that functional responses of native and invasive species are linked to impact on specific prey, these findings are used to assess the impact of marbled crayfish and to provide a more mechanistic explanation of why this is the case. Additionally, functional response experiments were repeated with individuals taken from field sites, where competitors, predator and prey had time to adapt behaviorally (or genetically) for several generations. It was tested whether changes in functional responses can be observed in comparison with experimental trials on naïve individuals. Since marbled crayfish are parthenogenetic, all changes should be referred to individual behavioral differences.

Furthermore, Chapter 3 asks how behavior mediates the interaction between two species and how individual behavioral types and eco–evolutionary experience (naïve aquarium vs co-occurring) influence foraging behavior. Therefore, aggressiveness, boldness and shelter use of individual crayfish was measured. These traits determine key intra- and interspecific interactions like interference competition that underlie the competitive displacement and invasion success of exotic crayfish (Pintor et al. 2008, Weis 2010). The results of these behavioral essays were then related to the individual functional responses from Chapter 2 to fathom the relationship between behavior and functional response. For example, are the all-female populations of marbled crayfish inferior to spiny-cheek crayfish males in fights? Do more bold or aggressive individuals have higher consumption rates?

Finally, Chapter 4 investigates invaded food webs with marbled crayfish and spiny-cheek crayfish in Germany. Prey-choice between the two species was studied by combining laboratory experiments with long-term diet data from stable isotope analysis of the naturalized marbled crayfish. Trophic function of different crayfish species has often been labeled as equivalent (Twardochleb et al. 2013), but reassembling the community after introduction of a functionally equivalent species should lead to niche partitioning (Winemiller et al. 2015). Consequently, these differences in trophic niches between species can substantially alter their impacts (Jackson et al. 2014, Jackson et al. 2016, Larson et al. 2017). Thus, Chapter 4 examines species-specific data on diet and trophic position of marbled crayfish to identify differences in synecological traits and assess whether the over-invasion of marbled crayfish has the potential for wider food-web consequences.

Literature cited

Alexander, J. M., J. M. Diez, and J. M. Levine. 2015. Novel competitors shape species' responses to climate change. Nature **525**:515–518.

Aquiloni, L., S. Brusconi, E. Cecchinelli, E. Tricarico, G. Mazza, A. Paglianti, and F. Gherardi. 2010. Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a predator of *Procambarus clarkii*. Biological Invasions **12**:3817–3824.

Bateson, M. 2002. Recent advances in our understanding of risk-sensitive foraging preferences. Proceedings of the Nutrition Society **61**:509–516.

Bernauer, D., and W. Jansen. 2006. Recent invasions of alien macroinvertebrates and loss of native species in the upper Rhine River, Germany. Aquatic Invasions **1**:55–71.

Bezemer, T. M., J. A. Harvey, and J. T. Cronin. 2014. Response of Native Insect Communities to Invasive Plants. Annual Review of Entomology **59**:119–141.

Bohman, P., L. Edsman, P. Martin, and G. Scholtz. 2013. The first Marmorkrebs (Decapoda: Astacida: Cambaridae) in Scandinavia. BioInvasions Records **2**:227–232.

Boyle, R. A., N. J. Dorn, and M. I. Cook. 2014. Importance of crayfish prey to nesting white ibis (*Eudocimus albus*). Waterbirds **37**:19–29.

Burdon, F. J., M. Reyes, A. C. Alder, A. Joss, C. Ort, K. Räsänen, J. Jokela, R. I. Eggen, and C. Stamm. 2016. Environmental context and magnitude of disturbance influence trait-mediated community responses to wastewater in streams. Ecology and Evolution **6**:3923–3939.

Burlakova, L. E., B. L. Tulumello, A. Y. Karatayev, R. A. Krebs, D. W. Schloesser, W. L. Paterson, T. A. Griffith, M. W. Scott, T. Crail, and D. T. Zanatta. 2014. Competitive Replacement of Invasive Congeners May Relax Impact on Native Species: Interactions among Zebra, Quagga, and Native Unionid Mussels. Plos One **9**:e114926.

Carthey, A. J., and P. B. Banks. 2014. Naïveté in novel ecological interactions: lessons from theory and experimental evidence. Biological Reviews **89**:932–949.

Carthey, A. J. R., and P. B. Banks. 2012. When Does an Alien Become a Native Species? A Vulnerable Native Mammal Recognizes and Responds to Its Long-Term Alien Predator. Plos One **7**:e31804.

Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances **1**:e1400253.

Chapple, D. G., S. M. Simmonds, and B. B. Wong. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? Trends in Ecology & Evolution **27**:57–64.

Chucholl, C. 2015. Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion pathway. Pages 83–114 *in* T. Kawai, Z. Faulkes, and G. Scholtz, editors. Freshwater crayfish: a global overview. CRC Press, Boca Raton, USA.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Chucholl, C., and M. Pfeiffer. 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., H. B. Stich, and G. Maier. 2008. Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. Fundamental and Applied Limnology / Archiv für Hydrobiologie **172**:27–36.

Chucholl, C., and F. Wendler. 2017. Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. Biological Invasions **19**:197–208.

Clavero, M., and E. García-Berthou. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology & Evolution **20**:110.

Clout, M. N., and J. C. Russell. 2008. The invasion ecology of mammals: a global perspective. Wildlife Research **35**:180–184.

Colautti, R. I., and S. C. H. Barrett. 2013. Rapid Adaptation to Climate Facilitates Range Expansion of an Invasive Plant. Science **342**:364–366.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters **14**:419–431.

Dick, J. T., M. E. Alexander, J. M. Jeschke, A. Ricciardi, H. J. MacIsaac, T. B. Robinson, S. Kumschick, O. L. Weyl, A. M. Dunn, and M. J. Hatcher. 2014. Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. Biological Invasions **16**:735–753.

Dick, J. T., K. Gallagher, S. Avlijas, H. C. Clarke, S. E. Lewis, S. Leung, D. Minchin, J. Caffrey, M. E. Alexander, C. Maguire, C. Harrod, N. Reid, N. R. Haddaway, K. D. Farnsworth, M. Penk, and A. Ricciardi. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions **15**:837–846.

Dick, J. T., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, and N. G. Taylor. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology **54**:1259–1267.

Doherty-Bone, T. M., A. M. Dunn, C. Liddell, and L. E. Brown. 2018. Transformation of detritus by a European native and two invasive alien freshwater decapods. Biological Invasions **20**:1799–1808.

Dunoyer, L., L. Dijoux, L. Bollache, and C. Lagrue. 2014. Effects of crayfish on leaf litter breakdown and shredder prey: are native and introduced species functionally redundant? Biological Invasions **16**:1545–1555.

Einfalt, L. M., and D. H. Wahl. 1997. Prey selection by juvenile walleye as influenced by prey morphology and behavior. Canadian Journal of Fisheries and Aquatic Sciences **54**:2618–2626.

Ercoli, F., T. J. Ruokonen, H. Hämäläinen, and R. I. Jones. 2014. Does the introduced signal crayfish occupy an equivalent trophic niche to the lost native noble crayfish in boreal lakes? Biological Invasions **16**:2025–2036.

Essl, F., S. Dullinger, W. Rabitsch, P. E. Hulme, K. Hülber, V. Jarošík, I. Kleinbauer, F. Krausmann, I. Kühn, and W. Nentwig. 2011. Socioeconomic legacy yields an invasion debt. Proceedings of the National Academy of Sciences **108**:203–207.

Estes, J., M. Riedman, M. Staedler, M. Tinker, and B. Lyon. 2003. Individual variation in prey selection by sea otters: patterns, causes and implications. Journal of Animal Ecology **72**:144–155.

Estonian Research Council. 2018. *Invasive marbled crayfish found in Narva power plant cooling canal* (8 June 2018). https://phys.org/news/2018-06-invasive-marbled-crayfish-narva-power.html, accessed 6 June 2019.

Evangelista, C., J. Cucherousset, and A. Lecerf. 2019. Contrasting ecological impacts of geographically close invasive populations. Oecologia **189**:529–536.

Fischer, D., P. Pavluvčík, F. Sedláček, and M. Šálek. 2009. Predation of the alien American mink, *Mustela vison* on native crayfish in middle-sized streams in central and western Bohemia. Folia Zoologica **58**:45–56.

Gallardo, B., M. Clavero, M. I. Sánchez, and M. Vilà. 2016. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology **22**:151–163.

Gherardi, F. 2007. Understanding the impact of invasive crayfish. Pages 507–542 *in* F. Gherardi, editor. Biological invaders in inland waters: Profiles, distribution, and threats. Springer, Dordrecht, The Netherlands.

Gherardi, F., L. Aquiloni, J. Diéguez-Uribeondo, and E. Tricarico. 2011. Managing invasive crayfish: is there a hope? Aquatic Sciences **73**:185–200.

Gherardi, F., B. Renai, and C. Corti. 2001. Crayfish predation on tadpoles: A comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). Bulletin Français de la Pêche et de la Pisciculture **361**:659–668.

Guillemain, M., C. Arzel, P. Legagneux, J. Elmberg, H. Fritz, M. Lepley, C. Pin, A. Arnaud, and G. Massez. 2007. Predation risk constrains the plasticity of foraging behaviour in teals, *Anas crecca*: a flyway-level circumannual approach. Animal Behaviour **73**:845–854.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, and F. Lyko. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nature Ecology & Evolution **2**:567–573.

Haddaway, N. R., R. H. Wilcox, R. E. Heptonstall, H. M. Griffiths, R. J. Mortimer, M. Christmas, and A. M. Dunn. 2012. Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. Plos One **7**:e32229.

Hansen, G. J. A., M. J. Vander Zanden, M. J. Blum, M. K. Clayton, E. F. Hain, J. Hauxwell, M. Izzo, M. S. Kornis, P. B. McIntyre, A. Mikulyuk, E. Nilsson, J. D. Olden, M. Papeş, and S. Sharma. 2013. Commonly Rare and Rarely Common: Comparing Population Abundance of Invasive and Native Aquatic Species. Plos One **8**:e77415.

Herbst, J. F. W., F. Guimpel, P. Haas, J. D. Heidenreich, J. R. Schellenberg, and L. Schmidt. 1790. Allgemeine Naturgeschichte der Krebse. Versuch einer Naturgeschichte der Krabben und Krebse - Nebst einer systematischen Beschreibung ihrer verschiedenen Arten, Bd. 1. Gottlieb August Lange, Berlin/Stralsund.

Hobbs, R. J., E. Higgs, C. M. Hall, P. Bridgewater, F. S. Chapin III, E. C. Ellis, J. J. Ewel, L. M. Hallett, J. Harris, K. B. Hulvey, S. T. Jackson, P. L. Kennedy, C. Kueffer, L. Lach, T. C. Lantz, A. E. Lugo, J. Mascaro, S. D. Murphy, C. R. Nelson, M. P. Perring, D. M. Richardson, T. R. Seastedt, R. J. Standish, B. M. Starzomski, K. N. Suding, P. M. Tognetti, L. Yakob, and L. Yung. 2014. Managing the whole landscape: historical, hybrid, and novel ecosystems. Frontiers in Ecology and the Environment **12**:557–564.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599–605.

Holdich, D. M. 1999. The negative effects of established crayfish introductions. Crustacean Issues **11**:31–48.

Holdich, D. M., and K. Crandall. 2002. Biology of freshwater crayfish. Blackwell Science, Oxford, UK.

Holdich, D. M., J. D. Reynolds, C. Souty-Grosset, and P. J. Sibley. 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.

Holling, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. The Canadian Entomologist **91**:293–320.

Höner, O. P., B. Wachter, M. L. East, and H. Hofer. 2002. The response of spotted hyaenas to long-term changes in prey populations: functional response and interspecific kleptoparasitism. Journal of Animal Ecology **71**:236–246.

Hossain, M. S., J. Patoka, A. Kouba, and M. Buřič. 2018. Clonal crayfish as biological model: a review on marbled crayfish. Biologia **73**:841–855.

Hubbell, S. P. 2005. Neutral theory in community ecology and the hypothesis of functional equivalence. Functional Ecology **19**:166–172.

Hudina, S., N. Galić, I. Roessink, and K. Hock. 2011. Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. Biological Invasions **13**:1791–1803.

Hufbauer, R. A., and M. E. Torchin. 2007. Integrating Ecological and Evolutionary Theory of Biological Invasions. Pages 79–96 *in* W. Nentwig, editor. Biological Invasions. Springer, Berlin/Heidelberg.

Ings, T. C., J. M. Montoya, J. Bascompte, N. Blüthgen, L. Brown, C. F. Dormann, F. Edwards, D. Figueroa, U. Jacob, J. I. Jones, R. B. Lauridsen, M. E. Ledger, H. M. Lewis, J. M. Olesen, F. J. F. Van Veen, P. H. Warren, and G. Woodward. 2009. Review: Ecological networks – beyond food webs. Journal of Animal Ecology **78**:253–269.

Jackson, M. C., J. Grey, K. Miller, J. R. Britton, and I. Donohue. 2016. Dietary niche constriction when invaders meet natives: evidence from freshwater decapods. Journal of Animal Ecology **85**:1098–1107.

Jackson, M. C., T. Jones, M. Milligan, D. Sheath, J. Taylor, A. Ellis, J. England, and J. Grey. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. Freshwater Biology **59**:1123–1135.

Jackson, M. C., R. J. Wasserman, J. Grey, A. Ricciardi, J. T. A. Dick, and M. E. Alexander. 2017. Novel and Disrupted Trophic Links Following Invasion in Freshwater Ecosystems. Pages 55–97 *in* D. A. Bohan, A. J. Dumbrell, and F. Massol, editors. Networks of Invasion: Empirical Evidence and Case Studies. Academic Press, Oxford, UK.

James, J., J. Thomas, A. Ellis, K. Young, J. England, and J. Cable. 2016. Over-invasion in a freshwater ecosystem: newly introduced virile crayfish (*Orconectes virilis*) outcompete established invasive signal crayfish (*Pacifastacus leniusculus*). Marine and Freshwater Behaviour and Physiology **49**:9–18.

Jeschke, J. M., and K. Hohberg. 2008. Predicting and testing functional responses: an example from a tardigrade–nematode system. Basic and Applied Ecology **9**:145–151.

Jeschke, J. M., F. Keesing, and R. S. Ostfeld. 2013. Novel Organisms: Comparing Invasive Species, GMOs, and Emerging Pathogens. Ambio **42**:541–548.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2002. Predator functional responses: discriminating between handling and digesting prey. Ecological Monographs **72**:95–112.

Jones, J. P., J. R. Rasamy, A. Harvey, A. Toon, B. Oidtmann, M. H. Randrianarison, N. Raminosoa, and O. R. Ravoahangimalala. 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. Biological Invasions **11**:1475–1482.

Kawai, T., G. Scholtz, S. Morioka, F. Ramanamandimby, C. Lukhaup, and Y. Hanamura. 2009. Parthenogenetic alien crayfish (Decapoda: Cambaridae) spreading in Madagascar. Journal of Crustacean Biology **29**:562–567.

Kawai, T., and M. Takahata. 2010. Biology of Crayfish. Hokkaido University Press, Sapporo, Japan.

Kéfi, S., E. L. Berlow, E. A. Wieters, S. A. Navarrete, O. L. Petchey, S. A. Wood, A. Boit, L. N. Joppa, K. D. Lafferty, and R. J. Williams. 2012. More than a meal… integrating non-feeding interactions into food webs. Ecology Letters **15**:291–300.

Keller, R. P., J. Geist, J. M. Jeschke, and I. Kühn. 2011. Invasive species in Europe: ecology, status, and policy. Environmental Sciences Europe **23**:23.

Kouba, A., A. Petrusek, and P. Kozák. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems **413**:05.

Kueffer, C. 2015. Ecological novelty: towards an interdisciplinary understanding of ecological change in the Anthropocene. Pages 19–37 *in* H. Greschke and J. Tischler, editors. Grounding Global Climate Change. Springer, Dordrecht, The Netherlands.

Kueffer, C., and C. A. Kull. 2017. Non-native Species and the Aesthetics of Nature. Pages 311–324 *in* M. Vilà and P. E. Hulme, editors. Impact of Biological Invasions on Ecosystem Services. Springer International Publishing, Cham, Switzerland.

Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. Johnson, A. M. Kuris, and D. J. Marcogliese. 2008. Parasites in food webs: the ultimate missing links. Ecology Letters **11**:533–546.

Larson, E. R., L. A. Twardochleb, and J. D. Olden. 2017. Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. Limnology **18**:275–286.

Leuven, R. S., G. van der Velde, I. Baijens, J. Snijders, C. van der Zwart, H. R. Lenders, and A. bij de Vaate. 2009. The river Rhine: a global highway for dispersal of aquatic invasive species. Biological Invasions **11**:1989.

Levin, N. E. 2015. Environment and climate of early human evolution. Annual Review of Earth and Planetary Sciences **43**:405–429.

Levin, P. S., N. Tolimieri, M. Nicklin, and P. F. Sale. 2000. Integrating individual behavior and population ecology: the potential for habitat-dependent population regulation in a reef fish. Behavioral Ecology **11**:565–571.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology **68**:619–640.

Lipták, B., A. Mrugała, L. Pekárik, A. Mutkovič, D. Gruľa, A. Petrusek, and A. Kouba. 2016. Expansion of the marbled crayfish in Slovakia: beginning of an invasion in the Danube catchment? Journal of Limnology **75**:305–312.

Lockwood, J. L., P. Cassey, and T. M. Blackburn. 2009. The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. Diversity and Distributions **15**:904–910.

Lodge, D. M., A. Deines, F. Gherardi, D. C. Yeo, T. Arcella, A. K. Baldridge, M. A. Barnes, W. L. Chadderton, J. L. Feder, and C. A. Gantz. 2012. Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. Annual Review of Ecology, Evolution, and Systematics **43**:449–472.

Lőkkös, A., T. Müller, K. Kovács, L. Várkonyi, A. Specziár, and P. Martin. 2016. The alien, parthenogenetic marbled crayfish (Decapoda: Cambaridae) is entering Kis-Balaton (Hungary), one of Europe's most important wetland biotopes. Knowledge and Management of Aquatic Ecosystems **417**:16.

Lopezaraiza–Mikel, M. E., R. B. Hayes, M. R. Whalley, and J. Memmott. 2007. The impact of an alien plant on a native plant–pollinator network: an experimental approach. Ecology Letters **10**:539–550.

Lukhaup, C. 2001. *Procambarus* sp. The Marbled crayfish. Aquaristik Aktuell **7–8**:48–51.

Luna, A. J. F., J. I. Hurtado-Zavala, T. Reischig, and R. Heinrich. 2009. Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. Journal of Biological Rhythms **24**:64–72.

Lurgi, M., B. C. López, and J. M. Montoya. 2012. Novel communities from climate change. Philosophical Transactions of the Royal Society B: Biological Sciences **367**:2913–2922.

Lyko, F. 2017. The marbled crayfish (Decapoda: Cambaridae) represents an independent new species. Zootaxa **4363**:544–552.

Marten, M., C. Werth, and D. Marten. 2004. The Marbled crayfish (Cambaridae, Decapoda) in Germany - another neozoan in the Rhein basin. Lauterbornia **50**:17–23.

Martin, P., N. J. Dorn, T. Kawai, C. van der Heiden, and G. Scholtz. 2010. The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). Contributions to Zoology **79**:107–118.

Marzano, F. N., M. Scalici, S. Chiesa, F. Gherardi, A. Piccinini, and G. Gibertini. 2009. The first record of the marbled crayfish adds further threats to fresh waters in Italy. Aquatic Invasions **4**:401–404.

McCarthy, J. M., C. L. Hein, J. D. Olden, and M. Jake Vander Zanden. 2006. Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. Freshwater Biology **51**:224–235.

McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. Trends in Ecology & Evolution **21**:178–185.

Mitchell, C. E., A. A. Agrawal, J. D. Bever, G. S. Gilbert, R. A. Hufbauer, J. N. Klironomos, J. L. Maron, W. F. Morris, I. M. Parker, and A. G. Power. 2006. Biotic interactions and plant invasions. Ecology Letters **9**:726–740.

Mollot, G., J. Pantel, and T. Romanuk. 2017. The Effects of Invasive Species on the Decline in Species Richness: A Global Meta-Analysis. Pages 61–83 *in* D. A. Bohan, A. J. Dumbrell, and F. Massol, editors. Advances in Ecological Research. Elsevier, Oxford, UK.

Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences **98**:5446–5451.

Moore, J. W., S. M. Carlson, L. A. Twardochleb, J. L. Hwan, J. M. Fox, and S. A. Hayes. 2012. Trophic tangles through time? Opposing direct and indirect effects of an invasive omnivore on stream ecosystem processes. Plos One **7**:e50687.

Naef-Daenzer, L., B. Naef-Daenzer, and R. G. Nager. 2000. Prey selection and foraging performance of breeding Great Tits *Parus major* in relation to food availability. Journal of Avian Biology **31**:206–214.

Novitsky, R., and M. Son. 2016. The first records of Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] (Crustacea, Decapoda, Cambaridae) in Ukraine. Ecologica Montenegrina **5**:44–46.

Nyström, P., O. Svensson, B. Lardner, C. Brönmark, and W. Granéli. 2001. The influence of multiple introduced predators on a littoral pond community. Ecology **82**:1023–1039.

Olsen, T. M., D. M. Lodge, G. M. Capelli, and R. J. Houlihan. 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. Canadian Journal of Fisheries and Aquatic Sciences **48**:1853–1861.

Olsson, K., and P. Nyström. 2009. Non-interactive effects of habitat complexity and adult crayfish on survival and growth of juvenile crayfish (*Pacifastacus leniusculus*). Freshwater Biology **54**:35–46.

Ottonello, D., S. Salvidio, and E. Rosecchi. 2005. Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). Amphibia-Reptilia **26**:562–565.

Pârvulescu, L., A. Togor, S. Lele, S. Scheu, D. Șinca, and J. Panteleit. 2017. First established population of marbled crayfish *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Decapoda, Cambaridae) in Romania. BioInvasions Records **6**:357–362.

Patoka, J., M. Buřič, V. Kolář, M. Bláha, M. Petrtýl, P. Franta, R. Tropek, L. Kalous, A. Petrusek, and A. Kouba. 2016. Predictions of marbled crayfish establishment in conurbations fulfilled: Evidences from the Czech Republic. Biologia **71**:1380–1385.

Peckarsky, B. L., S. D. Cooper, and A. R. McIntosh. 1997. Extrapolating from individual behavior to populations and communities in streams. Journal of the North American Benthological Society **16**:375–390.

Pejchar, L., H. A. Mooney, and evolution. 2009. Invasive species, ecosystem services and human well-being. Trends in Ecology & Evolution **24**:497–504.

Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos **117**:1629–1636.

Pocock, M. J., D. M. Evans, and J. Memmott. 2012. The robustness and restoration of a network of ecological networks. Science **335**:973–977.

Procheş, Ş., J. R. Wilson, D. M. Richardson, and M. Rejmánek. 2008. Searching for phylogenetic pattern in biological invasions. Global Ecology and Biogeography **17**:5–10.

Pulkkinen, K., T. J. Ruokonen, M. Mykrä, G. Tambe, J. Karjalainen, and H. Hämäläinen. 2013. Indirect effects of invasive crayfish on native fish parasites. Ecosphere **4**:50.

Puth, L. M., and D. M. Post. 2005. Studying invasion: have we missed the boat? Ecology Letters **8**:715–721.

Ricciardi, A., M. F. Hoopes, M. P. Marchetti, and J. L. Lockwood. 2013. Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs **83**:263–282.

Rodriguez, L. F. 2006. Can Invasive Species Facilitate Native Species? Evidence of How, When, and Why These Impacts Occur. Biological Invasions **8**:927–939.

Roth, B. M., C. L. Hein, and M. J. Vander Zanden. 2006. Using bioenergetics and stable isotopes to assess the trophic role of rusty crayfish (*Orconectes rusticus*) in lake littoral zones. Canadian Journal of Fisheries and Aquatic Sciences **63**:335–344.

Ruokonen, T. J., J. Karjalainen, and H. Hämäläinen. 2014. Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific. Freshwater Biology **59**:12–25.

Russell, J. C., S. Caut, S. H. Anderson, and M. Lee. 2015. Invasive rat interactions and over-invasion on a coral atoll. Biological Conservation **185**:59–65.

Russell, J. C., N. S. Sataruddin, and A. D. Heard. 2014. Over-invasion by functionally equivalent invasive species. Ecology **95**:2268–2276.

Samardžić, M., A. Lucić, I. Maguire, and S. Hudina. 2014. The first record of the marbled crayfish (*Procambarus fallax* (Hagen, 1870) f. *virginalis*) in Croatia. Crayfish news **36**:4-4.

Saul, W. C., and J. M. Jeschke. 2015. Eco-evolutionary experience in novel species interactions. Ecology Letters **18**:236–245.

Scholtz, G., A. Braband, L. Tolley, A. Reimann, B. Mittmann, C. Lukhaup, F. Steuerwald, and G. Vogt. 2003. Ecology: Parthenogenesis in an outsider crayfish. Nature **421**:806–806.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, and M. Arianoutsou. 2017. No saturation in the accumulation of alien species worldwide. Nature Communications **8**:14435.

Seitz, R., K. Vilpoux, U. Hopp, S. Harzsch, and G. Maier. 2005. Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. Journal of Experimental Zoology Part A: Comparative Experimental Biology **303**:393–405.

Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution **17**:170–176.

Sih, A. 1993. Effects of ecological interactions on forager diets: competition, predation risk, parasitism and prey behaviour. Pages 182–211 *in* R. N. Hughes, editor. Diet selection: an interdisciplinary approach to foraging behaviour. Blackwell, Oxford, UK.

Sih, A., D. I. Bolnick, B. Luttbeg, J. L. Orrock, S. D. Peacor, L. M. Pintor, E. Preisser, J. S. Rehage, and J. R. Vonesh. 2010. Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. Oikos **119**:610–621.

Sih, A., J. Cote, M. Evans, S. Fogarty, and J. Pruitt. 2012. Ecological implications of behavioural syndromes. Ecology Letters **15**:278–289.

Sih, A., M. C. Ferrari, and D. J. Harris. 2011. Evolution and behavioural responses to human-induced rapid environmental change. Evolutionary Applications **4**:367–387.

Simberloff, D. 2004. Community ecology: is it time to move on? The American Naturalist **163**:787–799.

Simberloff, D., J.-L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyšek, R. Sousa, E. Tabacchi, and M. Vilà. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution **28**:58–66.

Snyder, W. E., and E. W. Evans. 2006. Ecological Effects of Invasive Arthropod Generalist Predators. Annual Review of Ecology, Evolution, and Systematics **37**:95–122.

Souty-Grosset, C., D. M. Holdich, P. Y. Noël, J. D. Reynolds, and P. Haffner. 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France.

Strayer, D. L., V. T. Eviner, J. M. Jeschke, and M. L. Pace. 2006. Understanding the long-term effects of species invasions. Trends in Ecology & Evolution **21**:645–651.

Svoboda, J., A. Mrugała, E. Kozubíková-Balcarová, and A. Petrusek. 2017. Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. Journal of Fish Diseases **40**:127–140.

Thuiller, W., L. Gallien, I. Boulangeat, F. De Bello, T. Münkemüller, C. Roquet, and S. Lavergne. 2010. Resolving Darwin’s naturalization conundrum: a quest for evidence. Diversity and Distributions **16**:461–475.

Tigreros, N., E. H. Wang, and J. S. Thaler. 2018. Prey nutritional state drives divergent behavioural and physiological responses to predation risk. Functional Ecology **32**:982–989.

Toscano, B. J., N. J. Gownaris, S. M. Heerhartz, and C. J. Monaco. 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. Oecologia **182**:55–69.

Traveset, A., R. Heleno, S. Chamorro, P. Vargas, C. K. McMullen, R. Castro-Urgal, M. Nogales, H. W. Herrera, and J. M. Olesen. 2013. Invaders of pollination networks in the Galápagos Islands: emergence of novel communities. Proceedings of the Royal Society B: Biological Sciences **280**:20123040.

Twardochleb, L. A., J. D. Olden, and E. R. Larson. 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science **32**:1367–1382.

Usio, N., K. Suzuki, M. Konishi, and S. Nakano. 2006. Alien vs. endemic crayfish: roles of species identity in ecosystem functioning. Archiv für Hydrobiologie **166**:1–21.

Usio, N., and C. R. Townsend. 2004. Roles of crayfish: consequences of predation and bioturbation for stream invertebrates. Ecology **85**:807–822.

van der Wal, J. E., M. Dorenbosch, A. K. Immers, C. V. Forteza, J. J. Geurts, E. T. Peeters, B. Koese, and E. S. Bakker. 2013. Invasive crayfish threaten the development of submerged macrophytes in lake restoration. Plos One **8**:e78579.

van Kleunen, M., E. Weber, and M. Fischer. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters **13**:235–245.

Vander Zanden, M. J., and J. D. Olden. 2008. A management framework for preventing the secondary spread of aquatic invasive species. Canadian Journal of Fisheries and Aquatic Sciences **65**:1512–1522.

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. 2012. Ageing and longevity in the Decapoda (Crustacea): a review. Zoologischer Anzeiger - A Journal of Comparative Zoology **251**:1–25.

Vogt, G. 2017. Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: insights from clonal, invasive, polyploid, and domesticated animals. Environmental Epigenetics **3**:dvx002.

Vogt, G., N. J. Dorn, M. Pfeiffer, C. Lukhaup, B. W. Williams, R. Schulz, and A. Schrimpf. 2018. In-depth investigation of the species problem and taxonomic status of marbled crayfish, the first asexual decapod crustacean. bioRxiv:356170.

Vogt, G., C. Falckenhayn, A. Schrimpf, K. Schmid, K. Hanna, J. Panteleit, M. Helm, R. Schulz, and F. Lyko. 2015. The marbled crayfish as a paradigm for saltational speciation by autopolyploidy and parthenogenesis in animals. Biology Open **4**:1583–1594.

Weinländer, M., and L. Füreder. 2012. Associations between stream habitat characteristics and native and alien crayfish occurrence. Hydrobiologia **693**:237–249.

Weis, J. S. 2010. The role of behavior in the success of invasive crustaceans. Marine and Freshwater Behaviour and Physiology **43**:83–98.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**:1083–1100.

Westman, K., R. Savolainen, and M. Julkunen. 2002. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: a 30-year study. Ecography **25**:53–73.

Williams, J. W., and S. T. Jackson. 2007. Novel climates, no-analog communities, and ecological surprises. Frontiers in Ecology and the Environment **5**:475–482.

Winemiller, K. O., D. B. Fitzgerald, L. M. Bower, and E. R. Pianka. 2015. Functional traits, convergent evolution, and periodic tables of niches. Ecology Letters **18**:737–751.

Wollrab, S., S. Diehl, and A. M. Roos. 2012. Simple rules describe bottom-up and top-down control in food webs with alternative energy pathways. Ecology Letters **15**:935–946.

Wootton, J. T., and M. Emmerson. 2005. Measurement of Interaction Strength in Nature. Annual Review of Ecology, Evolution, and Systematics **36**:419–444.

Zeder, M. A. 2015. Core questions in domestication research. Proceedings of the National Academy of Sciences **112**:3191–3198.

CHAPTER 1

A TROPHIC INTERACTION FRAMEWORK FOR IDENTIFYING THE INVASIVE CAPACITY OF NOVEL ORGANISMS

Published as:
Penk, M., W.-C. Saul, J. T. A. Dick, I. Donohue, M. E. Alexander, **S. Linzmaier**, and J. M. Jeschke. 2017. A trophic interaction framework for identifying the invasive capacity of novel organisms. Methods in Ecology and Evolution **8**:1786–1794. https://doi.org/10.1111/2041-210X.12817

Abstract

1. The likelihood and impacts of invasions by novel organisms (e.g. non-native species, genetically modified organisms) on the composition and functioning of receiving biological communities hinges on their capacity to exploit resources and/or avoid predation relative to resident counterparts. While assessment of invasion risk based on the comparison of functional responses (per-capita consumption rate as a function of resource density) of novel species with native analogues has been gaining popularity, it may be undermined if alternative prey and potential predators are not represented realistically.

2. Here, we propose a conceptual framework that enables rigorous identification of trophic traits conducive to invasion success by novel organisms—irrespective of their trophic position—and their likely ecological impacts, given their arrival and establishment. We focus on consumption here, but our framework can also be used for autotrophic energy acquisition, and extended to non-trophic and indirect interactions.

3. The framework enables a structured and prioritized selection of subsets of trophic links for invasion risk assessment. It is based on foraging theory and advances in comparative functional responses in invasion ecology. It can even be used in the absence of a resident comparator organism and when resources or predators are only partly known.

4. Our approach enhances the predictive power of species screening, and thus advances prevention and management of invasions under a common framework for all types of novel organisms.

Keywords

alien species; dietary generalism; ecological novelty; functional responses; GMO; invasion success; predator–prey trophic interactions; risk assessment

Introduction

Predicting biological invasions (i.e. the spread of non-native species beyond the point of introduction) and managing their impacts (i.e. quantifiable alterations of the receiving ecosystem) remain key challenges in ecology (Simberloff et al. 2013). This demands improved understanding of the mechanisms of invasions. Human-assisted species translocations entail transfers across barriers that limit natural dispersal, and thus between environments which can have substantially different eco-evolutionary histories. Thus, introduced organisms can impart a high degree of ecological novelty to a system, which is conducive to invasiveness (Saul et al. 2013). Organisms arriving in new environments enter resident ecological interaction networks, and the identification of their interactions within resident communities is important for understanding community dynamics. Predicting the attributes of these novel interactions is crucial for prioritizing management of existing and anticipated invasions, and for assessing the side effects of intended introductions. Novel organisms (including translocated, but also range-expanding, genetically modified, synthesized or resurrected organisms; Jeschke et al. (2013)), whose ecological traits contrast with the eco-evolutionary experience of their resident interaction partners (Saul and Jeschke 2015), can potentially transform resident interaction networks through, for example, altering strengths, spatio-temporal patterns or other functional attributes of interactions (Mitchell et al. 2006, Downing et al. 2012, Mayer et al. 2013, Penk et al. 2015).

Predation is a particularly important interaction type that can have strong impacts on community dynamics. This is primarily because it affects both predator fitness and prey biomass directly, potentially causing trophic cascades (Terborgh and Estes 2010, O'Connor et al. 2013) and food limitations for competitors (Strayer and Malcom 2007). Introduced predators can therefore affect resident prey populations significantly (Hays and Conant 2007, Strayer 2009, Downing et al. 2012), with efficient exploitation of resources being conducive to high population growth, likely invasion success, and potentially considerable ecological impacts. Resource density is a key determinant of the feeding rate, and this relationship is characterized by “functional response” curves (Holling 1959). For instance, decreasing prey density can reduce predator encounter rates with prey and thus offer a density-dependent prey refuge in a predator–prey system with a sigmoidal (i.e. type III) functional response, but not with alternative functional response shapes where high proportions of prey are killed at low prey densities (types I, II or their variants; Holling (1959), Jeschke et al. (2004), Jeschke and Tollrian (2005)). Thus, the height —in particular the maximum feeding rate (i.e. curve asymptote)— and the shape of functional responses can reveal characteristics of consumer-resource interactions that are important for community dynamics and composition.

Invading predators with high ecological impact on their prey populations often have elevated functional responses compared to ecologically similar native species (Dick et al. 2017). Using comparative functional responses as an empirical screening method is thus rapidly gaining popularity among invasion biologists (Dick et al. 2013, Alexander et al. 2014, Barrios-O'Neill et al. 2014b, Rosewarne et al. 2016, Xu et al. 2016). The method typically infers invasion success and/or potential impacts from a limited number of prey species (frequently just one). However, biological invasions tend to lead to replacement of niche specialists by generalists (Clavel et al. 2011), and numerous studies have identified a positive association between dietary generalism and invasion success (Bessa-Gomes et al. 2003, Jeschke and Strayer 2006, Romanuk et al. 2009, Clavel et al. 2011, Arbačiauskas et al. 2013; but see Cassey et al. 2004, Jackson et al. 2016). Thus, the inefficient use of one particular resident prey species does not necessarily preclude invasion success or impact upon ecological networks with realistic complexity. In other cases, a resident organism may be an inferior predator on a particular prey species, and falsely appear a weaker overall interactor, compared to an introduced predator solely as a result of differing specialization, despite apparent ecological similarity (Rosenfeld 2002, Dunoyer et al. 2014). Investigating functional responses with multiple prey, thereby taking into account the potential role of generalism and specialism, can buffer against such biases and at the same time improve the much needed representation of whole-ecosystem impacts of novel organisms (Ehrenfeld 2010, Simberloff 2011, Penk et al. 2015). Furthermore, novel organisms can themselves be controlled by resident predators (Romanuk et al. 2009, MacNeil et al. 2013, Pintor and Byers 2015). Not accounting for top-down control experienced by introduced species, as has been typically the case in functional response-based screening methods, risks over-estimating their consumptive impacts and invasion success (but see Barrios-O'Neill et al. 2014a, Alexander et al. 2015).

Both top-down and bottom-up trophic interactions can thus directly affect the survival, fitness and ecological impacts of novel organisms. The complexity of these trophic links, including diet breadth and number of enemies, is an important determinant of invasion success (Romanuk et al. 2009). However, the logistics of incorporating multiple prey and predators into comparative functional responses may be demanding and frequently prohibitive.

We propose a conceptual framework for identifying and selecting a prioritized subset of trophic links to empirically assess the capacity for invasion success and ecological impacts of novel organisms (Figure 1.1). We provide a worked example of the application of the framework for an intermediate consumer, the marbled crayfish (*Procambarus fallax* forma *virginalis*[3]) in German low-land lakes (Figure A.1). This includes selection of both predators and prey, and can therefore instruct assessment of organisms of any trophic position. In addition, the marbled crayfish does not have any known native populations, and thus it is exemplary of quite complex assessment scenarios. For clarity, our arguments relate to predation, which includes true predation, herbivory, parasitism and parasitoidism. However, detritivory can also be an important dietary subsidy of generalist consumers (Wise et al. 2006, Jackson et al. 2016) and should be carried through the assessment if it contributes to the diet of

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[3] Marbled crayfish are now officially recognized as a species – *Procambarus virginalis* (Lyko, F. 2017. The marbled crayfish (Decapoda: Cambaridae) represents an independent new species. Zootaxa **4363**:544-552).

the novel (“focal”) organism. Although we focus on consumption, our framework can be applied to autotrophic energy acquisition, and extended to non-trophic and indirect interactions. Whereas the non-empirical steps of our framework are readily applicable to any type of interaction, non-trophic interactions may require different empirical methods.

Mapping potential interaction partners in the target community

Unless interaction with a particular resident organism is an a priori focus of assessment, an initial step of comprehensively mapping a potential network of direct consumptive interactions of the focal organism in the receiving (“target”) community should enable minimization of selection biases that may impede realistic assessment of the impact of the novel organism. This can be achieved by first listing all partners in direct consumptive interactions of the focal organism in its established range. This is then followed by matching all resident organisms in the target community that conform to the archetypes of these interaction partners and are likely to at least partly share spatio-temporal distribution patterns with the focal organism (Figure 1.1). We define an archetype as organisms that have a similar set of morphological and behavioral traits that can condition a given type of interaction (Cox and Lima 2006, Winemiller et al. 2015), for example, feeding or defense strategy.

Observed trophic interactions in a given environment may not fully represent the feeding preferences of an organism (Futuyma and Moreno 1988, Devictor et al. 2010), and trophic interaction strength with a particular prey may depend on its availability in comparison to other prey rather than on the true preference of the consumer (Jaworski et al. 2013, Davis et al. 2015, Hanmer et al. 2017), as well as on environmental drivers. Thus, interaction partners of the focal organism as well as interaction strengths may vary among communities that differ in species composition and densities. If the focal organism is already established in the target environment, site-specific data about interaction partners should be given precedence above data from other areas. Otherwise, information from multiple communities within the distribution range of the focal species may improve control for context-dependencies. Assigning preference attributes based on how frequent and dominant interaction partners are throughout the established range (e.g. Kissling et al. 2014) can then help prioritize the selection of interaction partners for assessment. Considering ontogenetic stages of the focal organism with contrasting interaction partners (e.g. size class, identity or trophic guild of prey or enemies) could further improve predictions because limitation at any single stage preceding reproduction could constrict population dynamics (Werner and Gilliam 1984, Rudolf and Lafferty 2011).

For focal organisms that do not yet occur in nature, such as genetically modified, resurrected, synthetic, hybridized or selectively bred organisms, interaction partners of phylogenetically or functionally closest (“quasi-focal”) organisms may provide reasonable approximation. For example, the marbled crayfish, introduced recently to German freshwaters (Chucholl et al. 2012), originated in the aquarium trade and does not have any known native populations (Vogt et al. 2015). However, it is morphologically and functionally similar to the spiny-cheek crayfish (*Orconectes*[4] *limosus*), a well-established earlier invader in Germany which can be considered a quasi-focal organism. The spiny-cheek crayfish is an omnivore that feeds on benthic invertebrates and macrophytes, and itself falls prey to fish, waterfowl and mustelids. Interaction partners of the spiny-cheek crayfish are thus good candidate prey and predators of the marbled crayfish (Figure A.1).

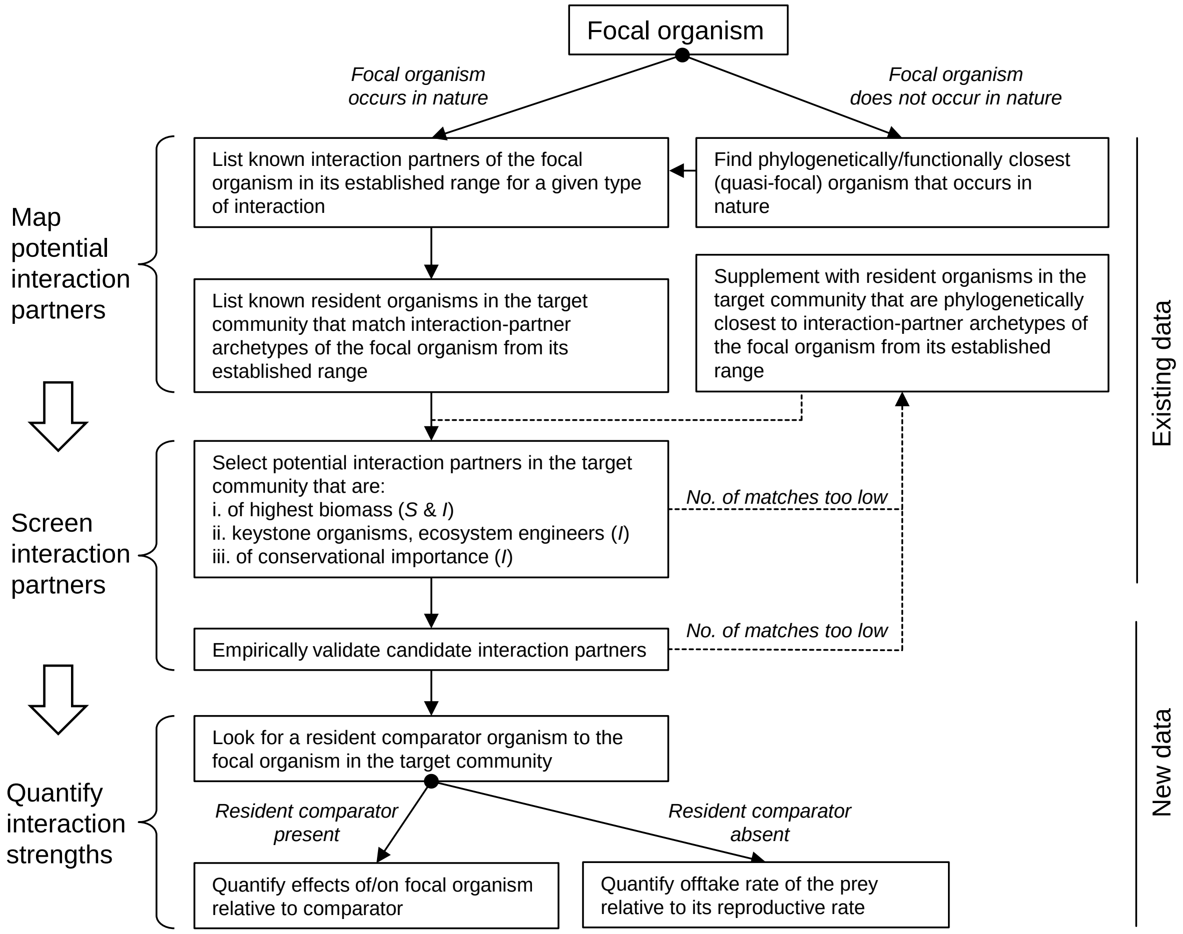

Figure 1.1 – Framework for quantifying interaction strength of a focal organism with multiple resident organisms in the target community for a given type of interaction. Nodes and broken links indicate alternative and supplementary paths, respectively. *S* and *I* indicate interaction-partner categories relevant for the assessment of invasion success and ecological impacts, respectively.

Screening for a prioritized subset of the mapped interaction network

Should assessment be restricted to a subset of potential interaction partners, then criteria for selecting them depend on the goal of the assessment—invasion success, impact, or both (Figure 1.1). The colonization of areas beyond the point of introduction, synonymous with invasion success (Blackburn et al. 2011), is likely if an organism is able to utilize abundant resources, and/or if it can avoid high

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[4] *Faxonius limosus* underwent a reclassification in August 2017, changing the genus of *Orconectes* to *Faxonius* (Crandall, K.A. and S. De Grave. 2017. An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. Journal of Crustacean Biology **37**:615-653).

extrinsic mortality. Thus, we recommend focusing on potential resources with the highest biomass in the target ecosystem when selecting a prioritized subset of all identified potential interactions for the assessment of the likelihood of invasion. However, attention should also be paid to potential predators that are expected to have the highest predation pressure on the focal organism. Notably, high predation on the focal organism could come from consumers with high individual predation rates, or those that are not necessarily individually voracious but occur in high abundance (Dick et al. 2017).

Interactions of the focal organism with dominant predators and prey have the potential to affect major energy conduits within ecological networks and are thus conducive to strong ecosystem-level impacts, such as altered diversity, structure and functioning of target communities (Lockwood et al. 2007, Penk et al. 2015, Jackson et al. 2016). Further, interactions with keystone species or ecosystem engineers (Jones et al. 1994, Power et al. 1996, Angelini et al. 2015), which are not necessarily very abundant, could amplify the indirect impact of invaders and convey ecosystem-level impacts. They should also be considered. It is also important to consider interactions with individual predator and prey organisms of conservation importance, regardless of whether such interactions have the potential to affect the whole community (Figure 1.1).

In general, we advise selecting multiple prey and predator species of the focal organism for empirical assessment. However, their number and distribution among the interactor groups discussed above (i.e. those of highest biomass, keystone organisms and ecosystem engineers, and those of conservation importance; Figure 1.1), will depend on the purpose of the assessment, trophic level and niche breadth of the focal organism, food web complexity in the target ecosystem, management priorities and logistic constraints. For example, monophagous and oligophagous predators, including parasites and parasitoids, have inherently limited numbers of prey, while mesopredators typically have fewer predators than basal prey (Turney and Buddle 2016). Figure 1.2 shows exemplary hypothetical module structures for interaction settings between focal and resident organisms, indicating the diversity of interactions that need to be considered. Some of the interactor group categories will frequently overlap, and some may not be present in the target community. If the focal organism is already established, experimental trials or field data can be used to ascertain and prioritize interaction partners in the target community before engaging in full assessment.

Empirical examples of structured choices of prey in functional response studies of invasive species are rare (but see Dick et al. 2013, Barrios-O'Neill et al. 2016, Xu et al. 2016). In our worked example of the marbled crayfish (Figure A.1), the mussel *Dreissena* spp. has high abundance in the target community and is an ecosystem engineer. Dreissenids and other animal prey with poor escape response are key and preferred contributors to crayfish energy budgets (Momot 1995). The snails *Radix* spp. and *Bithynia tentaculata* are other important primary consumers in the target community that are readily consumed by crayfish (Olsen et al. 1991, Nystrom et al. 1999). Testing predation on these three mollusc taxa could thus inform the assessment of both invasion success and ecological impacts of the marbled crayfish (Figures A.1 and A.2). The quasi-focal organism (spiny-cheek crayfish) is a major prey of perch (*Perca fluviatilis*), which is a relatively abundant fish species in German low-land lakes and often holds key positions in food webs (Persson et al. 2000). Predation by perch is likely restricted to immature or post-molt crayfish because of gape size limitation and the formidable defenses of mature crayfish. Nonetheless, this predatory fish could depress crayfish population dynamics, and thus it is a potentially important interactor (Figures A.1 and A.2).

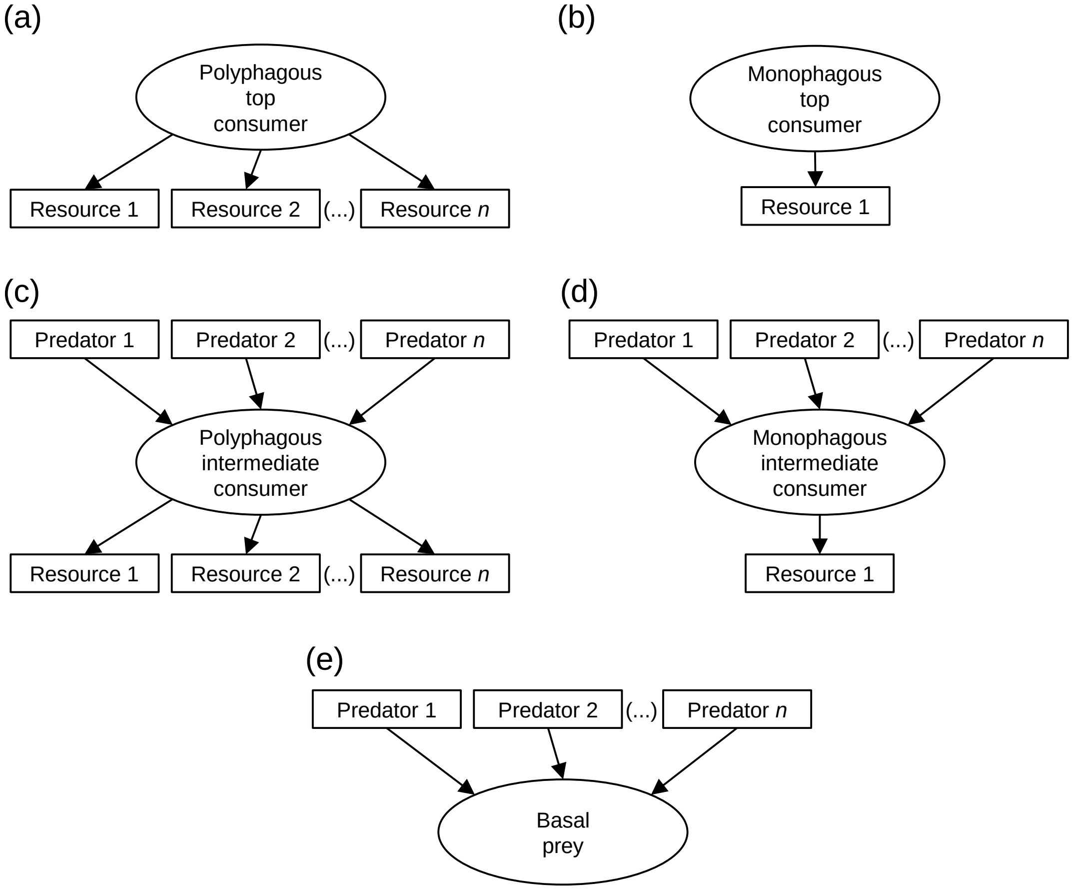


Figure 1.2 – Hypothetical interaction module structures for testing trophic interactions of a focal organism (oval shapes) representing top (a, b), intermediate (c, d) and basal (e) trophic positions, and two extrema on the diet-breadth continuum (polyphagous [a, c] and monophagous [b, d]).

Empirically testing trophic interactions for the prioritized interaction subset

Introduction of an organism that is of a predator or prey archetype already present in the resident community implies that resident prey or predators, respectively, are likely already familiar with such an archetype (Saul and Jeschke 2015). Because of such experience, it can be assumed that a novel organism can impact resident prey populations more strongly than their currently experienced predation pressure if its predatory traits toward a particular prey archetype are superior relative to its resident analogues. Similarly, a novel organism risks high impact from resident predators if its defenses are weaker than those of its resident analogues, thus promoting prey switching. Therefore, comparing the trophic interaction strengths of the focal organism with its prey or predators, with those of an ecologically similar resident, where such exists, provides a useful benchmark for gauging the magnitude of interaction strength (van Kleunen et al. 2010, Dick et al. 2014). By definition, no two species are identical (Ordonez 2014), but resident organisms that are of the same predator or prey archetype (Cox and Lima 2006, Winemiller et al. 2015) can offer a useful approximation of a reference baseline if any relevant functional differences between otherwise analogous species are acknowledged. In our worked example, the marbled crayfish co-occurs with other omnivorous crayfish (Chucholl et al. 2012) of a similar predator and prey archetype that can be used as comparators (Figure A.1).

On the other hand, a novel organism that does not have any resident comparator is likely to have characteristics largely unfamiliar to resident prey and predators and thus the potential to bypass their defenses and offences (Saul and Jeschke 2015). In such a case, the absolute, rather than comparative interaction strength of the focal novel organism with its prey and predators can be of primary interest, and offtake rate of prey in relation to its reproductive rate can be used to predict impact on prey populations (MacNeil et al. 2013; Figure 1.1). Qualitative pilot experiments can inform which degree of functional similarity can be assumed as a baseline.

Inference to real ecosystems

In situ measurements and manipulations provide realistic settings, but tend to allow poor control of confounding factors (but see Barrios-O'Neill et al. 2014b). Also, they cannot be carried out if the focal organism is not (yet) present in the target environment. Laboratory experiments, on the other hand, typically simplify biotic and abiotic contexts, and the applied relevance of their results depends on the degree to which experimental settings facilitate natural offensive and defensive behavior. For example, sheltering or camouflage may alter the shape of density-dependent predation, in that individual organisms devoid of their typical protective settings during experiments are more exposed to predation (Whittingham and Markland 2002, Horppila et al. 2003, Alexander et al. 2013, Barrios-O'Neill et al. 2015). Both ambient temperatures and environmental hypoxia can also affect activity level, and moderate predator–prey interactions (Englund et al. 2011, Laverty et al. 2015, Penk et al. 2016). Laboratory-derived functional responses typically isolate an individual predator and single prey species (e.g. Dick et al. 2013, Barrios-O'Neill et al. 2014b, Xu et al. 2016; but see Alexander et al. 2013, Medoc et al. 2013, Wasserman et al. 2016), and thus rarely account for prey switching or interference among predators which could affect the outcome of an interaction (Amarasekare 2002, Tschanz et al. 2007, van Leeuwen et al. 2013). The degree of spatio-temporal overlap of habitat use by the focal organism and its interaction partners is another important consideration (Polis et al. 1997). For example, a potentially strong interactor may have only a small time window for realizing such interactions if it rarely encounters particular prey and predator species. Detailed propositions for ameliorating these problems are beyond the scope of this manuscript, but we emphasize that lack of their consideration can undermine inference.

The need to quantify absolute interaction strengths accurately is largely circumvented in comparative studies, which focus on consumption rates relative to a native analogue rather than on absolute values, with an underlying assumption that both comparators would be influenced similarly by experimental artefacts (Dick et al. 2014). Indeed, comparative functional responses derived from simple laboratory experiments can be highly successful in explaining real-ecosystem ecological impacts of invaders across taxonomic and trophic groups (Dick et al. 2017). Context-dependencies may thus be particularly influential in making inference from studies on a novel organism that does not have a resident comparator because they rely on quantification of absolute interaction strengths.

Our framework specifically focuses on biological interactions, but the importance of intrinsic characteristics of the novel organism also has to be considered. For example, prognoses of population and community dynamics require at least some information on the reproductive rates of the focal organism and its interaction partners. The reproductive rate of a consumer determines the degree to which it can capitalize numerically on its ability to exploit prey and cumulatively increase its impact on prey populations, whereas the reproductive rate of prey determines their capacity to persist under given predation pressure (Twardochleb et al. 2012). Both of these factors are key drivers of community dynamics.

Any model necessitates a trade-off between generality, realism and precision (Levins 1966). It is impossible to achieve all of these simultaneously to full extent, and the decision as to how to optimize this trade-off depends upon the focal system. We therefore present a basic framework here, which needs to be adjusted and extended on a case-by-case basis to make it useful for the particular focal system in question. For example, for many systems it will be useful to incorporate non-consumptive or indirect interactions, or impacts on ecosystem services into the basic framework.

Non-consumptive and indirect interactions

Consumptive interactions are the key focus of our framework (Figure 1.1). However, non-consumptive and indirect (trait-mediated) interactions, for example through interference, facilitation and inhibition, can have important implications for community dynamics and in some cases even take prominence over direct impacts (Suraci et al. 2016). Indirect interactions occur when one species alters the effects that another species has on a third, potentially confounding predicted impacts of a novel organism that are derived from two-species studies (White et al. 2006). For instance, changes to the foraging behavior of a resident intermediate consumer as a result of the presence of a novel higher-order predator may alter the strength of interactions with a basal prey resource, releasing it from predation pressure (Townsend 1996). Alternatively, the presence of a resident higher-order predator may result in an exacerbated effect of a non-resident intermediate species towards its prey in comparison to a resident consumer, again influencing impact of the focal organism (Barrios-O'Neill et al. 2014a). Quantification of beneficial and disadvantageous outcomes of such interactions, in particular regulation of feeding and mortality rates, could be readily integrated in the empirical steps of our framework.

Conclusions

Key theoretical progress on functional responses in invasion ecology has come from retrospective empirical attempts to explain invasion success and impacts of established invaders (Hooff and Bollens 2004, Radford et al. 2007, Bollache et al. 2008, Dick et al. 2013). Such attempts typically focus on isolated interactions in which the invader is clearly efficient and superior over a native comparator. However, biological invasions are highly dependent on biological contexts (Donohue et al. 2013, Ricciardi et al. 2013, Saul et al. 2013), and robust prospective applications require a more comprehensive assessment network with multiple interaction partners, including predators of the focal organism. Applications of functional responses in biocontrol frequently fail to explain impact on individual prey organisms (Lester and Harmsen 2002, Fernandez-Arhex and Corley 2003). The inclusion of predators and alternative prey, together with more realistic representation of key abiotic conditions and explicit discussion of the relevance of results to natural ecosystems can improve explanatory and predictive power of impact assessments. We focused here on predation in a broad sense, but the same assessment protocol and analogous empirical methods can be used for detritivorous and autotrophic energy acquisition (Radford et al. 2007, McNickle and Brown 2014).

Risk assessment based on performance in comparable environments, where such information exists, is less laborious than collecting new data. However, interaction partners in new and existing ranges should be compared in a structured way to minimize bias. The steps of our framework that are based on existing data can be used to inform such comparisons (Figure 1.1). Furthermore, novel organisms can be introduced to dissimilar communities or abiotic conditions in comparison to their existing ranges, or they can be absent in nature. Such scenarios preclude comparisons based on performance elsewhere and necessitate collection of new data (Figure 1.1). In the face of limiting resources, a compromise between experimental complexity and accuracy of risk assessments needs to be reached on a case-by- case basis. Notably, relevant empirical data can be collected in situ (Angerbjorn et al. 1999, Goss-Custard et al. 2006, Moustahfid et al. 2010, Barrios-O'Neill et al. 2014b), permitting empirical testing of organisms that do not lend themselves well to laboratory conditions, or should not be interfered with on ethical grounds. In any case, the broader interaction network in the focal ecosystem should be at least theoretically considered, even if just to critically scrutinize the assessment outcomes. The non-empirical steps of our framework can inform such exercises regardless of the scale or complexity of the system in question.

Acknowledgments

We appreciate helpful comments by the Associate Editor and reviewers. M.P. was supported by a COST Action TD1209 STSM grant (011015-062070). W.C.S. and J.M.J. were supported by the ERA-Net BiodivERsA (project FFII) with Deutsche Forschungsgemeinschaft (DFG; JE 288/7-1). J.M.J. was additionally supported by the DFG grant JE 288/9-1.

Literature cited

Alexander, M. E., J. T. Dick, O. L. Weyl, T. B. Robinson, and D. M. Richardson. 2014. Existing and emerging high impact invasive species are characterized by higher functional responses than natives. Biology Letters **10**:20130946.

Alexander, M. E., J. T. A. Dick, and N. E. O'Connor. 2013. Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. Oikos **122**:1521–1531.

Alexander, M. E., H. J. Raven, and T. B. Robinson. 2015. Foraging decisions of a native whelk, *Trochia cingulata* Linnaeus, and the effects of invasive mussels on prey choice. Journal of Experimental Marine Biology and Ecology **470**:26–33.

Amarasekare, P. 2002. Interference competition and species coexistence. Proceedings of the Royal Society B-Biological Sciences **269**:2541–2550.

Angelini, C., T. van der Heide, J. N. Griffin, J. P. Morton, M. Derksen-Hooijberg, L. P. M. Lamers, A. J. P. Smolders, and B. R. Silliman. 2015. Foundation species' overlap enhances biodiversity and multifunctionality from the patch to landscape scale in southeastern United States salt marshes. Proceedings of the Royal Society B-Biological Sciences **282**:9.

Angerbjorn, A., M. Tannerfeldt, and S. Erlinge. 1999. Predator-prey relationships: Arctic foxes and lemmings. Journal of Animal Ecology **68**:34–49.

Arbačiauskas, K., J. Lesutiene, and Z. R. Gasiunaite. 2013. Feeding strategies and elemental composition in Ponto-Caspian peracaridans from contrasting environments: can stoichiometric plasticity promote invasion success? Freshwater Biology **58**:1052–1068.

Barrios-O'Neill, D., J. T. A. Dick, M. C. Emmerson, A. Ricciardi, and H. J. MacIsaac. 2015. Predator-free space, functional responses and biological invasions. Functional Ecology **29**:377–384.

Barrios-O'Neill, D., J. T. A. Dick, M. C. Emmerson, A. Ricciardi, H. J. MacIsaac, M. E. Alexander, and H. C. Bovy. 2014a. Fortune favours the bold: a higher predator reduces the impact of a native but not an invasive intermediate predator. Journal of Animal Ecology **83**:693–701.

Barrios-O'Neill, D., J. T. A. Dick, A. Ricciardi, H. J. MacIsaac, and M. C. Emmerson. 2014b. Deep impact: in situ functional responses reveal context-dependent interactions between vertically migrating invasive and native mesopredators and shared prey. Freshwater Biology **59**:2194–2203.

Barrios-O'Neill, D., R. Kelly, J. T. A. Dick, A. Ricciardi, H. J. MacIsaac, and M. C. Emmerson. 2016. On the context-dependent scaling of consumer feeding rates. Ecology Letters **19**:668–678.

Bessa-Gomes, C., M. Danek-Gontard, P. Cassey, A. P. Moller, S. Legendre, and J. Clobert. 2003. Mating behaviour influences extinction risk: insights from demographic modelling and comparative analysis of avian extinction risk. Annales Zoologici Fennici **40**:231–245.

Blackburn, T. M., P. Pysek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarosik, J. R. U. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution **26**:333–339.

Bollache, L., J. T. Dick, K. D. Farnsworth, and W. I. Montgomery. 2008. Comparison of the functional responses of invasive and native amphipods. Biology Letters **4**:166–169.

Cassey, P., T. M. Blackburn, S. Sol, R. P. Duncan, and J. L. Lockwood. 2004. Global patterns of introduction effort and establishment success in birds. Proceedings of the Royal Society B-Biological Sciences **271**:405–408.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Clavel, J., R. Julliard, and V. Devictor. 2011. Worldwide decline of specialist species: toward a global functional homogenization? Frontiers in Ecology and the Environment **9**:222–228.

Cox, J. G., and S. L. Lima. 2006. Naivete and an aquatic-terrestrial dichotomy in the effects of introduced predators. Trends in Ecology & Evolution **21**:674–680.

Davis, N. E., D. M. Forsyth, B. Triggs, C. Pascoe, J. Benshemesh, A. Robley, J. Lawrence, E. G. Ritchie, D. G. Nimmo, and L. F. Lumsden. 2015. Interspecific and Geographic Variation in the Diets of Sympatric Carnivores: Dingoes/Wild Dogs and Red Foxes in South-Eastern Australia. Plos One **10**:e0120975.

Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, W. Thuiller, P. Venail, S. Villeger, and N. Mouquet. 2010. Defining and measuring ecological specialization. Journal of Applied Ecology **47**:15–25.

Dick, J. T., M. E. Alexander, J. M. Jeschke, A. Ricciardi, H. J. MacIsaac, T. B. Robinson, S. Kumschick, O. L. Weyl, A. M. Dunn, and M. J. Hatcher. 2014. Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. Biological Invasions **16**:735–753.

Dick, J. T., K. Gallagher, S. Avlijas, H. C. Clarke, S. E. Lewis, S. Leung, D. Minchin, J. Caffrey, M. E. Alexander, C. Maguire, C. Harrod, N. Reid, N. R. Haddaway, K. D. Farnsworth, M. Penk, and A. Ricciardi. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions **15**:837–846.

Dick, J. T., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, and N. G. Taylor. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology **54**:1259–1267.

Donohue, I., O. L. Petchey, J. M. Montoya, A. L. Jackson, L. McNally, M. Viana, K. Healy, M. Lurgi, N. E. O'Connor, and M. C. Emmerson. 2013. On the dimensionality of ecological stability. Ecology Letters **16**:421–429.

Downing, A. S., E. H. van Nes, J. H. Janse, F. Witte, I. J. M. Cornelissen, M. Scheffer, and W. M. Mooij. 2012. Collapse and reorganization of a food web of Mwanza Gulf, Lake Victoria. Ecological Applications **22**:229–239.

Dunoyer, L., L. Dijoux, L. Bollache, and C. Lagrue. 2014. Effects of crayfish on leaf litter breakdown and shredder prey: are native and introduced species functionally redundant? Biological Invasions **16**:1545–1555.

Ehrenfeld, J. G. 2010. Ecosystem Consequences of Biological Invasions. Annual Review of Ecology, Evolution, and Systematics **41**:59–80.

Englund, G., G. Öhlund, C. L. Hein, and S. Diehl. 2011. Temperature dependence of the functional response. Ecology Letters **14**:914–921.

Fernandez-Arhex, V., and J. C. Corley. 2003. The functional response of parasitoids and its implications for biological control. Biocontrol Science and Technology **13**:403–413.

Futuyma, D. J., and G. Moreno. 1988. The evolution of ecological specialization. Annual Review of Ecology and Systematics **19**:207–233.

Goss-Custard, J. D., A. D. West, M. G. Yates, R. W. G. Caldow, R. A. Stillman, L. Bardsley, J. Castilla, M. Castro, V. Dierschke, S. Durell, G. Eichhorn, B. J. Ens, K. M. Exo, P. U. Udayangani-Fernando, P. N. Ferns, P. A. R. Hockey, J. A. Gill, I. Johnstone, B. Kalejta-Summers, J. A. Masero, F. Moreira, R. V. Nagarajan, I. P. F. Owens, C. Pacheco, A. Perez-Hurtado, D. Rogers, G. Scheiffarth, H. Sitters, W. J. Sutherland, P. Triplet, D. H. Worrall, Y. Zharikov, L. Zwarts, and R. A. Pettifor. 2006. Intake rates and the functional response in shorebirds (Charadriiformes) eating macro-invertebrates. Biological Reviews **81**:501–529.

Hanmer, J., J. W. White, and J. R. Pawlik. 2017. Application of diet theory reveals context-dependent foraging preferences in an herbivorous coral reef fish. Oecologia **184**:127–137.

Hays, W. S. T., and S. Conant. 2007. Biology and impacts of Pacific Island invasive species. 1. A worldwide review of effects of the small Indian mongoose, *Herpestes javanicus* (Carnivora : Herpestidae). Pacific Science **61**:3–16.

Holling, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. The Canadian Entomologist **91**:293–320.

Hooff, R. C., and S. M. Bollens. 2004. Functional response and potential predatory impact of *Tortanus dextrilobatus*, a carnivorous copepod recently introduced to the San Francisco Estuary. Marine Ecology Progress Series **277**:167–179.

Horppila, J., A. Liljendahl-Nurminen, T. Malinen, M. Salonen, A. Tuomaala, L. Uusitalo, and M. Vinni. 2003. *Mysis relicta* in a eutrophic lake: Consequences of obligatory habitat shifts. Limnology and Oceanography **48**:1214–1222.

Jackson, M. C., J. Grey, K. Miller, J. R. Britton, and I. Donohue. 2016. Dietary niche constriction when invaders meet natives: evidence from freshwater decapods. Journal of Animal Ecology **85**:1098–1107.

Jaworski, C. C., A. Bompard, L. Genies, E. Amiens-Desneux, and N. Desneux. 2013. Preference and Prey Switching in a Generalist Predator Attacking Local and Invasive Alien Pests. Plos One **8**:e82231.

Jeschke, J. M., F. Keesing, and R. S. Ostfeld. 2013. Novel Organisms: Comparing Invasive Species, GMOs, and Emerging Pathogens. Ambio **42**:541–548.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2004. Consumer-food systems: why type I functional responses are exclusive to filter feeders. Biological Reviews **79**:337–349.

Jeschke, J. M., and D. L. Strayer. 2006. Determinants of vertebrate invasion success in Europe and North America. Global Change Biology **12**:1608–1619.

Jeschke, J. M., and R. Tollrian. 2005. Effects of predator confusion on functional responses. Oikos **111**:547–555.

Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as Ecosystem Engineers. Oikos **69**:373–386.

Kissling, W. D., L. Dalby, C. Flojgaard, J. Lenoir, B. Sandel, C. Sandom, K. Trojelsgaard, and J. C. Svenning. 2014. Establishing macroecological trait datasets: digitalization, extrapolation, and validation of diet preferences in terrestrial mammals worldwide. Ecology and Evolution **4**:2913–2930.

Laverty, C., J. T. A. Dick, M. E. Alexander, and F. E. Lucy. 2015. Differential ecological impacts of invader and native predatory freshwater amphipods under environmental change are revealed by comparative functional responses. Biological Invasions **17**:1761–1770.

Lester, P. J., and R. Harmsen. 2002. Functional and numerical responses do not always indicate the most effective predator for biological control: an analysis of two predators in a two-prey system. Journal of Applied Ecology **39**:455–468.

Levins, R. 1966. Strategy of model building in population biology. American Scientist **54**:421–431.

Lockwood, J. L., M. F. Hoopes, and M. P. Marchetti. 2007. Invasion ecology. Blackwell Publishing, Oxford, UK.

MacNeil, C., J. Dick, M. Alexander, J. Dodd, and A. Ricciardi. 2013. Predators vs. alien: differential biotic resistance to an invasive species by two resident predators. NeoBiota **19**:1–19.

Mayer, C. M., L. E. Burlakova, P. Eklöv, D. Fitzgerald, A. Y. Karatayev, S. A. Ludsin, S. Millard, E. L. Mills, A. P. Ostapenya, L. G. Rudstam, B. Zhu, and T. V. Zhukova. 2013. Benthification of freshwater lakes. Pages 575–586 *in* T. F. Nalepa and D. W. Schloesser, editors. Quagga and Zebra Mussels: Biology, Impacts, and Control. CRC Press, Boca Raton, USA.

McNickle, G. G., and J. S. Brown. 2014. When Michaelis and Menten met Holling: towards a mechanistic theory of plant nutrient foraging behaviour. AoB Plants **6**:plu066.

Medoc, V., T. Spataro, and R. Arditi. 2013. Prey: predator ratio dependence in the functional response of a freshwater amphipod. Freshwater Biology **58**:858–865.

Mitchell, C. E., A. A. Agrawal, J. D. Bever, G. S. Gilbert, R. A. Hufbauer, J. N. Klironomos, J. L. Maron, W. F. Morris, I. M. Parker, and A. G. Power. 2006. Biotic interactions and plant invasions. Ecology Letters **9**:726–740.

Momot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science **3**:33–63.

Moustahfid, H., M. C. Tyrrell, J. S. Link, J. A. Nye, B. E. Smith, and R. J. Gamble. 2010. Functional feeding responses of piscivorous fishes from the northeast US continental shelf. Oecologia **163**:1059–1067.

Nystrom, P., C. Bronmak, and W. Graneli. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. Oikos **85**:545–553.

O'Connor, N. E., M. C. Emmerson, T. P. Crowe, and I. Donohue. 2013. Distinguishing between direct and indirect effects of predators in complex ecosystems. Journal of Animal Ecology **82**:438–448.

Olsen, T. M., D. M. Lodge, G. M. Capelli, and R. J. Houlihan. 1991. Mechanisms of impact of an introduced crayfish (*Orconectes rusticus*) on littoral congeners, snails, and macrophytes. Canadian Journal of Fisheries and Aquatic Sciences **48**:1853–1861.

Ordonez, A. 2014. Functional and phylogenetic similarity of alien plants to co-occurring natives. Ecology **95**:1191–1202.

Penk, M., K. Irvine, and I. Donohue. 2015. Ecosystem-level effects of a globally spreading invertebrate invader are not moderated by a functionally similar native. Journal of Animal Ecology **84**:1628–1636.

Penk, M. R., J. M. Jeschke, D. Minchin, and I. Donohue. 2016. Warming can enhance invasion success through asymmetries in energetic performance. Journal of Animal Ecology **85**:419–426.

Persson, L., P. Bystrom, and E. Wahlstrom. 2000. Cannibalism and competition in Eurasian perch: population dynamics of an ontogenetic omnivore. Ecology **81**:1058–1071.

Pintor, L. M., and J. E. Byers. 2015. Individual variation in predator behavior and demographics affects consumption of non-native prey. Behavioral Ecology **26**:797–804.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289–316.

Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones: Identifying keystone species is difficult—but essential to understanding how loss of species will affect ecosystems. Bioscience **46**:609–620.

Radford, I. J., K. J. M. Dickinson, and J. M. Lord. 2007. Functional and performance comparisons of invasive *Hieracium lepidulum* and co-occurring species in New Zealand. Austral Ecology **32**:338–354.

Ricciardi, A., M. F. Hoopes, M. P. Marchetti, and J. L. Lockwood. 2013. Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs **83**:263–282.

Romanuk, T. N., Y. Zhou, U. Brose, E. L. Berlow, R. J. Williams, and N. D. Martinez. 2009. Predicting invasion success in complex ecological networks. Philosophical Transactions of the Royal Society B-Biological Sciences **364**:1743–1754.

Rosenfeld, J. S. 2002. Functional redundancy in ecology and conservation. Oikos **98**:156–162.

Rosewarne, P. J., R. J. G. Mortimer, R. J. Newton, C. Grocock, C. D. Wing, and A. M. Dunn. 2016. Feeding behaviour, predatory functional responses and trophic interactions of the invasive Chinese mitten crab (*Eriocheir sinensis*) and signal crayfish (*Pacifastacus leniusculus*). Freshwater Biology **61**:426–443.

Rudolf, V. H. W., and K. D. Lafferty. 2011. Stage structure alters how complexity affects stability of ecological networks. Ecology Letters **14**:75–79.

Saul, W.-C., J. Jeschke, and T. Heger. 2013. The role of eco-evolutionary experience in invasion success. NeoBiota **17**:57–74.

Saul, W. C., and J. M. Jeschke. 2015. Eco-evolutionary experience in novel species interactions. Ecology Letters **18**:236–245.

Simberloff, D. 2011. How common are invasion-induced ecosystem impacts? Biological Invasions **13**:1255–1268.

Simberloff, D., J.-L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyšek, R. Sousa, E. Tabacchi, and M. Vilà. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution **28**:58–66.

Strayer, D. L. 2009. Twenty years of zebra mussels: lessons from the mollusk that made headlines. Frontiers in Ecology and the Environment **7**:135–141.

Strayer, D. L., and H. M. Malcom. 2007. Effects of zebra mussels (*Dreissena polymorpha*) on native bivalves: the beginning of the end or the end of the beginning? Journal of the North American Benthological Society **26**:111–122.

Suraci, J. P., M. Clinchy, L. M. Dill, D. Roberts, and L. Y. Zanette. 2016. Fear of large carnivores causes a trophic cascade. Nature Communications **7**:10698.

Terborgh, J., and J. A. Estes. 2010. Trophic cascades: predators, prey, and the changing dynamics of nature. Island Press, Washington DC, USA.

Townsend, C. R. 1996. Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. Biological Conservation **78**:13–22.

Tschanz, B., L. F. Bersier, and S. Bacher. 2007. Functional responses: A question of alternative prey and predator density. Ecology **88**:1300–1308.

Turney, S., and C. M. Buddle. 2016. Pyramids of species richness: the determinants and distribution of species diversity across trophic levels. Oikos **125**:1224–1232.

Twardochleb, L. A., M. Novak, and J. W. Moore. 2012. Using the functional response of a consumer to predict biotic resistance to invasive prey. Ecological Applications **22**:1162–1171.

van Kleunen, M., W. Dawson, D. Schlaepfer, J. M. Jeschke, and M. Fischer. 2010. Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecology Letters **13**:947–958.

van Leeuwen, E., A. Brannstrom, V. A. A. Jansen, U. Dieckmann, and A. G. Rossberg. 2013. A generalized functional response for predators that switch between multiple prey species. Journal of Theoretical Biology **328**:89–98.

Vogt, G., C. Falckenhayn, A. Schrimpf, K. Schmid, K. Hanna, J. Panteleit, M. Helm, R. Schulz, and F. Lyko. 2015. The marbled crayfish as a paradigm for saltational speciation by autopolyploidy and parthenogenesis in animals. Biology Open **4**:1583–1594.

Wasserman, R. J., M. E. Alexander, T. Dalu, B. R. Ellender, H. Kaiser, and O. L. F. Weyl. 2016. Using functional responses to quantify interaction effects among predators. Functional Ecology **30**:1988–1998.

Werner, E. E., and J. F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics **15**:393–425.

White, E. M., J. C. Wilson, and A. R. Clarke. 2006. Biotic indirect effects: a neglected concept in invasion biology. Diversity and Distributions **12**:443–455.

Whittingham, M. J., and H. M. Markland. 2002. The influence of substrate on the functional response of an avian granivore and its implications for farmland bird conservation. Oecologia **130**:637–644.

Winemiller, K. O., D. B. Fitzgerald, L. M. Bower, and E. R. Pianka. 2015. Functional traits, convergent evolution, and periodic tables of niches. Ecology Letters **18**:737–751.

Wise, D. H., D. M. Moldenhauer, and J. Halaj. 2006. Using stable isotopes to reveal shifts in prey consumption by generalist predators. Ecological Applications **16**:865–876.

Xu, M., X. Mu, J. T. Dick, M. Fang, D. Gu, D. Luo, J. Zhang, J. Luo, and Y. Hu. 2016. Comparative functional responses predict the invasiveness and ecological impacts of alien herbivorous snails. Plos One **11**:e0147017.

Appendix A

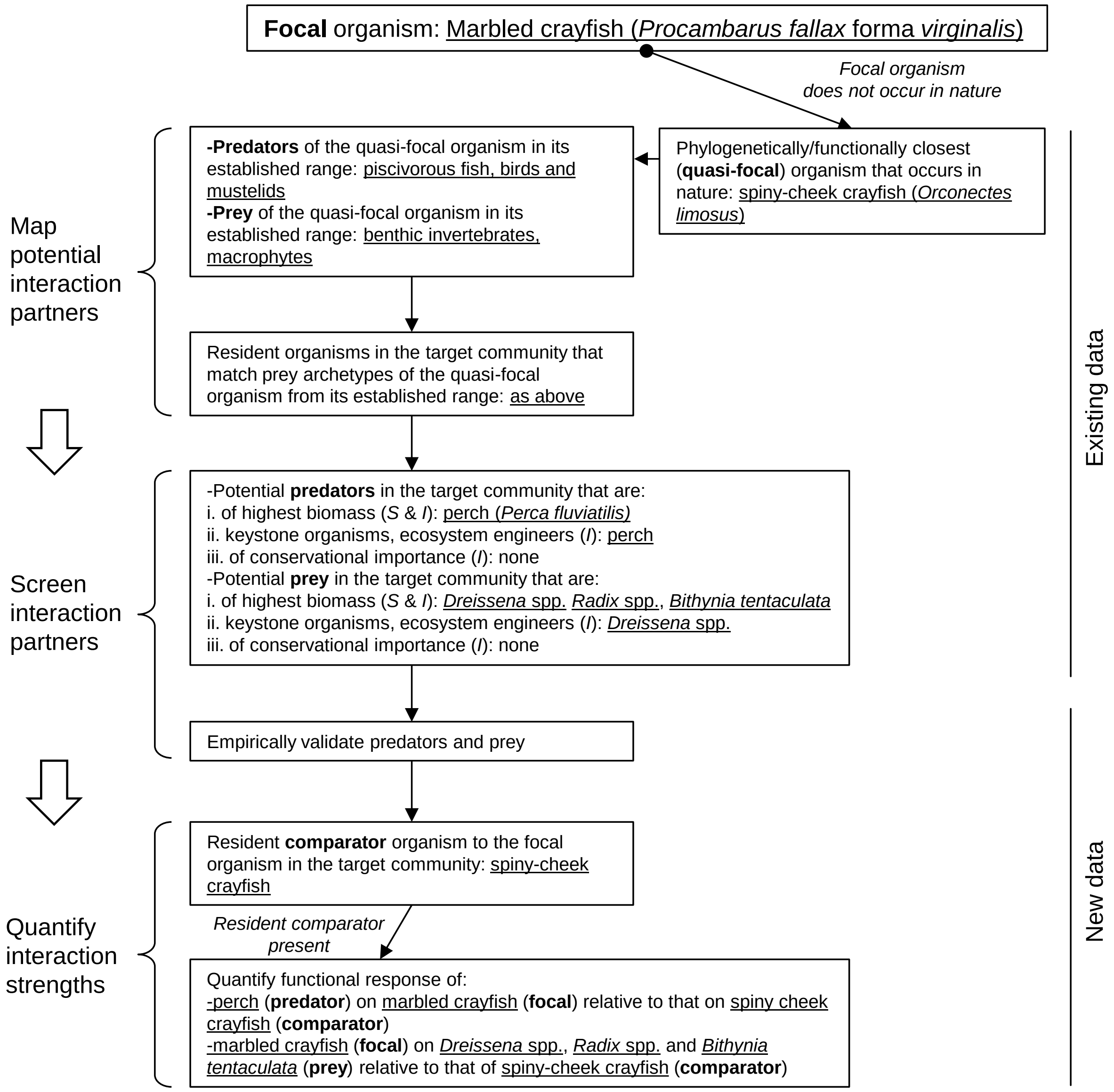


Figure A.1 – Illustration of the framework for quantifying interaction strength of a focal organism with resident organisms in the target community, using an example of predator and prey selection for the marbled crayfish (*Procambarus fallax* forma *virginalis*) in a German low-land lake. *S* and *I* indicate prey categories relevant for the assessment of invasion success and ecological impacts, respectively. Quasi-focal organism is also the resident comparator in this example.

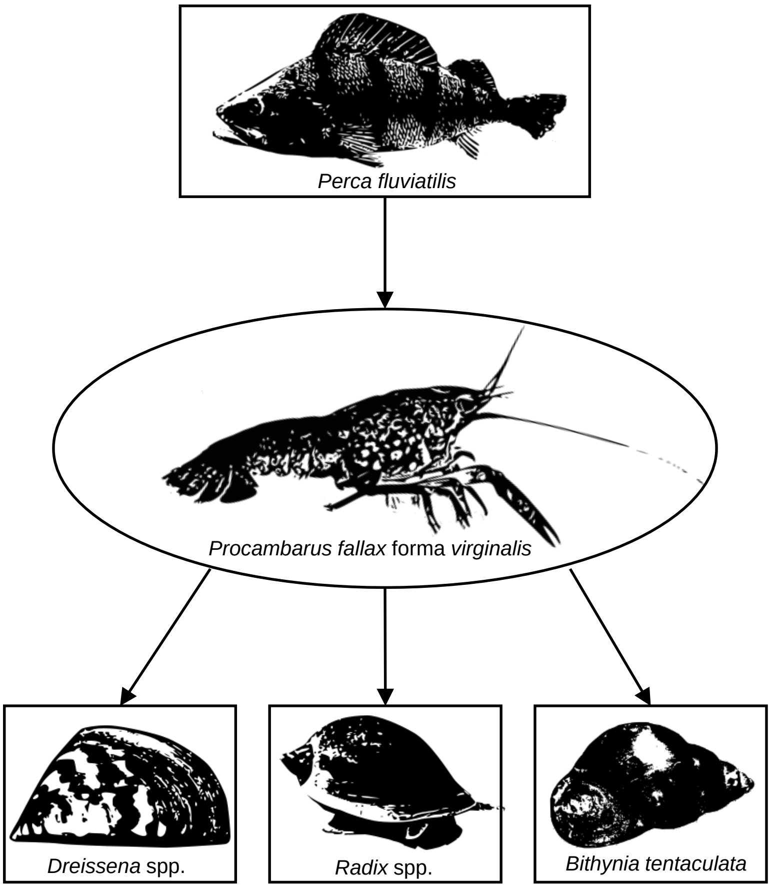


Figure A.2 – Illustration of the interaction module structure for testing using an example of predator and prey (top and bottom boxes, respectively) selected for the marbled crayfish (central box) in a German low-land lake. Organisms are not to scale.

CHAPTER 2

TOWARDS A MECHANISTIC UNDERSTANDING OF INDIVIDUAL-LEVEL FUNCTIONAL RESPONSES: INVASIVE CRAYFISH AS MODEL ORGANISMS

Submitted as:
Linzmaier, Stefan M. & Jonathan M. Jeschke: *Towards a mechanistic understanding of individual-level functional responses: invasive crayfish as model organisms*.

Abstract

1. In novel communities, a rising number of new and emerging invasive species interact with resident species, some of which are non-native themselves. We implemented an innovative trophic interaction framework for novel communities and quantified the interaction strength and impact potential of a truly novel species (marbled crayfish *Procambarus virginalis*) with a resident non-native counterpart (spiny-cheek crayfish *Faxonius limosus*). As prey, we used *Dreissena* mussels, which are non-native as well and now hold a key position in many European and North American aquatic ecosystems.
2. For both crayfish species, we predicted functional responses based on a mechanistic model that we parameterized with a set of experimental observations of foraging behavior and satiation. We compared these predicted functional responses to empirically observed responses. In addition, we incorporated behavioral traits like aggression, activity and boldness in the comparisons between the species and individuals to determine their influence on functional responses. We tested individuals from aquarium stocks as well as naturalized individuals from invaded water bodies.
3. Altogether, we performed 1095 experiments with 26 individual crayfish. We found that per capita predation of spiny-cheek crayfish exceeded that of marbled crayfish from aquaria and naturalized individuals. Functional responses differed between species and were mostly higher for spiny-cheek crayfish males. Marbled crayfish, however, were more voracious and reached satiation more slowly. Consumption rates correlated with aggression for marbled crayfish and with an aggressive threat response for spiny-cheek crayfish.
4. We conclude that spiny-cheek crayfish can outcompete marbled crayfish for abundant mussels, but both species probably do not substantially affect *Dreissena* mussel populations in the field. For marbled crayfish, high long-term consumption, interspecific aggression and reproduction rates can promote their establishment and spread. Risk assessments of these invaders should be improved by considering numerical responses, different prey organisms and predators.

Keywords

biological invasions; freshwater crayfish; foraging; functional response; mechanistic model; predator-prey interactions; trait variation

Introduction

As the number of species invasions increases, the understanding of novel communities becomes increasingly pressing (Pacifici et al. 2015, Seebens et al. 2017). These novel communities are often shaped by over-invasions, which take place when an invasive species is replaced by a functionally similar invader (Russell et al. 2014). When invasives and other novel organisms become part of native communities, they participate in predator-prey and other ecological interactions (Thuiller et al. 2010). Accurately predicting these interactions would enable us to focus management efforts on the most impactful species (Jeschke et al. 2013). Information on past interactions are often not available and lack the predictive power to quantify ecological impact and invasiveness of novel species (Kumschick et al. 2014); in addition, trait-based measures of impact are often context dependent and subject to significant variability among species (Parker et al. 2013, Leffler et al. 2014). But higher and more efficient resource utilization is a common characteristic of successful invaders when compared to functionally similar species – it may thus be useful for predicting their impact (Dick et al. 2014, Dick et al. 2017).

Functional responses

Trophic interactions and especially predation cause many of the far-reaching impacts that invasive species have, including altering community structure and population dynamics (David et al. 2017). The energy and matter in the form of prey or other resources that a novel organism can access is critical for its success in the new environment (Mooney and Cleland 2001, Funk and Vitousek 2007). This integral concept of ecology and evolution is ideally described by the functional response, i.e. the relationship of resource consumption at different densities per unit time (Holling 1959a, Oaten and Murdoch 1975, Berryman 1992). Functional responses directly and quantitatively measure per capita interaction strength in nonlinear interactions between a consumer and its resources (Jeschke et al. 2002, Kalinkat 2014). They are further linked to measures of energy flow (Marquet et al. 2004) and other trait-based characteristics of organisms like allometric relationships (Kalinkat et al. 2013b).

Three major types of functional response are typically discriminated: type I, II and III (Holling 1959a); where the type II is most frequently observed in experiments and modeled in theoretical studies (Jeschke et al. 2002, 2004):

$$y(N) = \frac{aN}{1+ahN} \qquad (1),$$

where y is the per capita consumption rate, N is prey density, a is attack rate (also known as: rate of successful search, success rate or capture rate) and h is handling time. Equation (1) is the most popular functional response model, Holling's (1959b) disc equation. It has an initial slope determined by a and rises towards an asymptote determined by h. However, these parameters are simplified. To allow for a biologically meaningful interpretation, they need to be further subdivided: a is the product of (i) predator-prey encounter rate, (ii) predator detection probability (iii) attack probability and (iv) attack efficiency; h is the time a predator needs to successfully attack and ingest prey for certain experimental conditions. Under natural conditions, digestion time can be very important as well (Jeschke et al. 2002, Li et al. 2018).

In a mechanistic framework, the values of these components of both a and h can be empirically measured (plus digestion time, given the experimental conditions; Jeschke et al. (2002), Li et al. (2018)), and then used to predict a predator's functional response by parameterizing a functional response model. If this predicted functional response reasonably matches the independently measured empirical functional response of the predator species, one can assume that the model includes the essential components of the focal system. Such a mechanistic approach is labor-intensive and rarely applied (exceptions include Holling (1966), Metz et al. (1988), Jeschke and Tollrian (2005a), Jeschke and Hohberg (2008), Gergs and Ratte (2009)). Instead, most studies on functional responses fit eq. (1) or a similar model to empirically measured functional responses. Parameter estimates for a and h derived in this way do not represent what their names suggest: for example, a parameter estimate for h returned by a regression fit of eq. (1) must not be confused with real handling time. Such parameter estimates cannot be interpreted biologically, as they are influenced by digestion, prey switching, learning or adaptive behavior in unknown ways (Jeschke and Tollrian 2005b, Geritz and Gyllenberg 2012). Conversely, mechanistic models allow for predictions of how the functional response would change if any of the parameters change.

Functional responses are a classic tool in community ecology and have been used in invasion biology for about a decade since Bollache et al. (2008) found higher functional responses in invasive *Dikerogammarus villosus* compared to native *Gammarus* species. Since then, higher efficiency in resource use by invaders has been found across taxonomic groups (Dick et al. 2013, Alexander et al. 2014, Xu et al. 2016). However, existing functional response studies focusing on biological invasions have not applied a mechanistic approach yet.

Behavior

Behavioral variation between individual consumers can substantially effect functional responses (Okuyama 2008). The variation in behavioral types of conspecifics is an important factor in structuring novel communities and changing population dynamics (Bolnick et al. 2011, Chapple et al. 2012, Sih et al. 2012) and entail important implications for invasion impact (Evangelista et al. 2019). Individual phenotypic variability generally manifests in traits that are important for surviving in a new environment (i.e. functional response traits) and traits that determine the ecological impact of invasive species (i.e. functional effects traits) (Raffard et al. 2017). Rapid increases in population size, as often observed in invasive species, as well as individual growth, require phenotypic traits that facilitate access to large amounts of resources (Pintor et al. 2009, Biro et al. 2014). For example, high feeding rates are coupled with high metabolic rates and thus with activity, gut retention time and maximum feeding rates (Rall et al. 2012). Individual activity patterns can predict foraging activity of consumers even when they are not feeding (Pruitt et al. 2011, McGhee et al. 2013). Furthermore, higher aggression and boldness are often correlated to higher foraging rates (Pintor et al. 2008). The relationships of behavioral types across a resource gradient (i.e. functional responses and/or their constituent parameters) are largely unexplored (Toscano et al. 2016). Some behavioral traits like explorative behavior could not be related to functional responses (Schröder et al. 2016), but others like aggressiveness (Hartley et al. 2019), activity and the response to predation threat (Toscano and Griffen 2014) strongly affected the functional response of individuals.

Invasive crayfish

Decapod crustaceans have invaded and subsequently altered freshwater ecosystems worldwide (Twardochleb et al. 2013). As polyphagous intermediate consumers, they can reduce macrophyte and invertebrate biomass, species diversity and richness (van der Wal et al. 2013, Mathers et al. 2016). In addition, they threaten native crayfish species by competition, intraguild predation and transmission of diseases, particularly crayfish plague (Aphanomyces astaci; Holdich et al. (2009).

In the wake of crayfish plague extinctions, a rising number of plague-resistant North American crayfish species have established populations and started spreading across Europe (Kouba et al. 2014). Several of these crayfish have been classified as invasive alien species of European Union concern under the Regulation 1143/2014. Two of them are the spiny-cheek crayfish (*Faxonius limosus*), which has become dominant in central and eastern European water bodies (Kouba, Petrusek & Kozák, 2014), and the marbled crayfish (*Procambarus virginalis*), which is a relatively recent newcomer. Due to the marbled crayfish’s popularity as a pet, aquarium releases have managed to establish a number of populations particularly in Germany (Chucholl et al. 2012). Marbled crayfish are estimated to become a problematic invader and spread beyond their current distribution (Chucholl et al. 2012, Chucholl and Wendler 2017). They often live in sympatry with spiny-cheek crayfish and attain almost similar sizes (Souty-Grosset et al. 2006, Chucholl and Pfeiffer 2010). Marbled crayfish do not naturally occur in the wild and were first described from the German pet trade (Lukhaup 2001). Its novelty and parthenogenetic mode of reproduction make the marbled crayfish especially interesting for biologists (Martin et al. 2016, Gutekunst et al. 2018). The ecology and behavior of this species in the field is virtually unknown, and new information could help estimate the risk of further spread and impact of this new species (Chucholl 2015, Linzmaier et al. 2018).

Introductions of such non-native species can alter species interactions, for example between predators and prey. Even if similar in size or density compared to resident species, non-native species may have a more flexible diet, be more efficient predators or have higher resource intake rates, thus exerting higher pressure on native communities than resident crayfish (Usio et al. 2006, Haddaway et al. 2012, Ercoli et al. 2014). Strong interspecific aggression and high activity of many invaders limit the accessibility of resources for competitors and puts additional pressure on potential prey organisms (Bubb et al. 2006, Pintor et al. 2008). The mechanisms behind the changes brought by new and novel crayfish can only be understood when individual interactions with other organisms are considered.

Aims of this study

We implemented the trophic interaction framework developed by Penk et al. (2017) and used comparative functional responses to predict changes in an invaded community by changes in interaction strength. We compared the focal novel organism (marbled crayfish) with the most similar species that is already present in the community of our reference lake (spiny-cheek crayfish in lake Müggelsee, Berlin, Germany). We then identified an important potential prey in the target community that has high biomass and represents a keystone organism. Mobile prey (e.g. Ephemeroptera species) are often not as much affected as non-mobile prey (e.g. mussels) (Hanson et al. 1990, Mathers et al. 2016). Also, invasion success is likely when highly abundant prey can be utilized (Tilman 2004). Thus, we chose *Dreissena* mussels as prey; they occur at extremely high biomasses and are readily consumed by crayfish (Perry et al. 2000, zu Ermgassen and Aldridge 2011). *Dreissena* mussels are ecosystem engineers and have massively reshaped ecosystems in Europe and North America by filtration and epibiosis (Ricciardi 2003); high predation rates on these mussels may thus substantially affect aquatic ecosystems (Karatayev et al. 2002). Marbled crayfish were expected to have a higher functional response due to their recent invasion success.

Further, we aimed for a mechanistic understanding of this process by identifying trophic traits that promote invasions and investigate the effect of individual behavioral types on these traits and the functional responses, bringing together research on animal personality and food-resource use (Toscano et al. 2016). In an additional set of experiments, we independently measured all stages of the predation cycle (functional response parameters) for individual crayfish. We then quantitatively compared the functional response to our model predictions. We hypothesized that these parameters would describe the functional response of both species. Finally, previously assessed behavioral types of the tested individuals were compared to functional responses and functional response parameters. We expected active, bold and aggressive individuals to have higher functional responses and higher functional response parameters.

Methods

Study sites

Individuals from three populations of crayfish were collected for the experiments. First, we caught spiny-cheek crayfish in lake Müggelsee close to our institute (52° 26' 6" N, 13° 38' 6" E), Germany, with baited (dogfood) crayfish traps (type “PIRAT”, 610 × 315 × 250 mm, mesh width 40 × 10 mm, Rapurosvo, Parainen, Finland) between April 2015 and June 2016. The traps were set over night and checked on the next day. Second, marbled crayfish were taken from aquarium stocks kept by Peer Martin (Comparative Zoology, Humboldt University, Berlin, Germany). And third, we caught naturalized marbled crayfish by hand (and to a minor degree, by traps) from stocks that live in sympatry with spiny-cheek crayfish and *Dreissena* mussels in the littoral zone from lakes (i) Moosweiher (48° 01' 51" N, 7° 48' 17" E) in Baden-Württemberg, Germany, and (ii) Krumme Lanke (52° 27' 0" N, 13° 13' 52" E) in Berlin, Germany.

We sexed and measured all crayfish manually with a sliding caliper to the nearest millimeter. Length was measured as carapace length (CL) from the tip of the rostrum to the posterior edge of the carapace.

Maintenance of test animals

Crayfish were maintained in a climate chamber (17 °C, 14 hours light: 10 hours dark). All crayfish were kept in the laboratory for at least one month before being used in experiments. All individuals used for measurements on behavior and feeding were single-housed in tanks (300 × 200 × 200 mm) that were filtered by air-driven sponge filters. Only naturalized marbled crayfish from the lake Moosweiher population were marked for differentiation among individuals, and kept in filtered single-species community tanks (800 × 400 × 200 mm). We marked them with a point-code on top of their carapace using a white outdoor marker (Edding 8055, Ahrensburg, Germany; (see Abrahamsson 1965). We waited about a week before remarking and measuring crayfish following molting events. One PVC pipe (150 mm, Ø 50 mm) was provided as shelter, and 30 mm of fine gravel were put in each tank as a substrate. Additional shelters (>2 per crayfish) were provided in the communal tanks to reduce agonistic interactions. Water was exchanged with fresh tap (~ 75%) water once a week. Half a ring of commercial crayfish food (Crabs Natural, sera, Heinsberg, Germany) was fed to each crayfish daily.

The protocol and procedures employed were ethically reviewed and approved by the Landesamt für Gesundheit und Soziales (LAGeSo), Berlin, Germany. All experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Setup and standard procedure

Experiments were conducted between June 2015 and April 2017. All trials took place in the climate chamber to reduce handling and guarantee minimal disturbance from outside. Each setup was sheathed by opaque, black plastic tarpaulin to further minimize disturbances. Handling time experiments, parameter measurements and satiation measurements were filmed by two network cameras (Dinion HP 1080p, Bosch, Stuttgart, Germany; one vertically above the tank and another one at the side of the tank). Videos were recorded under infrared illumination (except handling time) from infrared headlights under total darkness. VLC-player (version 2.2.1.0) was used to record and save video data.

Before crayfish were used in any experiments, we checked them for loss of appendages, obvious diseases, eggs, upcoming molting events and the current reproductive form (form I/II in spiny-cheek crayfish males). We tested randomly chosen male and female spiny-cheek crayfish (using a pair of ten-sided dice), as consumption is generally assumed to be independent of sex (Usio and Townsend 2002). Intact intermolt individuals (26 – 50 mm CL) were used in experiments only for one trial per day. Crayfish were excluded from experiments up to at least one week when either molts occurred or after the release of brood by egg-bearing female because crayfish reduce or cease feeding completely during ecdysis (Aiken and Waddy 1992).

Experimental tanks were filled with 20 mm of fine white sand and 150 mm of tap water of 15 °C temperature. Crayfish were released into the tank and allowed to acclimatize for 30 minutes prior to the start of experiment. Tanks were completely drained after each trial, and before setting up another experiment to avoid a potential bias by remaining pheromones in the water (Breithaupt 2011).

Dreissena mussels were collected at lake Müggelsee by hand-picking them off hard structures on a weekly basis. We refer to them as *Dreissena* mussels since meaningful differentiation between the two species present in lake Müggelsee, the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*D. rostriformis*), can only be done by genetic means (Beggel et al. 2015). After collection, mussels were kept separately in an aerated tank until required.

Size selection

To determine the optimal prey size for our experiments, we performed size-selection trials with spiny-cheek crayfish males (*n* = 7, CL = 34.2 ± 2.7), spiny-cheek crayfish females (*n* = 6, 37.2 ± 2.8) and marbled crayfish from aquaria (*n* = 12, 33.3 ± 3.3). We offered crayfish four different size classes of *Dreissena* mussels: 2 – 6 mm (*n* = 10), 7 – 11 mm (*n* = 10), 12 – 16 mm (*n* = 10) and 17 – 21 mm (*n* = 10). A single crayfish was placed into a tank measuring 400 × 400 × 200 mm with the mussels and a PVC-pipe for shelter. After 24 hours we counted the remaining mussels of each size class that were still alive.

Functional response model

As basic functional response model, we used the Royama-Rogers random predator equation (Royama 1971, Rogers 1972) which is an extension of Holling’s (1959b) disc equation (eq. 1) and accounts for prey depletion. For this model, we used the notation derived by Rosenbaum and Rall (2018) in R version 3.4.0:

$$y(N_0) = \frac{N_0 - W(ahN_0 * \exp(a(hN_0 - T)))}{ah} \qquad (2).$$

The model includes the number of mussels initially offered to the experimental predator (N_0), the LambertW function (W), attack rate (a), handling time (h) and total experimental time (T).

We derived these independent parameters for three treatment groups: spiny-cheek crayfish males (n = 12), spiny-cheek crayfish females (n = 7) and marbled crayfish from aquaria (n = 15). During the long course of experiments three marbled crayfish, three spiny-cheek crayfish females and two male spiny-cheek crayfish got sick or died during molts and thus had to be excluded from the study.

We decided to run short-term experiments of T = 1 hour, as the crayfish consumed many mussels per unit time within the restrictions of our tanks (Jeschke et al. 2002). For such a short time period, satiation effects should be minimal. We therefore decided not to include such effects in the model (cf. Jeschke et al. 2002, Jeschke and Tollrian 2005a, Jeschke and Hohberg 2008). We conducted separate experiments to look into the effects of satiation (see below). To parameterize the model and mechanistically predict crayfish consumption rates y, it was necessary to express a and h in more detail, so that they can be directly experimentally measured.

Attack rates

We followed Jeschke et al. (2002) to calculate attack rate (a):

$$a = \beta \times \gamma \times \delta \times \varepsilon \qquad (3).$$

Accordingly, we experimentally quantified each of the parameters in eq. 3 for individual spiny-cheek crayfish males (n = 10, CL = 35.4 ± 2.3), spiny-cheek crayfish females (n = 4, CL = 33.2 ± 3.8) and marbled crayfish from aquaria (n = 12, CL = 33.0 ± 3.9). The setup described in *size selection experiments* was used here again, and 20 mussels, each between 3 – 7 mm in size (shell length) and 4 – 40 mg in weight, were randomly distributed on the sandy bottom of the tank. The experiments were conducted in the dark when crayfish are most active and illuminated by infrared headlights (Holdich and Black 2007, Luna et al. 2009). We visually analyzed 17 hours of videos and measured parameters for 30 minutes after the first mussel was consumed. If the crayfish consumed less than five mussels during this time, the experiment was repeated on another day.

We calculated encounter rate β, probability of detection γ combined with probability of attack δ, and attack efficiency ε for (i) each trial, (ii) average values for each individual crayfish and (iii) average values for each species. Attack efficiency in each trial was calculated as the number of successful attacks (cracking and feeding of the mussel) divided by the total number of attacks. Probability of detection and probability of attack cannot be visually distinguished. The product of these two was calculated as the number of attacks divided by the number of encounters with the mussel. Generally, we observed that close proximity with the appendages of the crayfish seemed to be necessary for an attack under laboratory conditions (i.e. total darkness). An encounter was recorded when the mussel and the encounter field overlapped. The ellipsoid encounter field of a crayfish was approximated by the length between the end of the antennae and the end of the carapace, and the width of the first pereiopods with the base of the rostrum in the center (Giguère et al. 1982). To measure encounter rate β, we counted the number of encounters and divided them by the average number of mussels during the trial. This number was then multiplied by 60 (minutes) and divided by the time spent searching during the trial in minutes to yield encounter rate β per hour for that particular trial. The time spent searching was trial time minus time spent handling mussels which was defined as follows.

Handling times

We measured the components of handling time h as outlined in (Jeschke et al. 2002):

$$h = \frac{t_{att}}{\varepsilon} + t_{eat} \qquad (4),$$

where t_{att} is attacking time per prey item, t_{eat} is eating time per prey item and ε is attack efficiency. The latter was measured as outlined in the previous section and is included in eq. (4) because handling time includes time wasted through unsuccessful attacks (Jeschke et al. 2002).

We measured both mean t_{att} and t_{eat} (n = 3 – 12) of the aforementioned individuals of spiny-cheek crayfish males (CL = 33.4 ± 4), spiny-cheek crayfish females (CL = 33.0 ± 3.7) and marbled crayfish from aquaria (CL = 35.8 ± 2.6). Crayfish were put in a tank with the dimensions 300 × 200 × 200 mm. After acclimatization, a single crayfish was provided with 20 *Dreissena* mussels (same size range as in *attack rates*). Feeding was recorded under low light conditions for better resolution. We stopped recording after 20 minutes or until five mussels were consumed. The 11 hours and 20 minutes of recordings were later visually analyzed for the handling parameters according to the following definitions: Attacking time per mussel, the time spent during a predation attempt, was defined as the time from the moment of first contact with the mussel to the moment it is cracked. Eating time per mussel started when the crayfish feeds upon the mussel's flesh to the moment the mussel is abandoned (zu Ermgassen and Aldridge 2011).

Satiation

We estimated satiation per crayfish by recording a subset of individual spiny-cheek crayfish males (n = 8, CL = 38.3 ± 2.4), spiny-cheek crayfish females (n = 2, CL = 37.88 ± 2.7) and marbled crayfish from aquaria (n = 6, CL = 38.3 ± 2.5) feeding on 300 *Dreissena* mussels for three hours in the dark. We visually analyzed 90 hours of video recordings and noted the time at which the crayfish consumed a mussel in each trial. We presumed that crayfish become satiated with time, and the resulting cumulative consumption in a hungry crayfish will rise sharply with time at the beginning and less so when they had filled their guts (Elliott and Persson 1978, Jeschke and Hohberg 2008). After the experiment, we relocated the crayfish in its holding tank and sieved the sand with dip nets to remove all remaining mussels, feces and debris of feeding activity.

To estimate the point of transition between hunger and satiation, we performed breakpoint analysis in R version 3.4.0 (R Core Team (2017)) for the count of mussels and the time between two consumed mussels (command breakpoints from the package *strucchange* (Zeileis 2006). The breakpoint is the point after which the time period between mussel consumptions increases and which we equated with a full gut. The satiation per mussel s is the reciprocal value of the number of mussels in a full gut (Jeschke et al. 2002). For example, crayfish S9 had a full gut after having consumed 85 mussels; its satiation per mussel s was thus 0.012 (Table B.1). In the next step, we estimated maximum long-term consumption y_{max}, i.e. the maximum number of mussels consumed at an excess of prey including satiation, by fitting logarithmic models to the consumption rate over 15-min time intervals. Finally, we calculated gut retention time t_g as $1 / (y_{max,} \times s)$ (cf. Jeschke et al. (2002)).

Functional response experiments

Following the parameterization of the functional response model for each crayfish species, we empirically measured the functional responses in order to compare model predictions with observed consumption rates. These experiments were run in the same tanks under the same preconditions as the parameter experiments on a and h. We used the same individual spiny-cheek crayfish males (mean CL = 36.9 ± 1.9 mm), spiny-cheek crayfish females (mean CL = 35.1 ± 2.8 mm) and marbled crayfish from aquaria (mean CL = 34.1 ± 3.4 mm) tested for functional response parameters because these were later related to personality traits (2.10 Behavioral assay). In addition, we used naturalized marbled crayfish ($n = 7$, CL = 39.5 ± 6.6 mm), which were significantly larger than aquarium individuals (t-test: $df = 289$, $t = 289$, $p < 0.001$), to be able to also compare their functional responses to those of the groups of crayfish.

During the experiments, the crayfish were supplied with 20, 30, 40, 50, 60, 80 and 100 *Dreissena* mussels of the same size as for previous experiments. Three to five replicates at each density for each individual and within each treatment were measured in $n = 969$ feeding trials. Crayfish were left feeding on the mussels for one hour in darkness. We then removed the crayfish and sieved the sand for the feeding remains as described for the satiation experiment. The remaining mussels that were still alive or damaged but not consumed were then counted. If crayfish refused to feed and molted ($n = 140$) or had a new clutch ($n = 41$) following the experiment within one week, values were excluded from the dataset.

We calculated mean numbers of consumed mussels for each density for each individual. We then calculated species averages for spiny-cheek crayfish, marbled crayfish from aquarium and naturalized crayfish for each density and compared them to each other and the associated model. To account for size differences caused by molting events between the experiments, we also calculated size-corrected models using the mean sizes measured during functional response trials (Figure B.1).

Behavioral assay

In a previous study (Linzmaier et al. 2018), we performed personality experiments with the same individuals that have been used here in functional response experiments. Individuals were tested for differences in activity, aggressiveness and boldness: aggression during interspecific confrontations was scored with the system developed by Atema and Voigt (1995); activity was assessed as the proportion of time spent inside and outside a shelter during six hours of observation; and boldness was measured as the response of a crayfish to an approaching human hand. Further details on the behavioral assay can be found in Linzmaier et al. (2018). The individuals of both species were divided into two groups each by their average activity (low and high activity), aggression score (low and high aggression) and aggressive (positive score) or fearful threat response (negative score). We pooled male and female spiny-cheek crayfish here due to low sample size of the subgroups. We calculated means for each prey density from all individuals of the group. We then assessed if functional responses differed between these groups.

Statistics

Means of a, h, s and y_{max} were statistically compared by two-sided t-tests (Dick et al. 2013). The fit between predicted and measured consumption (i.e. the mechanistic model) was determined by Pearson's product-moment correlation. Functional response type of the measured functional responses was determined following Juliano (2001) by visual inspection of raw plotted data and the *frair_test* function, provided by the *frair* R-package (Pritchard et al. 2017) to test whether a type II or type III curve better fits the data. We further evaluated the measured functional responses by fitting regression models to the data of each group (based on Royama-Rogers random predator equation as above), and calculating 95% confidence intervals by bootstrapping (n = 999) (Pritchard et al. 2017). As starting values for each model fit, we used the measured parameter (a and h from 2.6 and 2.7) from each group for free model parameters and T = 1 h. We then looked at overlaps of the confidence intervals between marbled crayfish, spiny-cheek crayfish females and spiny-cheek crayfish males. We also compared aquarium with naturalized marbled crayfish in this way.

We looked at interspecific differences in feeding parameters by performing a non-metric multidimensional scaling (NMDS) analysis on measured feeding parameters in R (package *vegan* (Oksanen et al. 2018)). We included mean values of t_{att}, t_{eat}, β, γ, ε, y_{max} and s of all individuals where all variables including satiation were measured: spiny-cheek crayfish (male), n = 8; spiny-cheek crayfish (female), n = 2; marbled crayfish, n = 6). Female spiny-cheek crayfish were excluded from the NMDS analysis due to small sample size. Data were standardized by subtracting each value from the mean and dividing by standard deviation. Then data were centered on zero and shifted by the minimum to remove negative values. We performed the NMDS with k = 2 (number of dimensions) and a Gower dissimilarity index for mixed data (stress = 0.167). We checked the variables to see how they behave in the ordination space and added arrows to the variables if they were meaningfully representing a gradient. Finally, we tested for homogeneity of dispersion between species ($F = 0.157_1$, $p = 0.742$) and subsequently performed a non-parametric permutational MANOVA (PERMANOVA) to test for differences between the species. We visually checked the behavioral variables and feeding variable for normal distribution and performed a correlation matrix in R for all individuals mentioned above, including female spiny-cheek crayfish (package *Hmisc*; Harrell and Dupont (2018); method = Pearson).

Results

Models and observed consumption

The observed functional response was consistently higher for spiny-cheek crayfish males and females compared to marbled crayfish (Figure 2.1). This was also the case when correcting for size differences among crayfish (Figure B.1). Marbled crayfish had a significantly lower functional response than male spiny-cheek crayfish but not spiny-cheek crayfish females; spiny-cheek crayfish females had lower functional response than males (Figure B.2). Naturalized marbled crayfish had a slightly higher, although not significantly different functional response compared to aquarium marbled crayfish (Figure B.2). The curve progression we observed can best be described by a type II functional response (spiny-cheek males: $z = –14.80$, $p < 0.001$; spiny-cheek females: $z = –8.10$, $p < 0.001$; marbled crayfish: $z = –7.93$, $p < 0.001$). The predicted model fit was best for male spiny-cheek crayfish ($r = 0.87$, $t = 14.26$, $p < 0.001$), but also good for both female spiny-cheek crayfish ($r = 0.78$, $t = 6.32$, $p < 0.001$) and marbled crayfish ($r = 0.80$, $t = 11.80$, $p < 0.001$). The mechanistic models adequately predicted the functional responses but slightly overestimated it overall.

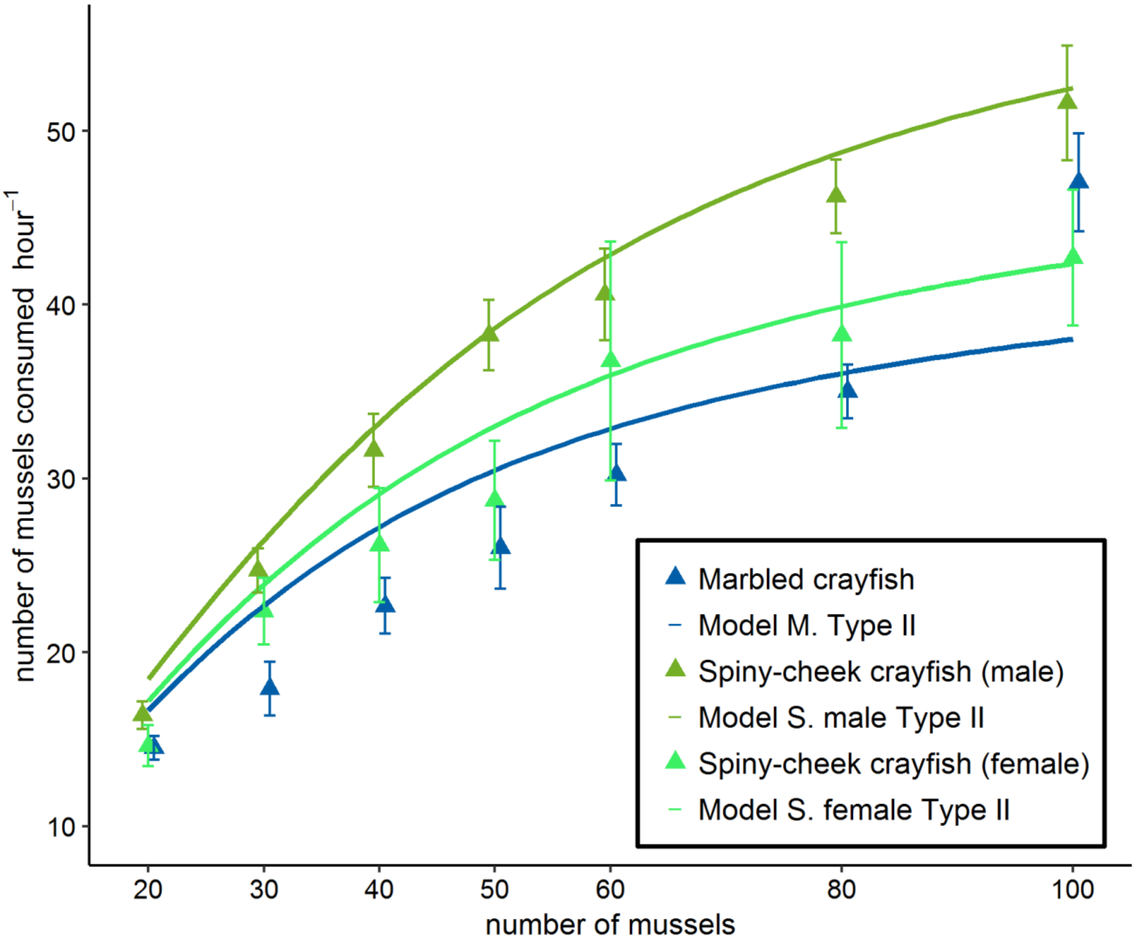


Figure 2.1 – Observed functional responses (means ± SE) for marbled crayfish, spiny-cheek crayfish females and spiny-cheek crayfish males and the functional response predicted from independently derived parameters. The number of mussels refers to one experimental area (0.16 m²).

Parameters

The handling time (*h*) of male spiny-cheek crayfish was significantly shorter than that of marbled crayfish (Table 2.1; *df* = 20, *t* = 2.75, *p* = 0.012). Female and male spiny-cheek crayfish (*df* = 12, *t* = 2.05, *p* = 0.063) as well as female spiny-cheek crayfish and marbled crayfish (*df* = 14, *t* = 0.63, *p* = 0.536) did not significantly differ in *h.* Attack rate (*a*) did not significantly differ between species or sexes (*df* = 20, *t* = 1.30,p = 0.207; *df* = 12, *t* = 0.77, *p* = 0.455; *df* = 14, *t* = 0.05, *p* = 0.961). A PERMANOVA comparing male spiny-cheek crayfish and marbled crayfish showed a significant difference between these groups in feeding parameters (PERMANOVA: $F = 2.64_1$; *p* = 0.018; cf. Figure 2.2). This comparison also includes satiation parameters: Long-term maximum feeding rates were higher for marbled crayfish (*df* = 12, *t* = 2.96, *p* = 0.012). Marbled crayfish did not have a higher gut capacity (*df* = 12, *t* = 2.04, *p* = 0.064) and therefore gut retention time than spiny-cheek crayfish males (Table 2.2).

Table 2.1. – Means (± SD) of measured and calculated predation parameters for marbled crayfish and spiny-cheek crayfish from foraging observations on *Dreissena* mussels.

	Marbled crayfish (n = 12)	Spiny-cheek crayfish (♀, n = 4)	Spiny-cheek crayfish (♂, n = 10)
Encounter rate β (h^{-1})†	6.84 ± 2.83	6.07 ± 3.20	7.62 ± 3.46
Detection probability γ × Attack probability attack δ†	0.48 ± 0.15	0.58 ± 0.19	0.54 ± 0.18
Attack efficiency ε†	0.93 ± 0.07	0.87 ± 0.14	0.93 ± 0.08
Attacking time t_{att} (sec)‡	21 ± 9	21 ± 6	17 ± 7
Eating time t_{eat} (sec)‡	56 ± 17	45 ± 8	36 ± 7
Handling time h (sec)§	79 ± 24	70 ± 14	54 ± 13
Total attack rate a (h^{-1})§	2.81 ± 1.09	2.84 ± 1.38	3.61 ± 1.78

† = Parameter experiment

‡ = Handling time experiment

§ = Calculated from parameter values above (see equations 3, 4)

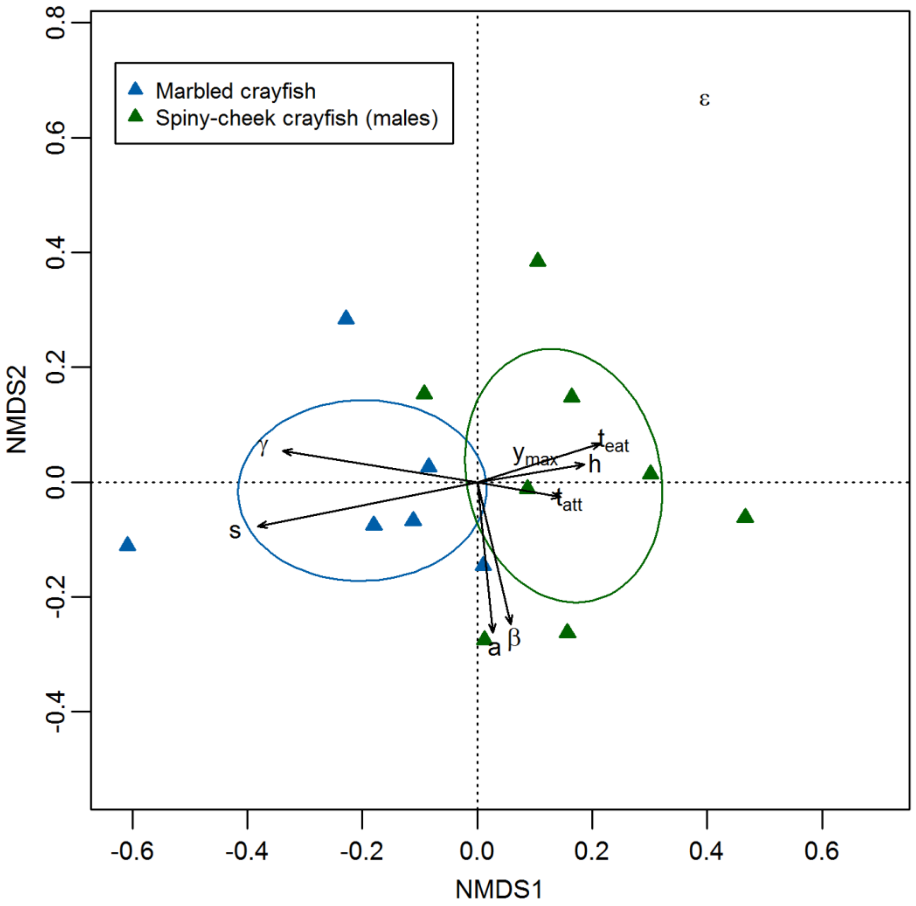


Figure 2.2 – Non-metric multidimensional scaling of individual crayfish (marbled crayfish and spiny-cheek crayfish males) and means of individual feeding parameters attacking time per prey item (t_{att}), eating time per prey item (t_{eat}), attack efficiency (ε), encounter rate (β), probability of detection (γ), handling time (h), attack rate (a), maximum long-term consumption (y_{max}) and satiation per mussel (s) with ellipsoid polygons around species centroids. The arrows represent gradients in the ordination space.

Table 2.2 – Means (± SD) of satiation parameters for marbled crayfish and spiny-cheek crayfish.

Parameter	Marbled crayfish (n = 6)	Spiny-cheek crayfish (♀, n= 2)	Spiny-cheek crayfish (♂, n = 8)
Number of prey items for satiation (g)†	96 ± 8	95 ± 2	80 ± 17
Maximum long-term consumption (y_{max} (d)†)	779 ± 128	612 ± 51	558 ± 145
Gut retention time (t_g (h)‡)	3.10 ± 0.59	3.77 ± 0.35	3.62 ± 0.56

† = Satiation Experiment

‡ = Calculated from satiation parameter values above (see main text)

Size selection

Both crayfish species preferred smaller mussels up to 11 mm over larger ones when given the choice between different size classes (Figure B.3). Marbled crayfish generally consumed fewer mussels than spiny-cheek crayfish.

Behavior and functional response

We found that highly vs. lowly active individuals of both marbled crayfish (Figure 2.3a; a: $df = 9$, $t = 1.71$, $p = 0.122$; h: $df = 9$, $t = 0.87$, $p = 0.409$) and spiny-cheek crayfish (Figure 2.3b; $df = 12$, $t = 0.39$, $p = 0.705$; $df = 12$, $t = 0.26$, $p = 0.800$) did not significantly differ in numbers of consumed mussels. Marbled crayfish with higher aggression scores had higher h ($df = 11$, $t = 2.21$, $p = 0.049$) and thus higher functional responses at high prey densities compared to individuals with low aggression scores (Figure 2.3c), whereas a did not significantly differ ($df = 11$, $t = 1.63$, $p = 0.131$). The predicted functional response models for the more aggressive individuals confirmed the measured values, slightly overestimating the consumption except for the highest prey numbers. No difference between the groups was found among spiny-cheek crayfish (Figure 2.3d; a: $df = 12$, $t = 0.27$, $p = 0.793$; h: $df = 12$, $t = 0.17$, $p = 0.864$). Bolder spiny-cheek crayfish with an aggressive threat response exhibited a higher functional response and had a significantly lower h (Figure 2.3f; $df = 12$, $t = 3.09$, $p = 0.009$), whereas a did not significantly differ (a: $df = 12$, $t = 0.13$, $p = 0.898$). The aggressive individuals were mostly male (one female) and the fearful individuals mostly female (one male; see Linzmaier et al. (2018)). The predicted functional response models confirmed these patterns for spiny-cheek crayfish (Figure 2.3f) and to a minor degree for marbled crayfish, while their measured data do not suggest this difference (Figure 2.3e).

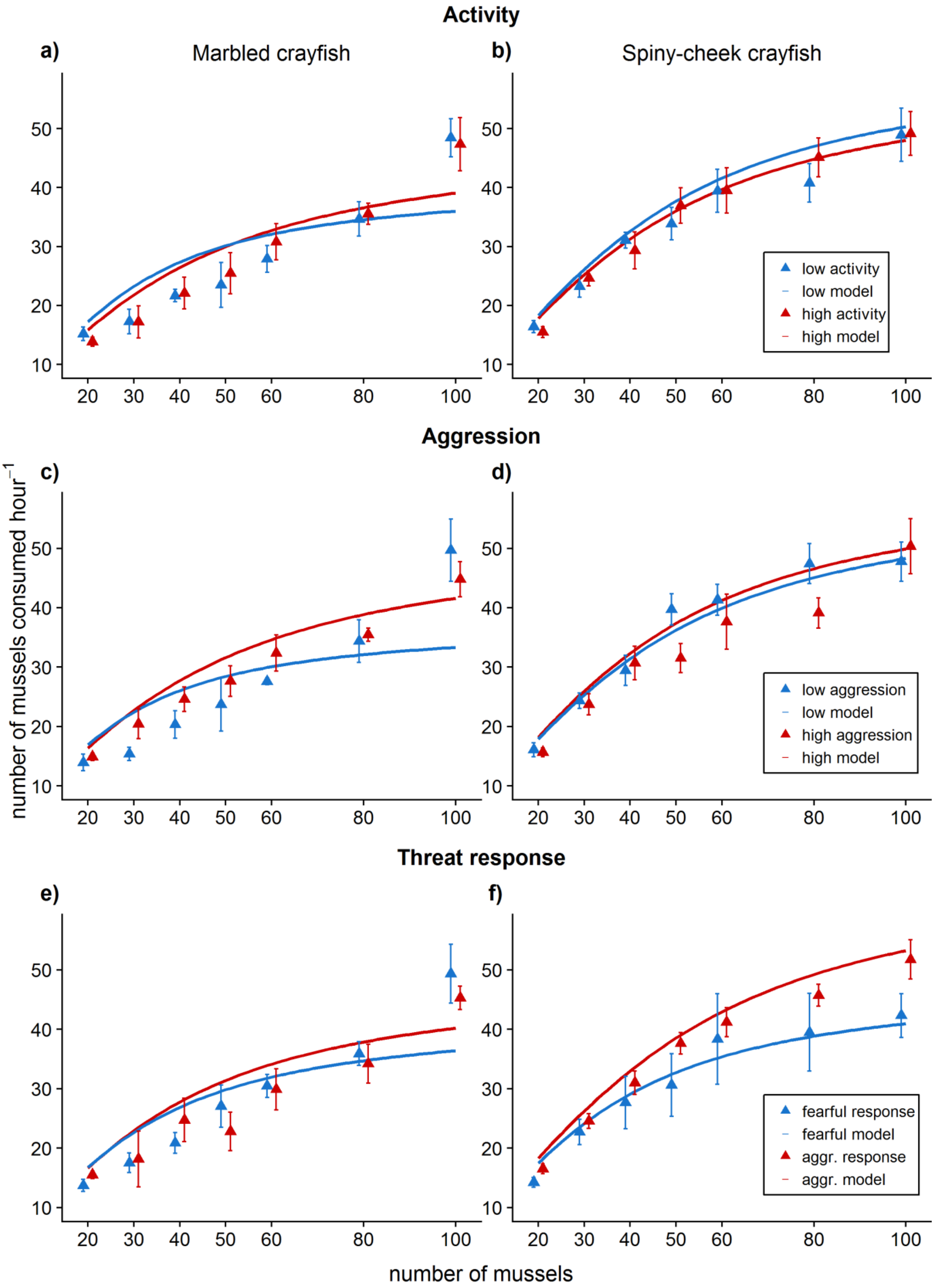
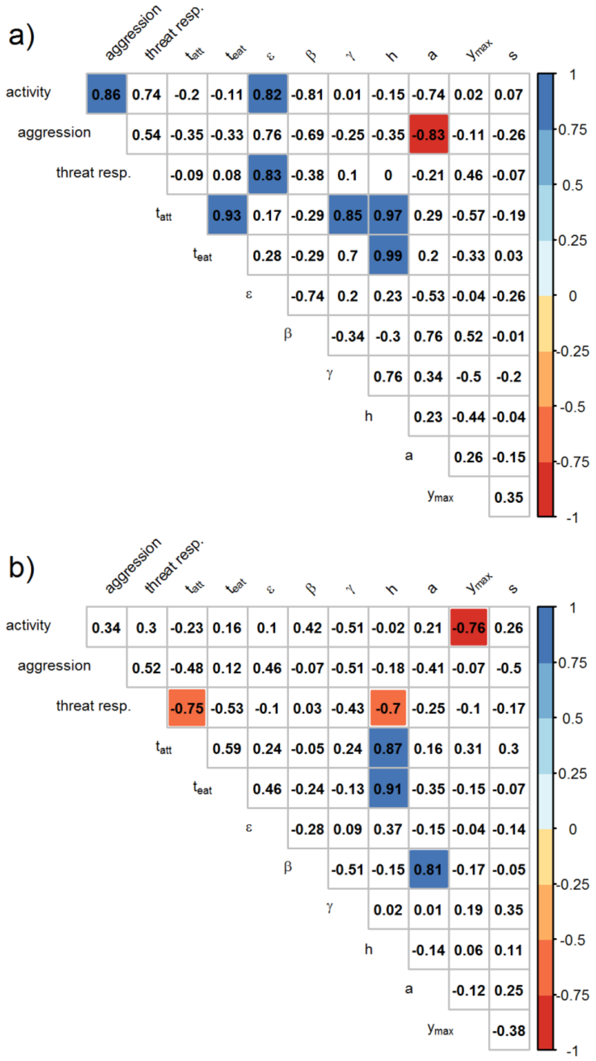


Figure 2.3 – Predicted and observed functional responses (means ± SE) for marbled crayfish (left column) and spiny-cheek crayfish (right column) of different behavioral types. The number of mussels refers to one experimental area (0.16 m²).

Bivariate Correlation

The multivariate correlations on the species level revealed similar patterns of significant correlations among variables in each species with a few exceptions (Figure 2.4 a & b). In individuals of marbled crayfish, aggression was negatively correlated with *a*. Activity was positively correlated with aggression and ε for marbled crayfish (see Linzmaier et al. 2018) and negatively correlated with y_{max} for spiny-cheek crayfish. More aggressive threat response scores were negatively correlated with *h* and t_{att} for spiny-cheek crayfish and positively with ε for marbled crayfish. The parameter *h* was naturally positively correlated with its constituent parameters, and *a* was positively correlated with β for spiny-cheek crayfish (see eqs. 3,4). The γ and t_{att} were positively correlated for marbled crayfish.



a)

	aggression	threat resp.	t_{att}	t_{eat}	ε	β	γ	h	a	y_{max}	s
activity	0.86	0.74	-0.2	-0.11	0.82	-0.81	0.01	-0.15	-0.74	0.02	0.07
aggression		0.54	-0.35	-0.33	0.76	-0.69	-0.25	-0.35	-0.83	-0.11	-0.26
threat resp.			-0.09	0.08	0.83	-0.38	0.1	0	-0.21	0.46	-0.07
t_{att}				0.93	0.17	-0.29	0.85	0.97	0.29	-0.57	-0.19
t_{eat}					0.28	-0.29	0.7	0.99	0.2	-0.33	0.03
ε						-0.74	0.2	0.23	-0.53	-0.04	-0.26
β							-0.34	-0.3	0.76	0.52	-0.01
γ								0.76	0.34	-0.5	-0.2
h									0.23	-0.44	-0.04
a										0.26	-0.15
y_{max}											0.35

b)

	aggression	threat resp.	t_{att}	t_{eat}	ε	β	γ	h	a	y_{max}	s
activity	0.34	0.3	-0.23	0.16	0.1	0.42	-0.51	-0.02	0.21	-0.76	0.26
aggression		0.52	-0.48	0.12	0.46	-0.07	-0.51	-0.18	-0.41	-0.07	-0.5
threat resp.			-0.75	-0.53	-0.1	0.03	-0.43	-0.7	-0.25	-0.1	-0.17
t_{att}				0.59	0.24	-0.05	0.24	0.87	0.16	0.31	0.3
t_{eat}					0.46	-0.24	-0.13	0.91	-0.35	-0.15	-0.07
ε						-0.28	0.09	0.37	-0.15	-0.04	-0.14
β							-0.51	-0.15	0.81	-0.17	-0.05
γ								0.02	0.01	0.19	0.35
h									-0.14	0.06	0.11
a										-0.12	0.25
y_{max}											-0.38

Figure 2.4 – Correlation matrices of behavioral variables (aggression, activity and threat response) and feeding variables (attacking time per prey item (t_{att}), eating time per prey item (t_{eat}), attack efficiency (ε), encounter rate (β), probability of detection (γ), handling time (*h*), attack rate (*a*), maximum long-term consumption (y_{max}) and satiation per mussel (*s*) in (**a**) a set of individual marbled crayfish and in (**b**) a set of individual spiny-cheek crayfish. Each cell contains the correlation coefficient and significant (α = 0.05) correlations are represented by colored squares. Red colors indicate negative correlations and blue colors positive correlations.

Discussion

We investigated the trophic interactions of two co-occurring invasive crayfish species and mussel prey with the framework developed by Penk et al. (2017). We looked at foraging and feeding parameters, the functional responses and behavioral types of single individual crayfish. Hereby, we gained a detailed mechanistic understanding of the studied predator-prey interaction. The observed interaction strength provides an estimate of relative impact potential of two invasive crayfish on an ecologically important invasive mussel. Further, we could adequately predict the relative interaction strength for two invasive crayfish on ubiquitous *Dreissena* mussel prey with our models. The detailed measurements showed that males of the widely established spiny-cheek crayfish outperformed naïve, aquarium as wells as sympatric, naturalized marbled crayfish on most aspects of feeding on *Dreissena* mussels. Feeding modalities and behavioral differences among individuals might explain some aspects of effectiveness in prey consumption.

Functional responses

The mechanistic models performed well in predicting the difference observed in the functional response between both species. Both species showed type II responses. This type of response has also been found in red swamp crayfish (*Procambarus clarkii*) feeding on *Dreissena polymorpha* (Gonçalves et al. 2017). The functional response curve can be influenced by many factors including habitat complexity (Kalinkat et al. 2013a). Structurally complex habitats provide shelter for prey, especially at low densities. Oyster shell habitats, for example, increased a differently for small and large mud crabs at low prey densities and resulted in type III functional responses whereas less complex habitats resulted in type II functional responses (Toscano and Griffen 2013, 2014). *Dreissena* mussels usually are easily accessible, settling on every hard surface available and live even on flat, soft and open sediments in large densities making a type II response also in the field very likely (Dermott and Munawar 1993). Some factors could still alter the predation process. *Dreissena* mussels seek refuge and form firm aggregations in response to predation (Kobak and Kakareko 2009, Naddafi and Rudstam 2013). Prey aggregations should lead to a decrease in β. This had been shown in signal crayfish (*Pacifastacus leniusculus*) feeding on *Dreissena* mussels which spent significantly more time foraging for single *Dreissena* mussels compared to druses of mussels (zu Ermgassen and Aldridge 2011). However, feeding rate was not different between single mussels and druses. We thus believe that our measurements realistically captured the foraging process on *Dreissena* mussels.

In contrast to our predictions, marbled crayfish had a lower functional response than male spiny-cheek crayfish. These might have been affected by egg production and care periods of marbled crayfish. Due to their parthenogenetic reproduction, our isolated marbled crayfish were still able to reproduce several times during the course of the experiments. This was not the case for spiny-cheek crayfish, which are sexually reproducing. Generally, marbled crayfish reduced or ceased feeding during reproduction, but we could also observe feeding individuals during reproduction. We do not know the exact times of reduced feeding, but the one week after release of the young that we set as limits before continuing measurements might have been too arbitrary.

Parameters and satiation

Feeding rates (this study) and boldness (Linzmaier et al. 2018) differed for male and female spiny-cheek crayfish. Usio and Townsend (2002) found that male *Paranephrops zealandicus* had higher feeding rates than females when feeding on leaf litter. Food conversion is usually the same for both sexes (Rodgers et al. 2006). In crayfish aquaculture, though, male crayfish of several species grow faster than females, especially in monosex-cultures (Lawrence et al. 2000). Hence, higher feeding rates for males could be assumed. However, in several cambarid crayfish feeding on *Dreissena* mussels, no differences or even higher feeding rates of females have been documented (MacIsaac 1994, Perry et al. 1997, Corkum and Cronin 2004). We think that behavioral or developmental differences could explain the differences we found between sexes.

Like all Cambaridae, spiny-cheek crayfish males and even females alternate between a sexually active form I and a non-reproductive form II (Hobbs 1974, Wetzel 2002). Higher aggression and sexual activity of form I males has been documented (e.g. Dunham and Guiasu 1997). Thus, form I males could have a higher energetic demand than form II males, and might consequently have a higher consumption rate. Our measurements were taken over two to three moltings and according form alterations. We recorded form alterations for male crayfish and found higher functional responses in form I compared to form II males, which in turn consumed more than females (Figure B.4). Hence, we think that feeding differences are highly dependent on reproductive stage and individual behavioral types.

Generally, spiny-cheek crayfish seemed to overcome marbled crayfish in most aspect of feeding. However, we found higher physiological capacity for marbled crayfish: they had a significantly higher maximum consumption rate y_{max}. Satiation models capture an important element of predation connecting ecological processes and physiology (Jeschke et al. 2002). How can we explain the apparently higher physiological capacity of marbled crayfish? In Linzmaier et al. (2018), we did not observe significant differences in activity between marbled and spiny-cheek crayfish, but metabolic demand of marbled crayfish could still be higher for marbled crayfish due to reproduction (see above). Also, marbled crayfish might have a more efficient conversion of resources to tissue or offspring as it has been found in other invaders (Byers 2000). This can, for example, come from increased enzyme activity. Johnston and Freeman (2005) found differences in enzyme activity of six species of shore crab, related to their preferential diet. Marbled crayfish seem to have an efficient metabolism that enables them to use feeding periods between rapidly alternating periods of molts and egg-bearing.

Impact

The observed per capita effect in the predatory impact of marbled crayfish on *Dreissena* mussels classifies them as a marginally impactful species according to the literature of comparative functional responses (Dick et al. 2013, Dick et al. 2014). Such relationships have often been related to impact of crayfish on their native counterparts. For example, the functional response of invasive signal crayfish on amphipod prey has been higher compared to white-clawed crayfish (*Austropotamobius pallipes*) (Haddaway et al. 2012, Taylor and Dunn 2018). In our case, however, we studied an established, invasive species and a recently arrived novel species (i.e. a co-invasion or even an over-invasion scenario (Russell et al. 2014)). This comparison reflects the ecological reality in many Central European water bodies and has much broader implications than comparisons against the ever rarer native noble crayfish (*Astacus astacus*) (Kouba et al. 2014). Also, comparisons at an early stage of the invasion or even before transport are more useful for risk assessment than a posteriori studies on higher impact of invaders (Leung et al. 2002).

The high maximum long-term consumption in marbled crayfish could have an effect on *Dreissena* mussel populations in the field, which impose an impactful invader by themselves. Some predators (native or invasive) potentially provide biotic resistance to invasive prey (Twardochleb et al. 2012). Blue crab (*Callinectes sapidus*), for example, significantly reduced zebra mussel populations in parts of the Hudson river (Carlsson et al. 2011). Also, field studies on rusty crayfish suggest that streams with moderate to high densities of crayfish will limit spread and densities of zebra mussels (Perry et al. 1997, 2000). While crabs also feed on larger, adult mussels, many crayfish species have been shown to prefer smaller prey (MacIsaac 1994, zu Ermgassen and Aldridge 2011). We could confirm that also spiny-cheek and marbled crayfish mostly fed on mussels that were smaller than 12 mm. This size class offers the greatest profitability for crayfish (Gonçalves et al. 2017). Thus, *Dreissena* mussels can reach a size refuge, and mostly small individuals will be impacted by crayfish predation. Additionally, *Dreissena* mussels adapt their behavior and morphology to predation by reducing feeding rate and increasing shell thickness at the cost of lower growth rates (Naddafi and Rudstam 2013, 2014).

Even in omnivores like crayfish, we can have different impacts on the food web by new invaders (Larson et al. 2017). In the field, the interaction strength between marbled and spiny-cheek crayfish plus their prey might be different due to the density of each species. New impact metrics for emerging invasives like the relative impact potential (RIP) consider species-specific predation rates and numerical responses in concert with functional responses (Dick et al. 2017). Marbled crayfish might have lower functional responses but have a faster reproduction and may thus reach higher abundances.

Besides the insight that we gained on the predator-prey relationship among crayfish and *Dreissena* mussels, the question remains whether the observed differences are conserved across prey types. Omnivore decapod crayfish feed on several types of prey, an abundant alternative prey, such as the invasive amphipod *Dikerogammarus villosus*, might shift the pattern of the overall response (Gergs and Rothhaupt 2008, Smout et al. 2010). Also, macrophytes or detritus might be differently affected, as they are consumed and provide refuge at the same time (Médoc et al. 2018). Ideally, future comparisons include multiple prey items as well as (average) abundance data of prey organisms in water bodies threatened by invasion.

Finally, invasive species sometimes do not even have to be more aggressive, be better in acquiring resources or exploit a new niche but have less intraspecific competition also called ‘friendly release’ to be successful (Warren et al. 2019). The almost genetically identical, all-female populations of marbled crayfish, for example, have the advantage that they do not have to compete for partners and that genetic similarity reduces aggression (Vogt 2008, Carazo et al. 2014).

Behavior

Intraspecific variability in prey consumption plays an important role in community dynamics (Raffard et al. 2017, Des Roches et al. 2018). For example, Evangelista et al. (2019) found that the effect of intraspecific variability among invasive red swamp crayfish on prey community responses, especially on leaf litter and snails, was higher than the effect of species presence (invasion) alone. Our expectations on the effects of behavioral types on functional responses and their parameters were partially met. Bolder spiny-cheek crayfish, and to a lesser degree also aggressive marbled crayfish, showed consistently higher functional responses which was confirmed by the model predictions of these individuals. Bolder spiny-cheek crayfish were mostly male and females that fled more often (Linzmaier et al. 2018). In general, females have smaller claws and are less able to defend themselves (Stein and Magnuson 1976). However, we also had a very fearful male and a very bold female, and believe that individual boldness can affect sex-based models. Marbled crayfish generally did not show a truly aggressive threat response. They were either ducking away from the approaching hand or fleeing (Linzmaier et al. 2018). Thus, the bolder individuals were classified by “not fleeing”, whereas spiny-cheek crayfish showed a meral spread and an aggressive approach.

Elevated activity levels seemed not to affect crayfish feeding patterns, but aggression slightly elevated feeding rates by lower *h* in marbled crayfish. Behavioral correlations or syndromes (Bell and Sih 2007) like aggression syndromes can couple activity and aggressive behaviors, which are also important for population establishment of invasive species, with feeding rates (Pintor et al. 2009). In our case such a syndrome has been found for marbled crayfish but not for spiny-cheek crayfish (Linzmaier et al. 2018). Higher aggression, however, does not necessarily mean higher food uptake. Invasive yabby (*Cherax destructor*), for example, won less agonistic encounters with the native fitzroy falls crayfish (*Euastacus dharawalus*), but were more efficient in feeding on a provided food source (Lopez et al. 2019). The same has been shown for invasive green crabs (*Carcinus maenas*) and their native comparators, the blue crabs (MacDonald et al. 2007). Therefore, the relationship of aggression and higher feeding rates could indeed be negative. High aggression and lower functional responses of marbled crayfish might thus be retained in the face of direct competition.

We showed that some behavioral types might per se be related to individual differences in feeding parameters. In most studies, values for feeding parameters (*a* and *h*) are not mechanistically measured but instead calculated from fitting functional response models (Toscano and Griffen 2014, Schröder et al. 2016, Hartley et al. 2019). As outlined above, these values must not be confused with real success rate and handling time. Thus, their relationship with measured behaviors cannot be mechanistically interpreted. Here we measured real individual-level functional responses and mechanistic parameters separately and linked them to important behavioral traits. Schröder et al. (2016) could not find such a relationship of parameters derived by phenomenological models in *Heterandria formosa* killifish feeding on *Artemia salina* nauplii and suggested that a connection of behavioral traits with feeding might be required. In our study, these patterns of elevated functional responses in more aggressive or bolder individuals could not be seen as a general pattern within both species but only in some instances. However, the predicted models from parameter measurements usually reflected the hypothesized patterns. Maybe these differences get more pronounced when feeding takes place under field conditions. Competition for food will influence interspecific aggression, and interference by predators affects activity and boldness (Pintor et al. 2008). Assessments like ours should therefore be extended by including higher-order predators or direct competition (see Penk et al. 2017).

Looking at the relationship of certain parameters and behavioral traits, we found that bolder and aggressive individuals (aggression against predators and competitors) had shorter *h*, favoring food uptake at high prey densities. Voracious feeding (high *a* and short *h*) was expected for both behavioral types, but this correlation could only be confirmed for the boldness scores. Such relationships have been found in, for example, signal crayfish were aggression, voracity and boldness were positively correlated with prey consumption rates (Pintor et al. 2008). Bolder individuals are usually higher-ranking in social hierarchies. These hierarchies can imply higher feeding rates in bolder individuals, as they eat more food than subordinate ones (Gherardi and Daniels 2003, Ahvenharju and Ruohonen 2006) and have higher functional responses (Hartley et al. 2019). The crayfish in our studies have been kept in isolation to exclude such dominance effects (Moore 2007). However, it could be that lower feeding rates are retained even in the absence of competition. For example, subordinate three-spined sticklebacks (*Gasterosteus aculeatus*) chose the “inferior” diet even in the absence of dominant individuals (Milinski 1982). Thus, the rank of each tested individual in a prior dominance hierarchy among the individuals might have reflected their consumption rates.

The locomotor crossover hypothesis states that predators exhibiting higher activity levels consume more prey when preying on low-activity prey (Huey and Pianka 1981). However, we could not find these correlations for *a* and *h*. We observed that faster moving crayfish were often not foraging but running along the aquarium pane, thereby ignoring mussels on their path. Activity, as we measured it, might not accurately reflect foraging activity, and periods of real foraging activity should be better distinguished from non-feeding activities to find the presumed positive links shown for other species (e.g. Pruitt et al. 2011, McGhee et al. 2013, Toscano and Griffen 2014).

Conclusions

The link of certain behavioral types with food consumption is probably not consistent across contexts but depends on the way these traits are measured. We could, however, demonstrate that marbled crayfish and spiny-cheek crayfish differ in their functional response and in several parameters related to their feeding behavior on mussels. Our detailed account of feeding parameters was able to mechanistically predict real consumption rates, and our data suggest stronger effects of spiny-cheek crayfish on mussel prey. However, if we look at the invasion history and population development of the invasive *Dreissena* mussels and the invasive spiny-cheek crayfish, we believe that crayfish cannot substantially reduce the mussels in most invaded systems. We further provide important data on the trophic ecology of marbled crayfish which still are at an early stage of invasion. Both the spiny-cheek crayfish and marbled crayfish are in the “List of Invasive Alien Species of Union Concern” (EU Regulation 1143/2014), banning the trade with these species and enforcing monitoring and management. The few systems that have confirmed self-sustaining populations of marbled crayfish seem to favor sympatry of both species (Chucholl and Pfeiffer 2010, Chucholl and Wendler 2017) and communities with multiple crayfish will probably increase in number. High long-term consumption, interspecific aggression (and low intraspecific aggression) and reproduction rates, however, can promote further establishment and spread of marbled crayfish. Thus, known marbled crayfish populations, which are mostly restricted to small lakes (Chucholl 2015), should be monitored and contained, as combined effects on ecosystems might be additive or even amplified (Jackson et al. 2014). Finally, behaviors in general are an integral part of biodiversity and essential to conservation but have not yet received much attention (Cordero-Rivera 2017). Marbled crayfish will therefore bring a change to European aquatic systems that might be less obvious.

Acknowledgments

We thank Michael Pfeiffer and Christoph Chucholl for sharing their knowledge on crayfish and helping us in the field. We further thank Peer Martin for the provisioning of marbled crayfish and Larissa Goebel for taking care of the crayfish and assisting us during the study. We also like to thank Maud Bernard-Verdier, Gregor Kalinkat, Angus McIntosh and anonymous reviewers for their advice on the manuscript. Financial support was provided by the Deutsche Forschungsgemeinschaft (DFG; JE 288/9-1, JE 288/9-2) and IGB.

Literature cited

Abrahamsson, S. 1965. A method of marking crayfish *Astacus astacus* Linné in population studies. Oikos **17**:228–231.

Ahvenharju, T., and K. Ruohonen. 2006. Unequal division of food resources suggests feeding hierarchy of signal crayfish (*Pacifastacus leniusculus*) juveniles. Aquaculture **259**:181–189.

Aiken, D., and S. Waddy. 1992. The growth process in crayfish. Reviews in Aquatic Sciences **6**:335–381.

Alexander, M. E., J. T. Dick, O. L. Weyl, T. B. Robinson, and D. M. Richardson. 2014. Existing and emerging high impact invasive species are characterized by higher functional responses than natives. Biology Letters **10**:20130946.

Atema, J., and R. Voigt. 1995. Behavior and sensory biology. Pages 313–348 *in* J. R. Factor, editor. The Biology of the Lobster. Academic Press, San Diego, USA.

Beggel, S., A. Cerwenka, J. Brandner, and J. Geist. 2015. Shell morphological versus genetic identification of quagga mussel (*Dreissena bugensis*) and zebra mussel (*Dreissena polymorpha*). Aquatic Invasions **10**:93–99.

Bell, A. M., and A. Sih. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters **10**:828–834.

Berryman, A. A. 1992. The Orgins and Evolution of Predator-Prey Theory. Ecology **73**:1530–1535.

Biro, P. A., B. Adriaenssens, and P. Sampson. 2014. Individual and sex-specific differences in intrinsic growth rate covary with consistent individual differences in behaviour. Journal of Animal Ecology **83**:1186–1195.

Bollache, L., J. T. Dick, K. D. Farnsworth, and W. I. Montgomery. 2008. Comparison of the functional responses of invasive and native amphipods. Biology Letters **4**:166–169.

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution **26**:183–192.

Breithaupt, T. 2011. Chemical communication in crayfish. Pages 257–276 *in* T. Breithaupt and M. Thiel, editors. Chemical communication in crustaceans. Springer, New York/Dordrecht/Heidelberg/London.

Bubb, D. H., T. J. Thom, and M. C. Lucas. 2006. Movement, dispersal and refuge use of co-occurring introduced and native crayfish. Freshwater Biology **51**:1359–1368.

Byers, J. E. 2000. Competition between Two Estuarine Snails: Implications for Invasions of Exotic Species. Ecology **81**:1225–1239.

Carazo, P., C. K. Tan, F. Allen, S. Wigby, and T. Pizzari. 2014. Within-group male relatedness reduces harm to females in *Drosophila*. Nature **505**:672–675.

Carlsson, N. O., H. Bustamante, D. L. Strayer, and M. L. Pace. 2011. Biotic resistance on the increase: native predators structure invasive zebra mussel populations. Freshwater Biology **56**:1630–1637.

Chapple, D. G., S. M. Simmonds, and B. B. Wong. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? Trends in Ecology & Evolution **27**:57–64.

Chucholl, C. 2015. Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion pathway. Pages 83–114 *in* T. Kawai, Z. Faulkes, and G. Scholtz, editors. Freshwater crayfish: a global overview. CRC Press, Boca Raton, USA.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Chucholl, C., and M. Pfeiffer. 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., and F. Wendler. 2017. Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. Biological Invasions **19**:197–208.

Cordero-Rivera, A. 2017. Behavioral Diversity (Ethodiversity): A Neglected Level in the Study of Biodiversity. Frontiers in Ecology and Evolution **5**:1–7.

Corkum, L. D., and D. J. Cronin. 2004. Habitat complexity reduces aggression and enhances consumption in crayfish. Journal of Ethology **22**:23–27.

David, P., E. Thebault, O. Anneville, P.-F. Duyck, E. Chapuis, and N. Loeuille. 2017. Impacts of invasive species on food webs: a review of empirical data. Advances in Ecological Research **56**:1–60.

Dermott, R., and M. Munawar. 1993. Invasion of Lake Erie offshore sediments by *Dreissena*, and its ecological implications. Canadian Journal of Fisheries and Aquatic Sciences **50**:2298–2304.

Des Roches, S., D. M. Post, N. E. Turley, J. K. Bailey, A. P. Hendry, M. T. Kinnison, J. A. Schweitzer, and E. P. Palkovacs. 2018. The ecological importance of intraspecific variation. Nature Ecology & Evolution **2**:57–64.

Dick, J. T., M. E. Alexander, J. M. Jeschke, A. Ricciardi, H. J. MacIsaac, T. B. Robinson, S. Kumschick, O. L. Weyl, A. M. Dunn, and M. J. Hatcher. 2014. Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. Biological Invasions **16**:735–753.

Dick, J. T., K. Gallagher, S. Avlijas, H. C. Clarke, S. E. Lewis, S. Leung, D. Minchin, J. Caffrey, M. E. Alexander, C. Maguire, C. Harrod, N. Reid, N. R. Haddaway, K. D. Farnsworth, M. Penk, and A. Ricciardi. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions **15**:837–846.

Dick, J. T., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, and N. G. Taylor. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology **54**:1259–1267.

Dunham, D. W., and R. C. Guiasu. 1997. Agonistic Interactions in Male form II *Cambarus robustus* Girard, 1852 Crayfish (Decapoda, Cambaridae) and a Comparison Between Male form I and form II Intra-Form Contests. Crustaceana **70**:720–736.

Elliott, J., and L. Persson. 1978. The estimation of daily rates of food consumption for fish. Journal of Animal Ecology **47**:977–991.

Ercoli, F., T. J. Ruokonen, H. Hämäläinen, and R. I. Jones. 2014. Does the introduced signal crayfish occupy an equivalent trophic niche to the lost native noble crayfish in boreal lakes? Biological Invasions **16**:2025–2036.

Evangelista, C., J. Cucherousset, and A. Lecerf. 2019. Contrasting ecological impacts of geographically close invasive populations. Oecologia **189**:529–536.

Funk, J. L., and P. M. Vitousek. 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature **446**:1079.

Gergs, A., and H. T. Ratte. 2009. Predicting functional response and size selectivity of juvenile *Notonecta maculata* foraging on *Daphnia magna*. Ecological Modelling **220**:3331–3341.

Gergs, R., and K.-O. Rothhaupt. 2008. Effects of zebra mussels on a native amphipod and the invasive *Dikerogammarus villosus*: the influence of biodeposition and structural complexity. Journal of the North American Benthological Society **27**:541–548.

Geritz, S., and M. Gyllenberg. 2012. A mechanistic derivation of the DeAngelis–Beddington functional response. Journal of Theoretical Biology **314**:106–108.

Gherardi, F., and W. H. Daniels. 2003. Dominance hierarchies and status recognition in the crayfish *Procambarus acutus acutus*. Canadian Journal of Zoology **81**:1269–1281.

Giguère, L. A., A. Delâge, L. M. Dill, and J. Gerritsen. 1982. Predicting encounter rates for zooplankton: a model assuming a cylindrical encounter field. Canadian Journal of Fisheries and Aquatic Sciences **39**:237–242.

Gonçalves, V., F. Gherardi, and R. Rebelo. 2017. Bivalve or gastropod? Using profitability estimates to predict prey choice by *P. clarkii*. acta ethologica **20**:107–117.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, and F. Lyko. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nature Ecology & Evolution **2**:567–573.

Haddaway, N. R., R. H. Wilcox, R. E. Heptonstall, H. M. Griffiths, R. J. Mortimer, M. Christmas, and A. M. Dunn. 2012. Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. Plos One **7**:e32229.

Hanson, J. M., P. A. Chambers, and E. E. Prepas. 1990. Selective foraging by the crayfish *Orconectes virilis* and its impact on macroinvertebrates. Freshwater Biology **24**:69–80.

Harrell, F. E., and C. Dupont. 2018. Hmisc: Harrell Miscellaneous. (ver. 4.1-1) [R package]. Retrieved from https://CRAN.R-project.org/package=Hmisc.

Hartley, A., A. M. Shrader, and S. Chamaillé-Jammes. 2019. Can intrinsic foraging efficiency explain dominance status? A test with functional response experiments. Oecologia **189**:105–110.

Hobbs, H. H. J. 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smithsonians Contributions to Zoology **164**:1–32.

Holdich, D. M., and J. Black. 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquatic Invasions **2**:1–15.

Holdich, D. M., J. D. Reynolds, C. Souty-Grosset, and P. J. Sibley. 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.

Holling, C. S. 1959a. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. The Canadian Entomologist **91**:293–320.

Holling, C. S. 1959b. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist **91**:385–398.

Holling, C. S. 1966. The functional response of invertebrate predators to prey density. The Memoirs of the Entomological Society of Canada **98**:5–86.

Huey, R. B., and E. R. Pianka. 1981. Ecological consequences of foraging mode. Ecology **62**:991–999.

Jackson, M. C., T. Jones, M. Milligan, D. Sheath, J. Taylor, A. Ellis, J. England, and J. Grey. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. Freshwater Biology **59**:1123–1135.

Jeschke, J. M., and K. Hohberg. 2008. Predicting and testing functional responses: an example from a tardigrade–nematode system. Basic and Applied Ecology **9**:145–151.

Jeschke, J. M., F. Keesing, and R. S. Ostfeld. 2013. Novel Organisms: Comparing Invasive Species, GMOs, and Emerging Pathogens. Ambio **42**:541–548.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2002. Predator functional responses: discriminating between handling and digesting prey. Ecological Monographs **72**:95–112.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2004. Consumer-food systems: why type I functional responses are exclusive to filter feeders. Biological Reviews **79**:337–349.

Jeschke, J. M., and R. Tollrian. 2005a. Effects of predator confusion on functional responses. Oikos **111**:547–555.

Jeschke, J. M., and R. Tollrian. 2005b. Predicting herbivore feeding times. Ethology **111**:187–206.

Johnston, D., and J. Freeman. 2005. Dietary preference and digestive enzyme activities as indicators of trophic resource utilization by six species of crab. The Biological Bulletin **208**:36–46.

Juliano, S. A. 2001. Nonlinear curve fitting: predation and functional response curves. Pages 178–196 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiment. Oxford University Press, New York, USA.

Kalinkat, G. 2014. Bringing animal personality research into the food web arena. Journal of Animal Ecology **83**:1245–1247.

Kalinkat, G., U. Brose, and B. C. Rall. 2013a. Habitat structure alters top-down control in litter communities. Oecologia **172**:877–887.

Kalinkat, G., F. D. Schneider, C. Digel, C. Guill, B. C. Rall, and U. Brose. 2013b. Body masses, functional responses and predator–prey stability. Ecology Letters **16**:1126–1134.

Karatayev, A. Y., L. E. Burlakova, and D. K. Padilla. 2002. Impacts of zebra mussels on aquatic communities and their role as ecosystem engineers. Pages 433–446 *in* E. Leppäkoski, S. Gollasch, and S. Olenin, editors. Invasive aquatic species of Europe. Distribution, impacts and management. Springer, Dordrecht, The Netherlands.

Kobak, J., and T. Kakareko. 2009. Attachment strength, aggregation and movement of the zebra mussel (*Dreissena polymorpha*, Bivalvia) in the presence of potential predators. Fundamental and Applied Limnology/Archiv für Hydrobiologie **174**:193–204.

Kouba, A., A. Petrusek, and P. Kozák. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems **413**:05.

Kumschick, S., M. Gaertner, M. Vilà, F. Essl, J. M. Jeschke, P. Pyšek, A. Ricciardi, S. Bacher, T. M. Blackburn, and J. T. Dick. 2014. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. Bioscience **65**:55–63.

Larson, E. R., L. A. Twardochleb, and J. D. Olden. 2017. Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. Limnology **18**:275–286.

Lawrence, C. S., Y. W. Cheng, N. M. Morrissy, and I. H. Williams. 2000. A comparison of mixed-sex vs. monosex growout and different diets on the growth rate of freshwater crayfish (*Cherax albidus*). Aquaculture **185**:281–289.

Leffler, A. J., J. J. James, T. A. Monaco, and R. L. Sheley. 2014. A new perspective on trait differences between native and invasive exotic plants. Ecology **95**:298–305.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London B: Biological Sciences **269**:2407–2413.

Li, Y., B. C. Rall, and G. Kalinkat. 2018. Experimental duration and predator satiation levels systematically affect functional response parameters. Oikos **127**:590–598.

Linzmaier, S. M., L. S. Goebel, F. Ruland, and J. M. Jeschke. 2018. Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. Ecosphere **9**:e02385.

Lopez, L. K., K. Hendry, M. Y. L. Wong, and A. R. Davis. 2019. Insight into invasion: Interactions between a critically endangered and invasive crayfish. Austral Ecology **44**:78–85.

Lukhaup, C. 2001. *Procambarus* sp. The Marbled crayfish. Aquaristik Aktuell **7–8**:48–51.

Luna, A. J. F., J. I. Hurtado-Zavala, T. Reischig, and R. Heinrich. 2009. Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. Journal of Biological Rhythms **24**:64–72.

MacIsaac, H. J. 1994. Size-selective predation on zebra mussels (*Dreissena polymorpha*) by crayfish (*Orconectes propinquus*). Journal of the North American Benthological Society **13**:206–216.

Marquet, P. A., F. A. Labra, and B. A. Maurer. 2004. Metabolic ecology: linking individuals to ecosystems. Ecology **85**:1794–1796.

Martin, P., S. Thonagel, and G. Scholtz. 2016. The parthenogenetic Marmorkrebs (Malacostraca: Decapoda: Cambaridae) is a triploid organism. Journal of Zoological Systematics and Evolutionary Research **54**:13–21.

Mathers, K. L., R. P. Chadd, M. J. Dunbar, C. A. Extence, J. Reeds, S. P. Rice, and P. J. Wood. 2016. The long-term effects of invasive signal crayfish (*Pacifastacus leniusculus*) on instream macroinvertebrate communities. Science of the Total Environment **556**:207–218.

McGhee, K. E., L. M. Pintor, and A. M. Bell. 2013. Reciprocal behavioral plasticity and behavioral types during predator-prey interactions. The American Naturalist **182**:704–717.

Médoc, V., L. Thuillier, and T. Spataro. 2018. Opportunistic omnivory impairs our ability to predict invasive species impacts from functional response comparisons. Biological Invasions **20**:1307–1319.

Metz, J., M. Sabelis, and J. Kuchlein. 1988. Sources of variation in predation rates at high prey densities: an analytic model and a mite example. Experimental Applied Acarology **5**:187–205.

Milinski, M. 1982. Optimal foraging: the influence of intraspecific competition on diet selection. Behavioral Ecology and Sociobiology **11**:109–115.

Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences **98**:5446–5451.

Moore, P. A. 2007. Agonistic behavior in freshwater crayfish: the influence of intrinsic and extrinsic factors on aggressive behavior and dominance. Pages 90–112 *in* J. E. Duffy and M. Thiel, editors. Evolutionary ecology of social and sexual systems: Crustacea as model organisms. Oxford University Press, New York, USA.

Naddafi, R., and L. G. Rudstam. 2013. Predator-induced behavioural defences in two competitive invasive species: the zebra mussel and the quagga mussel. Animal Behaviour **86**:1275–1284.

Naddafi, R., and L. G. Rudstam. 2014. Predator-induced morphological defences in two invasive dreissenid mussels: implications for species replacement. Freshwater Biology **59**:703–713.

Oaten, A., and W. W. Murdoch. 1975. Functional response and stability in predator-prey systems. The American Naturalist **109**:289–298.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: Community Ecology Package (ver. 2.5-2) [R package]. Retrieved from https://CRAN.R-project.org/package=vegan.

Okuyama, T. 2008. Individual behavioral variation in predator–prey models. Ecological Research **23**:665–671.

Pacifici, M., W. B. Foden, P. Visconti, J. E. Watson, S. H. Butchart, K. M. Kovacs, B. R. Scheffers, D. G. Hole, T. G. Martin, and H. R. Akcakaya. 2015. Assessing species vulnerability to climate change. Nature Climate Change **5**:215.

Parker, J. D., M. E. Torchin, R. A. Hufbauer, N. P. Lemoine, C. Alba, D. M. Blumenthal, O. Bossdorf, J. E. Byers, A. M. Dunn, R. W. Heckman, M. Hejda, V. Jarošík, A. R. Kanarek, L. B. Martin, S. E. Perkins, P. Pyšek, K. Schierenbeck, C. Schlöder, R. van Klinken, K. J. Vaughn, W. Williams, and L. M. Wolfe. 2013. Do invasive species perform better in their new ranges? Ecology **94**:985–994.

Penk, M., W.-C. Saul, J. T. A. Dick, I. Donohue, M. E. Alexander, S. Linzmaier, and J. M. Jeschke. 2017. A trophic interaction framework for identifying the invasive capacity of novel organisms. Methods in Ecology and Evolution **8**:1786–1794

Perry, W. L., D. M. Lodge, and G. A. Lamberti. 1997. Impact of crayfish predation on exotic zebra mussels and native invertebrates in a lake-outlet stream. Canadian Journal of Fisheries and Aquatic Sciences **54**:120–125.

Perry, W. L., D. M. Lodge, and G. A. Lamberti. 2000. Crayfish (*Orconectes rusticus*) impacts on zebra mussel (*Dreissena polymorpha*) recruitment, other macroinvertebrates and algal biomass in a lake-outlet stream. The American Midland Naturalist **144**:308–316.

Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos **117**:1629–1636.

Pintor, L. M., A. Sih, and J. L. Kerby. 2009. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology **90**:581–587.

Pritchard, D. W., R. A. Paterson, H. C. Bovy, and D. Barrios-O'Neill. 2017. FRAIR: an R package for fitting and comparing consumer functional responses. Methods in Ecology and Evolution **8**:1528–1534.

Pruitt, J. N., J. J. Stachowicz, and A. Sih. 2011. Behavioral types of predator and prey jointly determine prey survival: potential implications for the maintenance of within-species behavioral variation. The American Naturalist **179**:217–227.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/.

Raffard, A., A. Lecerf, J. Cote, M. Buoro, R. Lassus, and J. Cucherousset. 2017. The functional syndrome: linking individual trait variability to ecosystem functioning. Proceedings of the Royal Society B: Biological Sciences **284**:20171893.

Rall, B. C., U. Brose, M. Hartvig, G. Kalinkat, F. Schwarzmüller, O. Vucic-Pestic, and O. L. Petchey. 2012. Universal temperature and body-mass scaling of feeding rates. Philosophical Transactions of the Royal Society B **367**:2923–2934.

Ricciardi, A. 2003. Predicting the impacts of an introduced species from its invasion history: an empirical approach applied to zebra mussel invasions. Freshwater Biology **48**:972–981.

Rodgers, L. J., P. I. Saoud, and D. B. Rouse. 2006. The effects of monosex culture and stocking density on survival, growth and yield of redclaw crayfish (*Cherax quadricarinatus*) in earthen ponds. Aquaculture **259**:164–168.

Rogers, D. 1972. Random search and insect population models. The Journal of Animal Ecology **42**:369–383.

Rosenbaum, B., and B. C. Rall. 2018. Fitting functional responses: Direct parameter estimation by simulating differential equations. Methods in Ecology and Evolution **9**:2076–2090.

Royama, T. 1971. A comparative study of models for predation and parasitism. Researches on Population Ecology **13**:1–91.

Russell, J. C., N. S. Sataruddin, and A. D. Heard. 2014. Over-invasion by functionally equivalent invasive species. Ecology **95**:2268–2276.

Schröder, A., G. Kalinkat, and R. Arlinghaus. 2016. Individual variation in functional response parameters is explained by body size but not by behavioural types in a poeciliid fish. Oecologia **182**:1129–1140.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, and M. Arianoutsou. 2017. No saturation in the accumulation of alien species worldwide. Nature Communications **8**:14435.

Sih, A., J. Cote, M. Evans, S. Fogarty, and J. Pruitt. 2012. Ecological implications of behavioural syndromes. Ecology Letters **15**:278–289.

Smout, S., C. Asseburg, J. Matthiopoulos, C. Fernández, S. Redpath, S. Thirgood, and J. Harwood. 2010. The functional response of a generalist predator. Plos One **5**:e10761.

Souty-Grosset, C., D. M. Holdich, P. Y. Noël, J. D. Reynolds, and P. Haffner. 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France.

Stein, R. A., and J. J. Magnuson. 1976. Behavioral response of crayfish to a fish predator. Ecology **57**:751–761.

Taylor, N., and A. Dunn. 2018. Predatory impacts of alien decapod Crustacea are predicted by functional responses and explained by differences in metabolic rate. Biological Invasions **20**:2821–2837.

Thuiller, W., L. Gallien, I. Boulangeat, F. De Bello, T. Münkemüller, C. Roquet, and S. Lavergne. 2010. Resolving Darwin’s naturalization conundrum: a quest for evidence. Diversity and Distributions **16**:461–475.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences **101**:10854–10861.

Toscano, B. J., N. J. Gownaris, S. M. Heerhartz, and C. J. Monaco. 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. Oecologia **182**:55–69.

Toscano, B. J., and B. D. Griffen. 2013. Predator size interacts with habitat structure to determine the allometric scaling of the functional response. Oikos **122**:454–462.

Toscano, B. J., and B. D. Griffen. 2014. Trait-mediated functional responses: predator behavioural type mediates prey consumption. Journal of Animal Ecology **83**:1469–1477.

Twardochleb, L. A., M. Novak, and J. W. Moore. 2012. Using the functional response of a consumer to predict biotic resistance to invasive prey. Ecological Applications **22**:1162–1171.

Twardochleb, L. A., J. D. Olden, and E. R. Larson. 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science **32**:1367–1382.

Usio, N., K. Suzuki, M. Konishi, and S. Nakano. 2006. Alien vs. endemic crayfish: roles of species identity in ecosystem functioning. Archiv für Hydrobiologie **166**:1–21.

Usio, N., and C. R. Townsend. 2002. Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. Oikos **98**:512–522.

van der Wal, J. E., M. Dorenbosch, A. K. Immers, C. V. Forteza, J. J. Geurts, E. T. Peeters, B. Koese, and E. S. Bakker. 2013. Invasive crayfish threaten the development of submerged macrophytes in lake restoration. Plos One **8**:e78579.

Vogt, G. 2008. The marbled crayfish: a new model organism for research on development, epigenetics and evolutionary biology. Journal of Zoology **276**:1–13.

Warren, R. J., K. Reed, A. Mathew, K. Krupp, M. Goodman, K. Archibald, and D. J. Spiering. 2019. Release from intraspecific competition promotes dominance of a non-native invader. Biological Invasions **21**:895–909.

Wetzel, J. E. 2002. Form alternation of adult female crayfishes of the genus *Orconectes* (Decapoda: Cambaridae). The American Midland Naturalist **147**:326–337.

Xu, M., X. Mu, J. T. Dick, M. Fang, D. Gu, D. Luo, J. Zhang, J. Luo, and Y. Hu. 2016. Comparative functional responses predict the invasiveness and ecological impacts of alien herbivorous snails. Plos One **11**:e0147017.

Zeileis, A. 2006. Implementing a Class of Structural Change Tests: An Econometric Computing Approach. Computational Statistics & Data Analysis **50**:2987–3008.

zu Ermgassen, P. S. E., and D. C. Aldridge. 2011. Predation by the invasive American signal crayfish, *Pacifastacus leniusculu*s Dana, on the invasive zebra mussel, *Dreissena polymorpha* Pallas: the potential for control and facilitation. Hydrobiologia **658**:303–315.

Appendix B

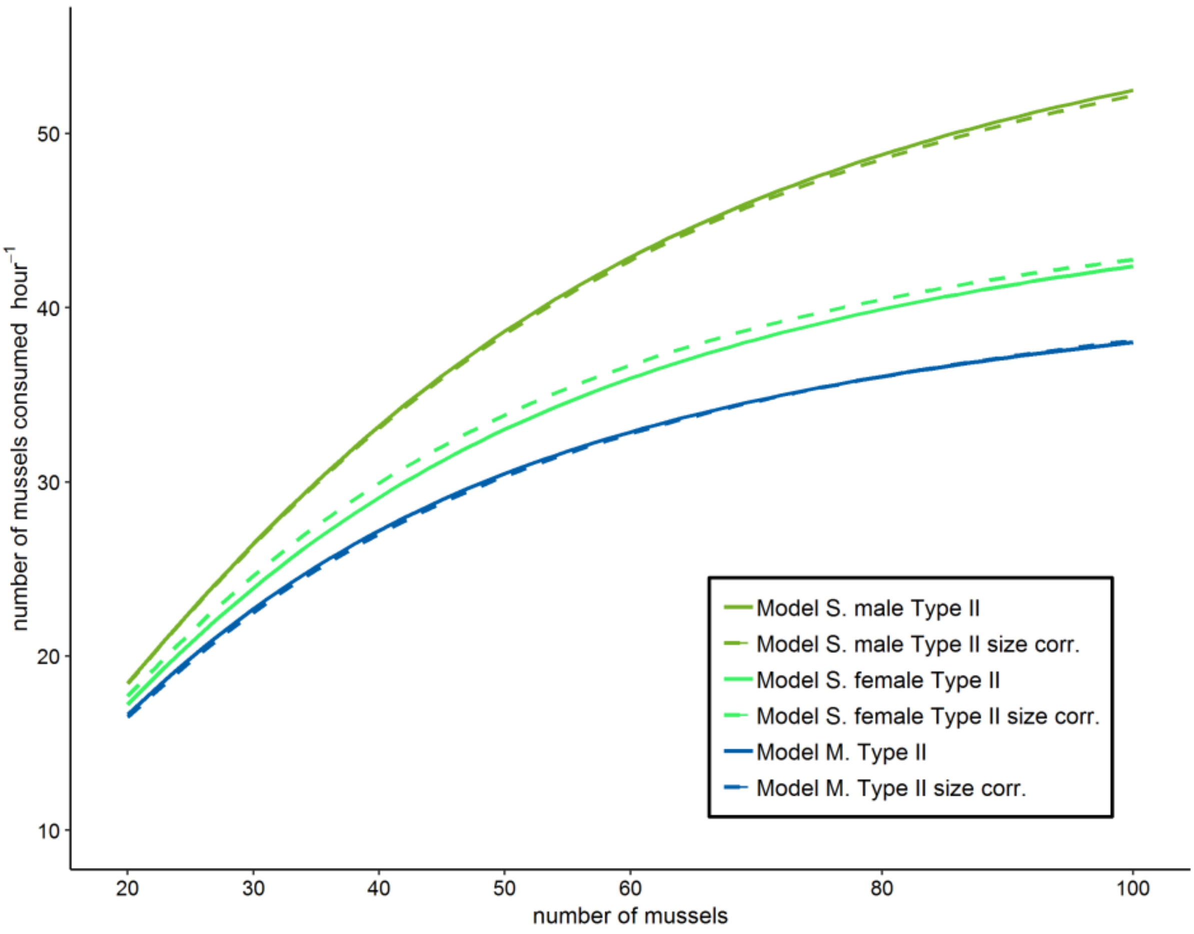


Figure B.1 – Size-corrected models from extrapolated parameters (*a* and *h*) corresponding to the mean size of each group that was measured in functional response experiments. The number of mussels refers to one experimental area (0.16 m²).

Table B.1 – Measured and calculated predation and satiation parameters for individual marbled crayfish and spiny-cheek crayfish from foraging observations on *Dreissena* mussels (density for handling-time and parameter experiments: 20 mussels; density for satiation experiments: 300 mussels).

					handling time experiment			parameter experiment					satiation experiment		
#	species	sex	form	CL	t_{att}	t_{eat}	b (s)	β (h^{-1})	γ (%)	δ (%)	ε (%)	a (h^{-1})	s	y_{max} (mussels/d)	t_g (h)
spiny-cheek crayfish															
S1	S	m	2	34	18	31	50	3.51	0.71	1.00	1.00	2.48	0.010	857	3.09
S2	S	m	2	33	15	35	50	8.92	0.50	1.00	1.00	4.46	0.013	432	4.39
S3	S	m	1	33	12	49	61	3.31	0.43	1.00	1.00	1.42	0.014	403	4.35
S4	S	m	2	36	35	44	78	7.08	0.68	1.00	0.89	4.27	0.019	619	3.13
S5	S	m	2	36	9	31	42	5.90	0.57	1.00	0.76	2.56	0.011	594	3.71
S6	S	m	1	34	16	35	55	12.82	0.60	1.00	1.00	7.69	N/A	N/A	N/A
S7	S	m	2	34	14	30	46	3.73	0.83	1.00	0.90	2.79	0.019	446	2.90
S8	S	m	1	40	20	44	64	11.83	0.37	1.00	0.92	4.05	0.011	547	3.86
S9	S	m	1	36	11	27	40	9.66	0.52	1.00	0.85	4.25	0.012	562	3.50
S10	S	m	2	38	15	34	49	9.45	0.23	1.00	1.00	2.13	N/A	N/A	N/A
S11	S	f	N/A	32	13	36	49	5.97	0.48	1.00	0.93	2.68	N/A	N/A	N/A
S12	S	f	N/A	29	27	56	83	3.01	0.79	1.00	1.00	2.37	0.011	576	4.02
S13	S	f	N/A	34	22	45	69	4.78	0.36	1.00	0.90	1.54	0.012	648	3.53
S14	S	f	N/A	38	22	43	78	10.52	0.68	1.00	0.67	4.78	N/A	N/A	N/A
marbled crayfish															
M1	M	f	N/A	31	23	49	72	13.12	0.50	1.00	0.83	5.47	0.010	811	3.41
M2	M	f	N/A	29	20	43	63	4.15	0.53	1.00	1.00	2.21	0.010	641	3.75
M3	M	f	N/A	29	47	94	140	5.52	0.69	1.00	1.00	3.82	0.011	672	3.35
M4	M	f	N/A	30	20	54	74	8.69	0.35	1.00	1.00	3.01	0.009	792	3.24
M5	M	f	N/A	43	16	51	69	7.85	0.33	1.00	0.89	2.32	0.013	756	2.79
M6	M	f	N/A	36	12	31	43	4.45	0.50	1.00	0.92	2.04	N/A	N/A	N/A
M7	M	f	N/A	33	29	74	103	7.24	0.45	1.00	1.00	3.26	N/A	N/A	N/A
M8	M	f	N/A	31	16	34	50	7.55	0.37	1.00	0.89	2.48	N/A	N/A	N/A
M9	M	f	N/A	33	18	60	81	7.87	0.29	1.00	0.86	1.97	N/A	N/A	N/A
M10	M	f	N/A	32	12	49	61	8.51	0.41	1.00	1.00	3.47	0.012	1003	2.08
M11	M	f	N/A	35	23	69	94	2.20	0.78	1.00	0.86	1.47	N/A	N/A	N/A
M12	M	f	N/A	34	21	58	82	4.93	0.52	1.00	0.88	2.16	N/A	N/A	N/A

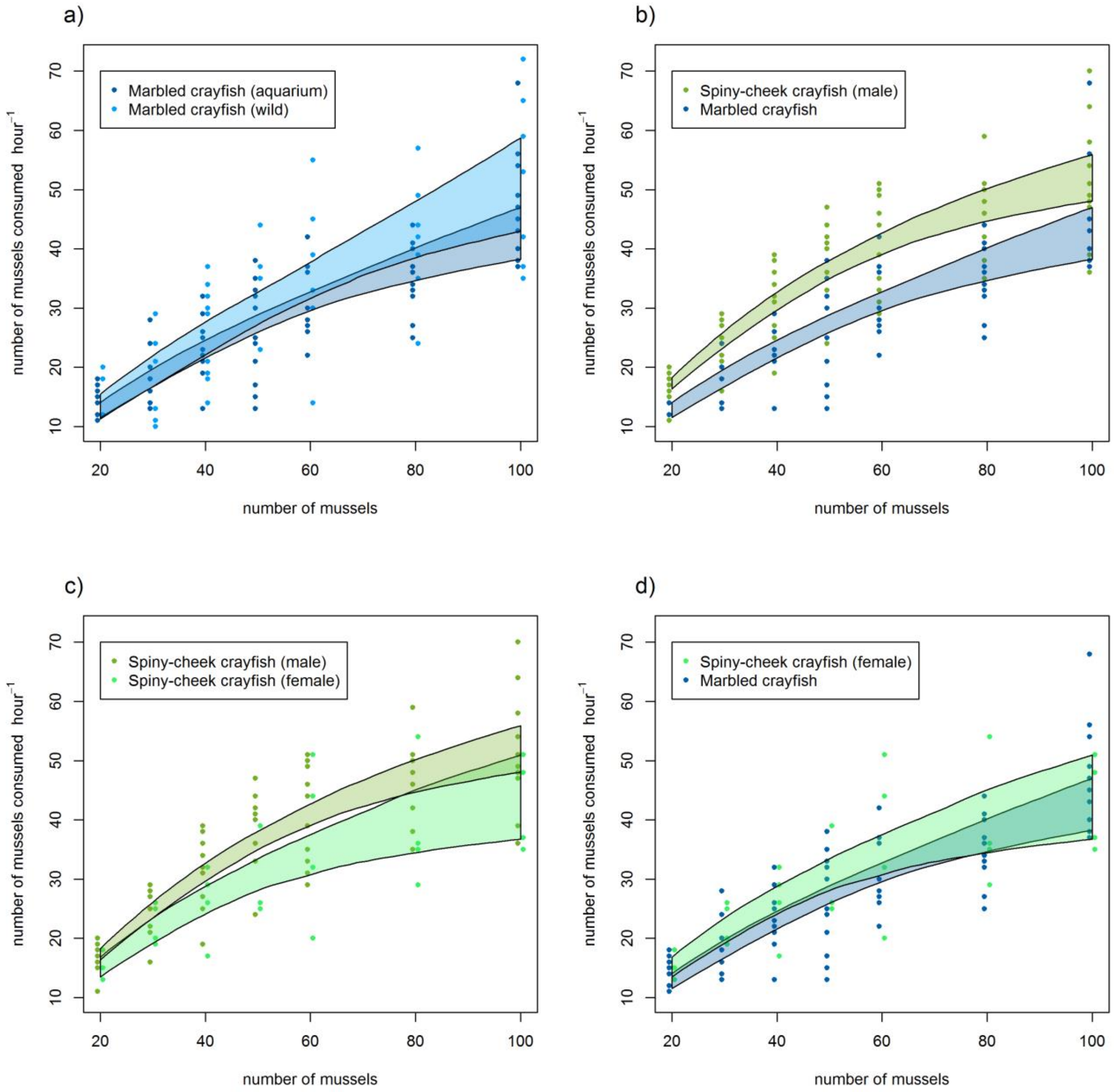


Figure B.2 – Empirical approximations of 95% confidence intervals based on bootstrapped model fits for the number of mussels consumed by (a) aquarium vs naturalized (wild) marbled crayfish, (b) marbled crayfish vs male spiny-cheek crayfish, (c) male vs female spiny-cheek crayfish and (d) marbled crayfish vs female spiny-cheek crayfish. The number of mussels refers to one experimental area (0.16 m^2).

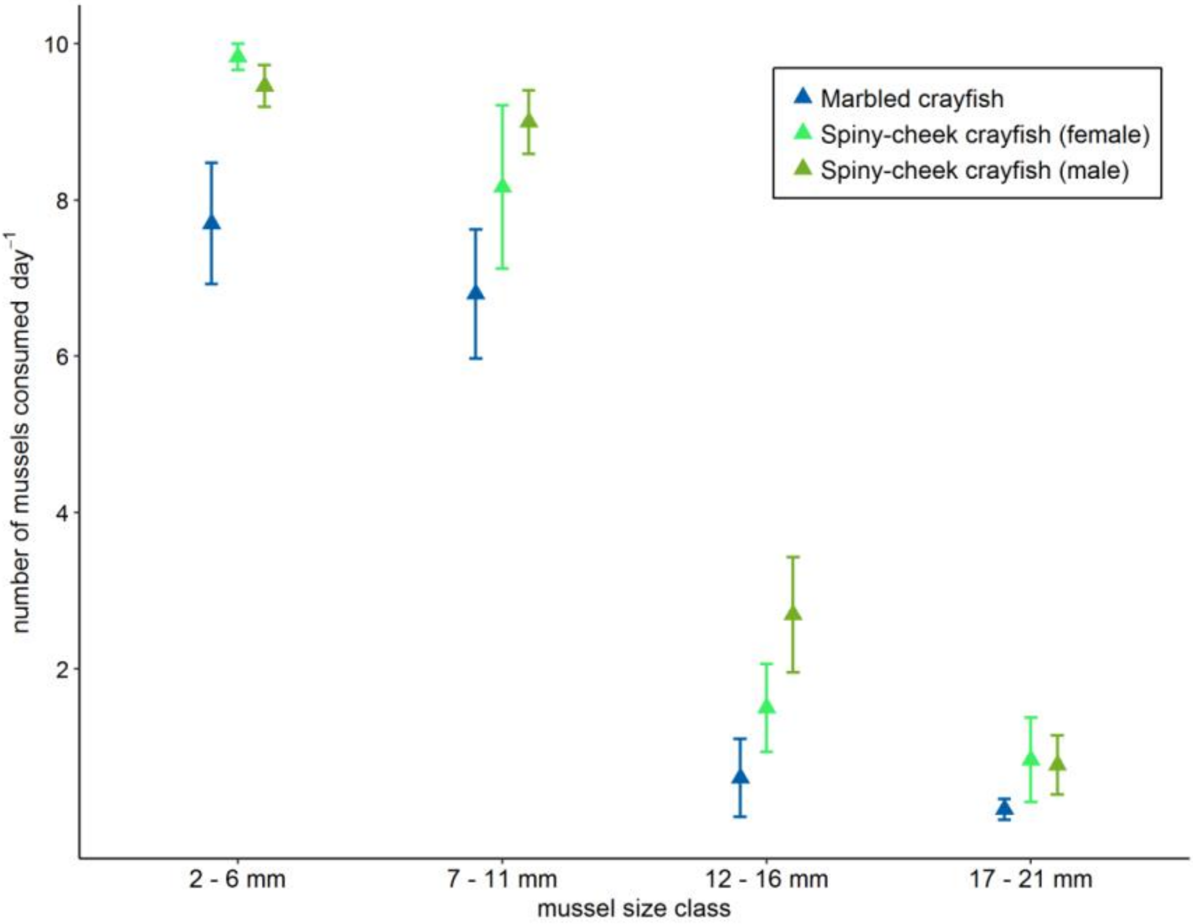


Figure B.3 – Number of mussels consumed in 24 hours during size selection experiments of marbled crayfish and spiny-cheek crayfish males and females. The number of consumed *Dreissena* mussels are given as mean number of mussel consumed (± SE) for the four given size classes.

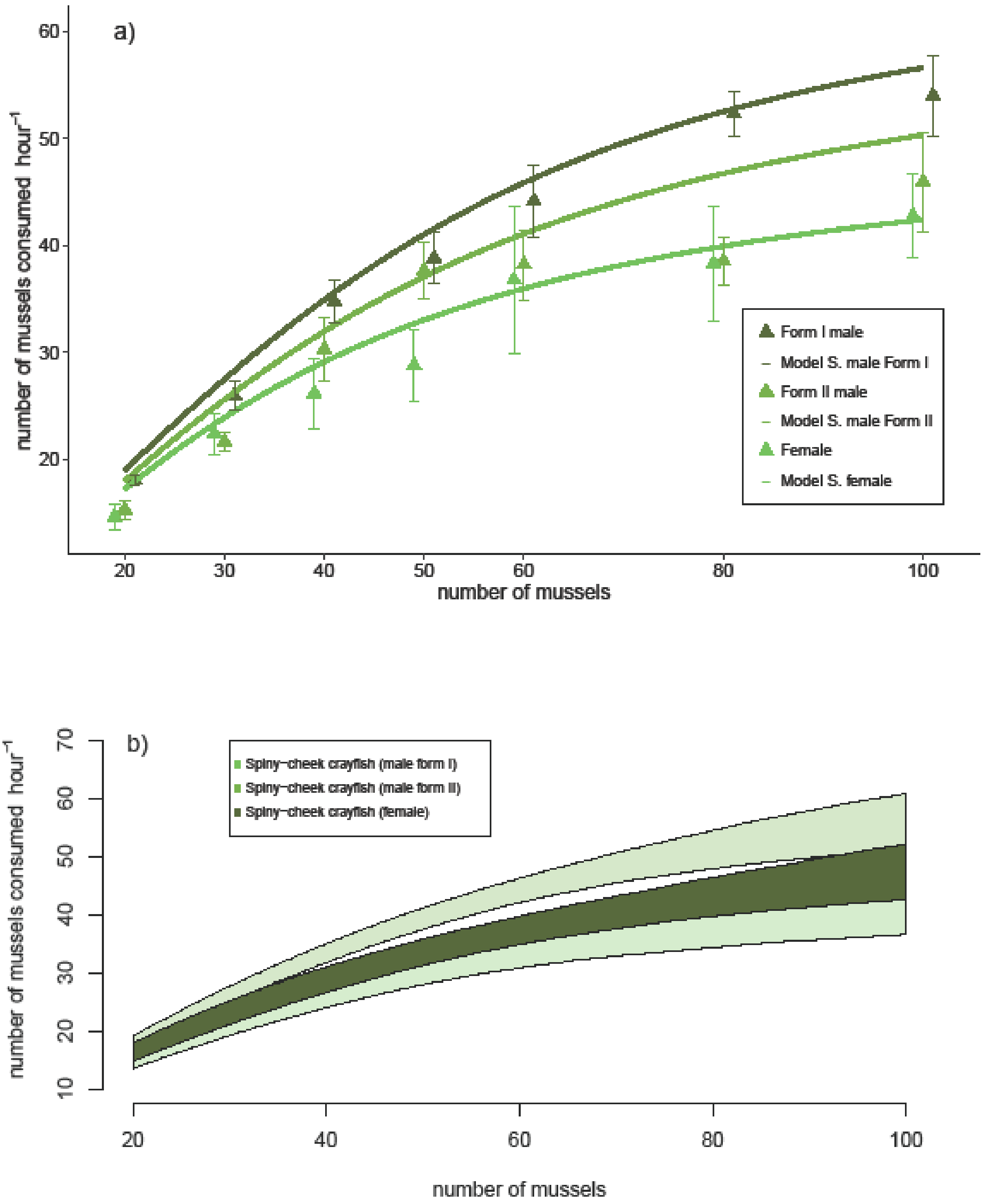


Figure B.4 – Functional responses of spiny-cheek crayfish females, males of reproductive form I and males of non-reproductive form II (the number of mussels refers to one experimental area, 0.16 m²): (a) observed functional responses (mean ± SE) and model predictions based on independently derived parameters; (b) bootstrapped regression fits with 95% confidence intervals.

CHAPTER 3

BEHAVIORAL DIFFERENCES IN AN OVER-INVASION SCENARIO:

MARBLED VS. SPINY-CHEEK CRAYFISH

Published as:
Linzmaier, S. M., L. S. Goebel, F. Ruland, and J. M. Jeschke. 2018. Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. Ecosphere **9**:e02385.
https://doi.org/10.1002/ecs2.2385

Abstract

New species often invade ecosystems already dominated by previous invaders. Ornamental freshwater crayfish, particularly parthenogenetic marbled crayfish (*Procambarus virginalis*), increasingly establish in European water bodies where they interact with resident native and non-native species. Behavioral traits and behavioral syndromes can influence the outcome of these species interactions. The behavior of non-native crayfish is often studied in notorious invaders but rarely in new and emerging species, although those provide the best opportunity for management. Activity, aggressiveness, and boldness have repeatedly been associated with invasion success and species displacement. Further, crayfish can adapt their behavior after they have established in the new range. We investigated whether marbled crayfish can displace the widely established spiny-cheek crayfish (*Orconectes*[5] *limosus*). Specifically, we compared their behavioral traits and evaluated whether these traits differ, using marbled crayfish populations from aquaria and the field and spiny-cheek crayfish from the field. We staged agonistic encounters, measured activity levels, and recorded the response to a simulated threat of both species and both origins (field and aquarium) in laboratory trials. We found that in agonistic encounters, marbled crayfish were on average more aggressive than spiny-cheek crayfish, even against larger opponents. Aggressiveness and activity were positively correlated, which is indicative for an aggression syndrome. Marbled crayfish from the field were less active than those from aquaria, but there was no difference in aggressiveness. Marbled crayfish often froze in response to a simulated threat, whereas spiny-cheek crayfish reacted either offensively or defensively. These results from the laboratory illustrate potentially important behavioral mechanisms behind crayfish over-invasions and show behavioral plasticity in a species where all known individuals are genetically identical. To better understand the invasion process in nature, the species' reproductive biology and interactions with other members of the community should be considered. We conclude that the recent success of marbled crayfish in establishing new populations could be influenced by their behavioral flexibility and their potential to competitively persist in the presence of established invasive crayfish.

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[5] Now in the genus *Faxonius* (see Chapter 1, p. 25).

Keywords

aggression; behavioral flexibility; behavioral syndromes; behavioral variability; biological invasions; freshwater crayfish; shelter use; threat response.

Introduction

Species invasions have already massively altered aquatic communities and are still increasing worldwide (Gallardo et al. 2016, Seebens et al. 2017). Consequently, more and more invasive species compete with already established, functionally similar invasive species, a process that has been termed over-invasion (Russell et al. 2014). In novel communities, such over-invasions and species introduction dates are of great relevance and allow for more detailed analyses than a simple, dichotomous distinction between native vs. non-native species that ignores species residence times (Dornelas et al. 2014).

The consequences of multiple species invasions are largely unknown (Hewitt and Huxel 2002), but the invasion outcome and interaction strength between invading and resident species can be assessed by behavioral differences and correlated suites of behavioral traits (i.e., behavioral syndromes; Chapple et al. 2012, Sih et al. 2012, Penk et al. 2017). Some behavioral traits such as activity, aggressiveness, and boldness have repeatedly been associated with invasion success (Weis 2010, Chapple et al. 2012). Furthermore, the ability to behaviorally adapt to a new environment, that is, behavioral flexibility, promotes invasion success (Wright et al. 2010). Naïve non-native species have to adapt to new prey, competitors, or predators by means of evolution or learning (Saul and Jeschke 2015, Wong and Candolin 2015). Comparative studies across invading species can help elucidate what makes some invaders more successful than others (van Kleunen et al. 2010).

Ornamental crayfish invasions

Particularly since the beginning of the 20th century, decapod crayfish invasions have resulted in a decline of native crayfish populations and severe changes to ecosystems, for example, in Europe (Holdich et al. 2009, Lodge et al. 2012). Nowadays, increasing numbers of new non-native crayfish species are imported by the pet trade from North America and Australasia to Europe, and some species have already been released in nature (Chucholl 2013, Chucholl and Wendler 2017). As more of these recently arrived species have started to establish populations, interactions with other invasive species will shape future crayfish distributions and novel species communities (Kouba et al. 2014). However, the propagule pressure of the new invaders and the incumbent advantage of the old invaders will be decisive for potential competitive displacement in these over-invasion scenarios (Lockwood et al. 2005, Russell et al. 2014). Crayfish from the pet trade have the disadvantage that they are naïve to prey, predators, or competitors when they are released from aquaria (Hazlett 1994, Martin 2014). For example, aquaria or other hatchery-reared fish are more vulnerable to predation than those that have experienced predation (Kellison et al. 2000, Yokota et al. 2007). Some studies looked at agonistic behaviors among competing old and new invasive crayfish species (Chucholl et al. 2008, Hudina et al. 2011, James et al. 2016), but broader behavioral comparisons are necessary to investigate the invasive potential of species before or at an early stage of invasion.

Invasive crayfish: behavioral differences and flexibility

Non-native crayfish are model organisms in invasion ecology and are also frequently used in behavioral studies (Gherardi et al. 2012, Lodge et al. 2012). Evidence suggests that highly invasive crayfish typically display stronger interspecific aggression toward resident congeners, in that way limiting access to critical resources for competitors (e.g. Gherardi and Cioni 2004, Klocker and Strayer 2004, Chucholl et al. 2008). Also, larger body and chela size are advantageous in these agonistic interactions (Garvey and Stein 1993, Vorburger and Ribi 1999). Invasive crayfish species are often more active (Bubb et al. 2006), perceive more predation cues (Hazlett et al. 2003), or avoid predation more effectively (Garvey et al. 1994) than native crayfish. Activity, aggressiveness, and boldness in crayfish are often correlated and thought to be part of an aggression syndrome (Pintor et al. 2008, 2009). These behavioral syndromes can be explained by state variables (such as growth) that often covary with sets of behaviors (Biro et al. 2014). Furthermore, invasive crayfish adapt behavioral traits after introduction in response to resident crayfish species and the community of invaded water bodies (Pintor et al. 2008, Hanshew and Garcia 2012). For example, native crayfish that had experience with an invasive competitor were more aggressive toward the opponent than naïve native individuals (Hayes et al. 2009). Also, the presence of predators alters the activity of invasive and native crayfish (Hirvonen et al. 2007, Aquiloni et al. 2010). It has been shown that invasive crayfish and crabs can learn how to respond to newly emerging threats after invading new territories (Hazlett et al. 2002, Roudez et al. 2008). By looking at multiple behavioral traits and integrating behavioral flexibility and new concepts like behavioral syndromes (Gherardi et al. 2012), species displacements and ecological invasions might be better understood and managed.

Model organisms

Spiny-cheek crayfish (*Orconectes limosus*) and marbled crayfish (*Procambarus virginalis*) are examples for invaders with a high functional similarity. They can thus be used as comparator organisms sensu Penk et al. (2017): Comparing marbled crayfish to resident spiny-cheek crayfish allows assessing the invasive capacity of marbled crayfish. Furthermore, both species are included in the List of Invasive Alien Species of Union Concern (EU Regulation 1143/2014). They co-occur in some lakes in Germany, but differ in their invasion history and morphology (Chucholl and Pfeiffer 2010, Chucholl et al. 2012).

Spiny-cheek crayfish have been spread across Central Europe since the late 19th century, now being one of the most common European crayfish species (Kouba et al. 2014). They display sexual dimorphism with males having larger chelae than females (Souty-Grosset et al. 2006). In parts of their native range, spiny-cheek crayfish were outcompeted by other invasive species from the genus *Orconectes* (Klocker and Strayer 2004). The interactions of spiny-cheek crayfish with other crayfish in their invasive range, however, have rarely been studied (Musil et al. 2010).

The peculiar marbled crayfish are triploid descendants of the sexually reproducing slough crayfish (*Procambarus fallax*; Martin et al. 2010, Lyko 2017, Gutekunst et al. 2018). Marbled crayfish represent the only known decapod crustacean capable of apomictic parthenogenesis (Scholtz et al. 2003, Seitz et al. 2005). What makes marbled crayfish even more unique is the fact that no native population has been recorded so far (summarized in Chucholl et al. 2012 and citations therein). The obscure origin of marbled crayfish lies in the tanks of traders or breeders of crayfish, and neither behavior nor ecology of the species within invaded lakes is yet understood (Chucholl et al. 2012). The first naturalized marbled crayfish population (i.e., in the field) was reported near Freiburg, Germany, in 2003 (Marten et al. 2004). In recent years, sightings from the Netherlands, Italy, Slovakia, Sweden, and other German lakes followed (see Chucholl et al. 2012 for review). Since these populations stem from marbled crayfish previously reared in aquaria, they can be considered to have been naïve to interspecific competition and predators before they were released. The aquarium origin and the beginning establishment of isogenic populations in pre-invaded lakes provide a unique opportunity to study behavioral mechanisms of species displacement and behavioral flexibility in the natural environment.

Goals and hypotheses

We compared the behavior of marbled and spiny-cheek crayfish to assess competitive interaction strength, flexibility in behavior of an invader, and possible species displacement in crayfish (over-)invasions. Specifically, we assessed interspecific aggressiveness, activity, and boldness of the two focal species. In addition, we compared naïve, aquarium, and naturalized populations of marbled crayfish that are sympatric to spiny-cheek crayfish with each other to elucidate changes in behavior that result from naturalization. Finally, we looked for correlations between aggressiveness and activity, associated with aggression syndromes in individuals of both crayfish species. We hypothesized that crayfish species differ in behavioral traits that are important for invasion success, for example, agonistic behavior. Resident spiny-cheek crayfish were expected to dominate marbled crayfish because their males have large chelae in contrast to the all-female marbled crayfish. The latter were thought to be more active than spiny-cheek crayfish and respond less appropriately to a threat since they originate from aquaria without natural selection regimes. Marbled crayfish should generally exhibit less variability in behavior since they are isogenic. We further hypothesized that after marbled crayfish came in contact with spiny-cheek crayfish and predators in a natural environment, they will adapt their behavior. Marbled crayfish from invaded water bodies were expected to be more aggressive than aquarium crayfish to compete and coexist with spiny-cheek crayfish. Finally, marbled crayfish experiencing predation in the field should be less active and more responsive to threats than aquarium marbled crayfish.

Material and Methods

Study sites

Spiny-cheek crayfish were collected in lake Müggelsee in front of the institute (52°26’06″ N, 13°38’06″ E), Germany, with crayfish traps (type PIRAT, 610 × 315 × 250 mm, mesh width 40 × 10 mm, Rapurosvo, Parainen, Finland) between April 2015 and June 2016. The traps were baited with dog food or dead fish and were set overnight and checked on the next day. Aquarium stocks of marbled crayfish were provided by Peer Martin (Comparative Zoology, Humboldt University, Berlin, Germany). Additional marbled crayfish that live in sympatry with spiny-cheek crayfish were mostly hand-collected or, to a minor degree, caught by traps in the littoral zone from lakes (1) Moosweiher (48°01’51″ N, 7°48’17″ E) in Baden-Württemberg, Germany, and (2) Krumme Lanke (52°2700″ N, 13°13’52″ E) in Berlin, Germany. Crayfish were transported in Styrofoam boxes filled with water 30 mm deep and macrophytes in excess.

Maintenance of test animals

All crayfish were sexed and measured manually with a sliding caliper to the nearest millimeter. The length was measured as carapace length (CL) from the tip of the rostrum to the posterior edge of the carapace. Tanks were set up on shelves in a climate chamber with a constant temperature at 17 °C under a photoperiod of 14:10 h light:dark. All crayfish were kept in the laboratory for at least one month before being used in experiments. All aquarium marbled crayfish, naturalized marbled crayfish from lake Krumme Lanke, and all spiny-cheek crayfish used for individual measurements (>3 replicates) in behavioral experiments were kept individually in tanks (300 × 200 × 200 mm) filtered by air-driven sponge filters. Naturalized marbled crayfish from lake Moosweiher and additional spiny-cheek crayfish that have been used only as opponents in agonistic encounters were marked and housed in filtered single-species community tanks separated by sex (800 × 400 × 200 mm). All housing tanks were filled with 30 mm of fine gravel, and PVC pipes (150 mm, diameter 50 mm) were provided for shelter. Communal tanks were provided with a surplus of shelters (>2 per crayfish) to minimize aggression. To differentiate among the crayfish kept in communal tanks, we used the non-invasive, numerical marking system of Abrahamsson (1965) where crayfish were marked with a point code on top of their carapace. The crayfish were marked with a white outdoor marker (Edding 8055, Ahrensburg, Germany). After molts, we waited for the exoskeleton to be hardened completely and measured the new length before remarking the animals. Tanks were cleaned once a week and around 75% of water was exchanged with fresh tap water. Individual crayfish were fed half a ring of commercial crayfish food (Crabs natural, sera, Heinsberg, Germany) daily. Dried and blanched oak leaves were provided ad libitum as additional food and environmental enrichment. After the end of the study, crayfish were used for further experiments on their prey choice and feeding mechanics.

The protocol and procedures employed were ethically reviewed and approved by the Landesamt für Gesundheit und Soziales (LAGeSo), Berlin, Germany. All experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Setup and standard procedure

All experiments were conducted in the climate chamber to reduce handling and guarantee minimal disturbance from outside. Two tanks measuring 400 × 400 × 200 mm were set up in the chamber, each filmed by two cameras (one vertically above the tank and another one at the side of the tank). All experiments were recorded or photographed with network cameras (Dinion HP 1080p, Bosch, Stuttgart, Germany) capable of recording under infrared illumination. Videos and photographs were recorded and saved with VLC player (version 2.2.1.0). Each setup was covered with an opaque, black plastic tarpaulin to further minimize disturbances.

All crayfish used for the experiments were in good condition (no obvious diseases, all appendages present and intact). Each crayfish was used only for one experimental trial per day. Intermolt individuals of both male and female sex (22 – 50 mm CL) were used in experiments. Females carrying eggs or larvae were excluded from experiments up to at least one week after the release of the brood. Test animals were randomly chosen among available crayfish with a pair of ten-sided dice.

In each experiment, the tanks were filled with 20 mm of fine gravel and 150 mm of tap water of 15 °C temperature. Crayfish were released into experimental tanks and allowed to acclimatize for 30 min prior to the experiment. After each trial, the tank was completely drained before setting up another experiment to avoid a potential bias by remaining pheromones in the water (Breithaupt 2011).

Allometry

Since larger chelae can be advantageous in agonistic encounters, we measured chela length of the right cheliped (in mm) for a random set of crayfish from three groups: male spiny-cheek crayfish (N = 52), female spiny-cheek crayfish (N = 28), and marbled crayfish (N = 81) with CLs between 20 and 50 mm. We fitted linear regression models (command lm()) in R version 3.4.0 (R Core Team 2017) to predict chela size depending on CL in each of these groups. To test for differences in intercept and slope in the three regression lines, we fitted three models for each pair of two of the three groups accounting for CL, the group, and their interaction.

Aggressiveness

We tested agonistic behavior against size-matched opponents (± 1 mm CL) in interspecific encounters of individual spiny-cheek crayfish males (N = 12), spiny-cheek crayfish females (N = 7), aquarium-reared marbled crayfish (N = 14), and naturalized marbled crayfish (N = 13). Three encounters were staged for each individual against three different opponents. The availability of matching pairs was reduced by egg-bearing females and molting individuals and resulted in uneven numbers of replicates. To better discriminate species and size effects, we later staged confrontations of the same individuals with smaller (<4 ± 2 mm CL [mean ± standard deviation, SD]; N = 15 for spiny-cheek crayfish, N = 13 for marbled crayfish) and larger opponents (>4 ± 2 mm CL [mean ± SD]; N = 16 for spiny-cheek crayfish, N = 14 for marbled crayfish; modified from Vorburger and Ribi 1999).

Experimental tanks were separated into two sides with a removable opaque divider (PVC). The corners were rounded with plastic glass to avoid that submissive animals become trapped. For each trial, one crayfish was transferred into each compartment. After acclimatization, the divider was lifted and the encounter recorded on video to later assess and score each interaction. The experiments were conducted in the dark when crayfish are most active and illuminated by infrared headlights (Holdich and Black 2007, Luna et al. 2009).

Each confrontation was recorded with both cameras. The recording time was set at 35 min. The first 30 min after opening the divider was analyzed for agonistic behavior, and 5 min was added as buffering time. If fewer than five interactions took place within the 30 min, the buffering time was checked for more interactions. If there were still fewer than five interactions including the buffering time, the experiment was repeated with another opponent for each crayfish on another day.

To quantify interaction strength during the confrontations, the observed behavior was scored with the system developed by Atema and Voigt (1995; Table 3.1). For every five-seconds, each member of the pair was assigned an aggression score. The scoring system was modified by giving ignoring, which was not originally included in the system, the score 0. Ignoring was observed when crayfish were within one body length of one another or had physical contact, but did not show any visible response (i.e., taxis) toward the opponent’s presence (e.g., crawling along the aquarium pane, crawling over or under the body of the opponent). The opponent could show another agonistic behavior at the same time and was scored, respectively. The term separate includes all situations where the individuals were apart for more than one body length and no score was applied. When more than one agonistic behavior was shown within five-seconds, higher scores outranked lower (positive) scores 0–5). Score −2 outranked −1 and both flight behaviors (scores −1 and −2) outranked score 0 or positive scores. The interactions ended with one crayfish fleeing or separating itself from the counterpart by more than one body length.

Table 3.1 – Definitions of agonistic behaviors observed in crayfish and their designated score (modified from Atema and Voigt (1995)).

Score	Behavior	Definition
−2	Fleeing	Walking away (rapidly), walking backwards (rapidly), tail-
−1	Avoidance	Walking away (slowly), walking backwards (slowly), turning
0	Ignoring	Indifference towards each other within less than one body-
1	No physical contact	Facing, approaching, turning towards, following
2	No physical contact	High on legs, claw open, meral spread, claw forward, antenna
3	Physical contact	Antenna touching, claw touching, claw tapping, claw pushing,
4	Physical contact	Claw lock
5	Unrestrained use of claws	Claw snapping, claw ripping
n/a	Separate	Opponents one body-length or more apart

For every individual and confrontation, we counted the total number of each observed score (for all five-second intervals) during the 30 min of confrontation for each crayfish. To see relative frequencies of certain scores among the groups, a standardized count was calculated by adding up the scores for each group and dividing it by the number of tested individuals. For every individual and confrontation, we calculated an aggression score by multiplying each score with the number of observations and adding them up for all behaviors (Karavanich and Atema 1998). We then adjusted the aggression score by dividing it by the number of interactions (5-s intervals) that were observed during 30 min (adj. AS). We did this adjustment to obtain a better measure of average aggression level since the time spent interacting with the other crayfish differed largely between trials. A negative or low aggression score represents a submissive individual or the loser of the encounter, whereas a high value indicates an aggressive individual or the winner of the encounter.

We performed analyses using linear mixed-effects models to detect agonistic score differences between the groups or species with individual as random factor (command lmer() from package *lme4*, (Bates et al. 2014)). As fixed effects, we used species, CL at the time of the fight (molting and therefore growth can occur between days of the experimental period), origin (aquarium or naturalized, only applicable to marbled crayfish), and sex (only applicable to spiny-cheek crayfish). All possible combinations of fixed effects and interactions between fixed effects were calculated—except between species, origin, and sex as these are confounded. Models were ranked by Akaike's information criterion (AIC) and Akaike's model weight. Marginal (fixed factors only) and conditional (fixed factors and random factor) R^2 values for the best model were calculated using the *MuMIn* package (Bartón 2013).

Activity

We tested the activity (time spent outside of the shelter) of individual spiny-cheek crayfish males (N = 11), spiny-cheek crayfish females (N = 5), aquarium-reared marbled crayfish (N = 13), and naturalized marbled crayfish (N = 14). Each individual crayfish was tested in three trials. The experimental tanks were filled with gravel 20 mm deep and completely divided by half with an opaque divider (PVC). A PVC pipe (l = 150 mm, diameter 50 mm) in each compartment was provided as shelter. A crayfish was transferred into each compartment. After acclimatization, photographs were taken in the dark under infrared light every 30 min for 6 h, starting 30 min after artificial nightfall. Photographs were later checked for the position of the crayfish in the tank. Crayfish were considered to be outside the shelter when all of the carapace and the pereopods were visible outside the PVC pipe, as viewed from above. We summed up the number of observations outside the shelter and the number of observations inside the shelter for each trail.

We then applied a generalized linear mixed-effects model (GLMM) for binary responses with R to detect differences in time spent outside and inside the shelter among spiny-cheek crayfish males, spiny-cheek crayfish females, aquarium-reared marbled crayfish, and naturalized marbled crayfish (command glmer; package *lme4*). The individual was included in the model as random factor. Similar to the aggression scores, we calculated all possible combinations of fixed effects and interactions between fixed effects— except between species, origin, and sex as these are confounded. Models were ranked by AIC and Akaike's model weight and we calculated marginal and conditional R^2 values for the best model using the *MuMIn* package (Bartón 2013).

Threat response

The response to a simulated threat as a measure of boldness was tested for spiny-cheek crayfish males (N = 15), spiny-cheek crayfish females (N = 15), aquarium-reared marbled crayfish (N = 13), and naturalized marbled crayfish (N = 19). Individual crayfish were placed in the experimental tank with 20 mm of sand as substrate and allowed to acclimatize. The crayfish were then approached from the upper front, using an angle of ~45°, by the hand of the experimenter in a steady but brisk movement. Threat responses were recorded under dim light conditions from above the tank. Each individual crayfish was tested three times but only once per day. The experiment followed the approach by Pintor et al. (2008), but with a modification since most crayfish in preliminary trials did not show a response to the hand if its movement was stopped above the surface. Thus, the movement of the hand was extended into the water, aiming for the front of the crayfish until a contact would occur. The hand was put through a hole in the tarpaulin when the crayfish was in a suitable position. Before the crayfish was approached, it needed to be at least one body length away from the aquarium pane, so it would not be constrained when displaying a flight reaction. Hands were washed with warm water after each trial to avoid a potential bias by remaining pheromones (Breithaupt 2011).

The first, initial response of the crayfish to the hand was assessed. Crayfish responded either by tail-flipping, that is, shooting backward (flight; score −1); stop moving and ducking (freezing; score 0); or by showing a threat display, that is, lifting their claws (fight; score 1). The scores of the three trials were summed up, and a general response score was given to each individual crayfish. A negative sum resulted in a general flight response, a positive score resulted in a fight response, and a sum of 0 was classified as freeze. We tested for differences between the groups with a chi-square test in R (command chisq.test) with 100,000 bootstrap simulations. We also compared all combinations of groups of crayfish and corrected for multiple testing using the Bonferroni-Holm method. Additionally, all groups of crayfish were checked for potential effects of CL using Spearman rank correlations.

Results

Allometry

Carapace length was a significant predictor of chela length for crayfish from all groups (Figure C.1). The average chela length was significantly smaller, and the slope was less steep for marbled than for spiny-cheek crayfish males (linear regression, $t = 8.75$, $P < 0.001$ and $t = -13.49$, $P < 0.001$). There were also significant differences in chela length and slope of the regression lines between spiny-cheek crayfish females and males (linear regression, $t = 4.60$, $P < 0.001$ and $t = -7.16$, $P < 0.001$). There was no significant difference in chela size between marbled crayfish and spiny-cheek crayfish females or slope of regression lines (linear regression, $t = 1.55$, $P = 0.12$ and $t = -1.3$, $P = 0.20$).

Aggression

All linear mixed-effects models were sorted according to their delta-AIC value and AIC weights. Models with an AIC weight above 0.05 are presented in Table 3.2 (see Table C.1 for all models). The best model includes species and individual CL (size) as predictors (marginal $R^2 = 0.16$; conditional $R^2 = 0.31$). All other models with a model weight above 0.05 also include species and size plus either origin, sex, or interaction terms. Species and size thus seem to be the most important predictors for aggressiveness, whereas other factors are less important. Carapace length was positively correlated with adj. AS. We considered individual as random factor in the analyses but found no statistical effect on aggression. In initial exploratory analyses, we also looked for an effect of the day of experiment (1st, 2nd, or 3rd) but did not find such an effect.

Table 3.2 – Linear mixed-effects models of adjusted aggression score (adj. AS) analysis.

Model (fixed effects)	**delta-AIC**	**AIC weight**
– species(SC) + size	0	0.221
species(SC) + size – species(SC):size	1.3	0.116
– species(SC) + size + origin(aq)	1.7	0.095
– species(SC) + size − sex(m)	2	0.082
– species(SC) + size + sex(m) – size:sex(m)	2.5	0.063
species(SC) + size + origin(aq) – species(SC):size	2.9	0.053

Notes: Listed are the best models according to Akaike's model weight (Akaike's information criterion [AIC] weight). Indicated positive or negative effects of variables relate to the values of these variables given in brackets (m, male; SC, spiny-cheek crayfish; aq, aquarium origin); these are compared to female marbled crayfish from the field as reference. All models include the individual (IND) as random factor (Adj. AS ~ intercept + fixed effects + (1|IND)).

The adj. AS of marbled crayfish was on average 0.67 ± 0.16 standard error (SE) higher than the adj. AS of spiny-cheek crayfish (Figure 3.1). The results of the mixed-effects models that neither (1) sex nor (2) origin is important predictor of aggression are also illustrated in Figure 3.1: adj. AS of (1) male and female spiny-cheek crayfish, and (2) aquarium-reared and naturalized marbled crayfish were similar.

Aggression encounters rarely escalated (scores 4 and 5 were rare; Figure C.2) and were mostly resolved by claw pushing or boxing (score 3). Marbled crayfish rarely initiated fights with a threat display or responded equally to spiny-cheek threat displays (score 2). Furthermore, marbled crayfish often ignored their opponent (score 0). Higher negative scores indicated that spiny-cheek crayfish lost more encounters than marbled crayfish.

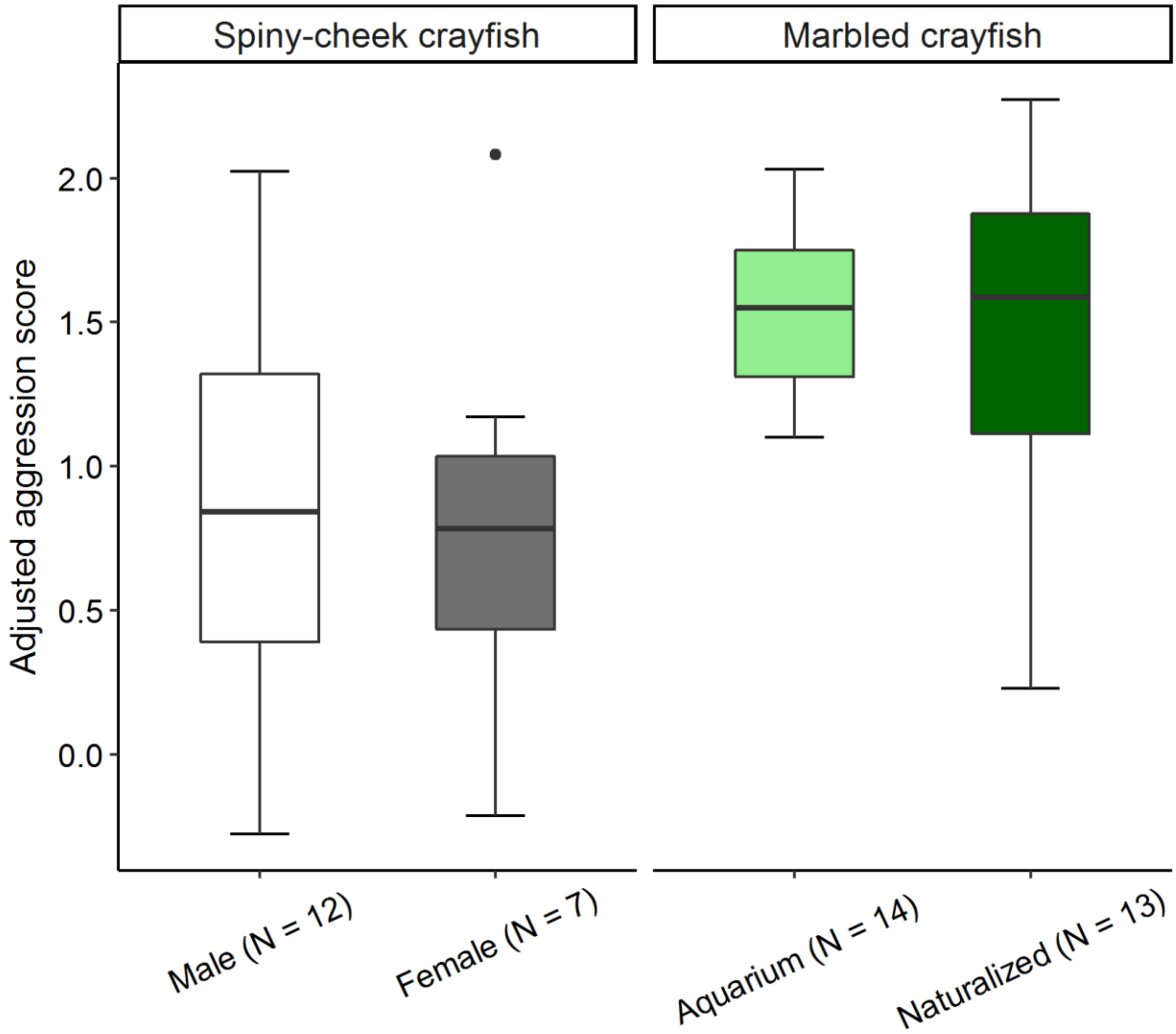

Figure 3.1 – Adjusted aggression scores in pairwise interspecific interactions among spiny-cheek crayfish males (open boxplot), spiny-cheek crayfish females (gray boxplot), and marbled crayfish from aquaria (light green boxplot) and naturalized populations (darkgreen boxplot).

In agonistic encounters against smaller opponents from either sex, marbled crayfish differed significantly from spiny-cheek crayfish and won all interactions, whereas spiny-cheek crayfish lost most interactions (Fisher's exact test, *df* = 25, *P* < 0.001; Figure 3.2). Against larger opponents, marbled crayfish similarly won 64% of encounters and spiny-cheek only 31%, but this difference was not statistically significant (Fisher's exact test, *df* = 28, *P* = 0.14).

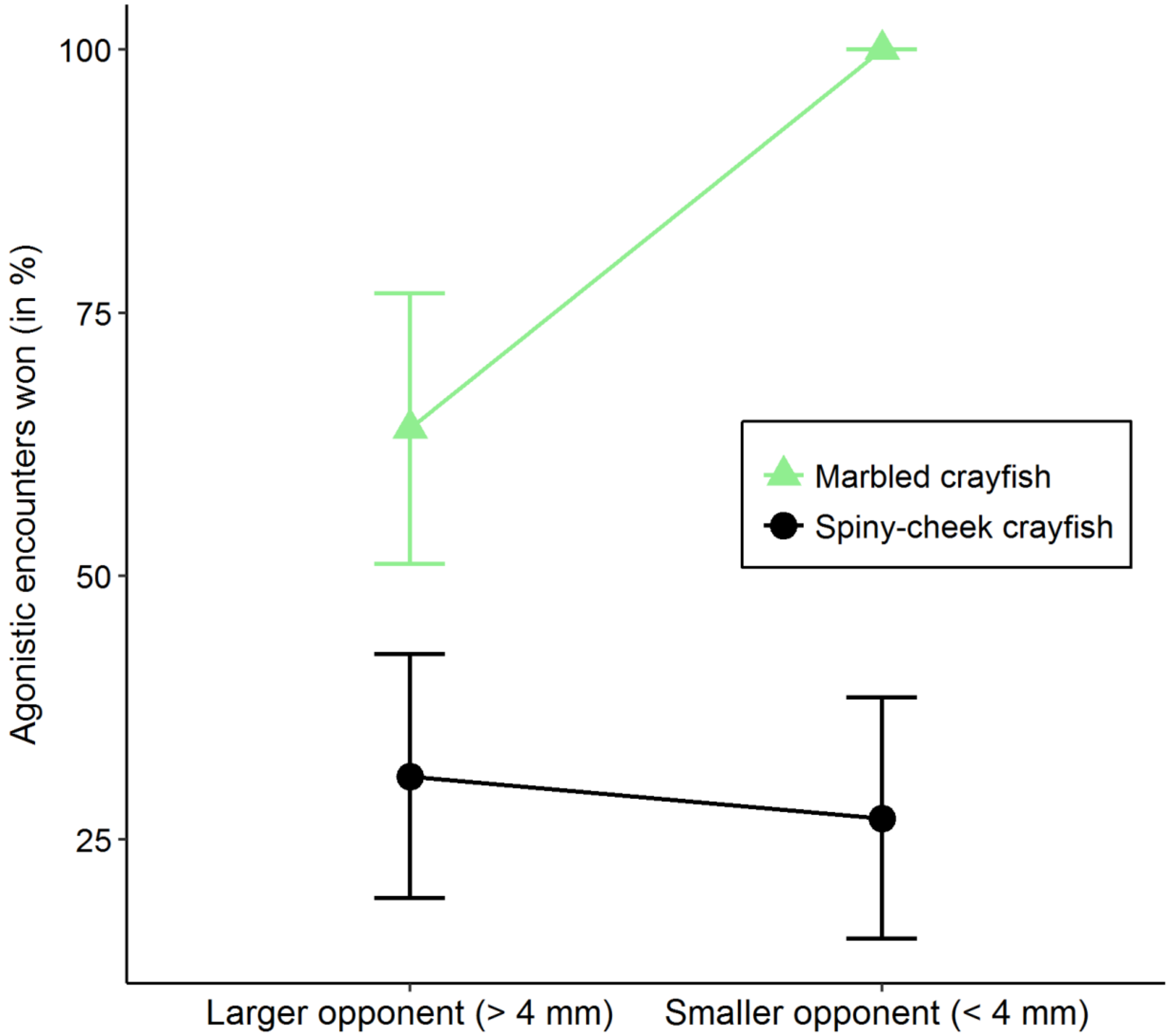

Figure 3.2 – Agonistic encounters won (in percent ± standard error) by marbled crayfish (triangles, light green) and spiny-cheek crayfish (both sexes; circles, black) with opponents of unequal size of the other species. The left side shows the outcomes against larger opponents (spiny-cheek crayfish, N = 16; marbled crayfish, N = 14) and the right side against smaller opponents (spiny-cheek crayfish, N = 15; marbled crayfish, N = 13).

Activity

The best model (marginal R^2 = 0.10; conditional R^2 = 0.21) uses origin and size as predictors: Aquarium marbled crayfish were more active than all other groups (Table 3.3, Figure 3.3; see Table C.2 for all models). Activity was negatively correlated with size for all crayfish. Spiny-cheek crayfish males, spiny-cheek crayfish females, and naturalized marbled crayfish spent more time inside than outside the shelter. All models using other predictors along origin were weaker than the one with origin and size as the sole predictors, and models not accounting for origin were negligible in explanatory power (AIC weights <0.001; Table 3.3). In conclusion, shelter use did not differ markedly among sex or species, but the rearing environment (origin) and size were meaningful predictors of activity. We considered individuals as random factor, but these had no effect on shelter use. In initial exploratory analyses, we also looked for an effect of the day of experiment but did not find one.

Table 3.3 – Generalized linear mixed-effects model results of activity analysis.

Model (fixed effects)	**delta-AIC**	**AIC weight**
origin(aq) – size	0.0	0.161
origin(aq)	0.4	0.131
origin(aq) – size – origin(aq):size	1.4	0.079
origin(aq) + sex(m) – size – sex(m):size	1.5	0.077
origin(aq) + sex(m) – size − sex(m):size – origin(aq):size	1.8	0.066
origin(aq) + sex(m) – size	1.9	0.062
origin(aq) – size – species(SC)	2.0	0.060
origin(aq) + sex(m)	2.3	0.051

Notes: Listed are the best models with decreasing Akaike's model weight. Indicated positive or negative effects of variables relate to the values of these variables given in brackets (m, male; SC, spiny-cheek crayfish; aq, aquarium origin); these are compared to female marbled crayfish from the field as reference. All models include the individual (IND) as random factor (ratio of time spent outside/inside the shelter ~ intercept + fixed effects + (1|IND)). AIC, Akaike's information criterion.

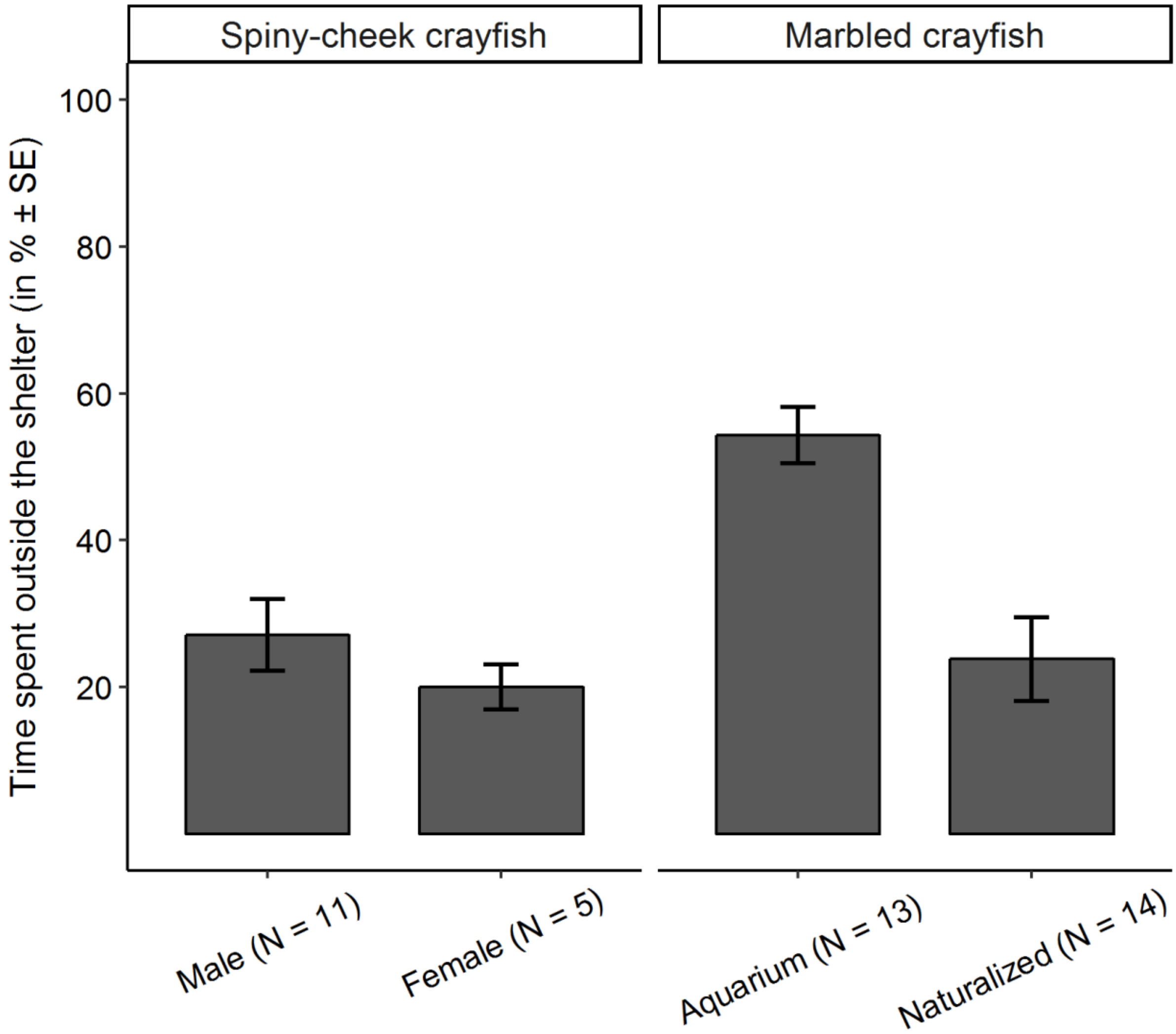

Figure 3.3 – Percentage of time spent outside the shelter (± standard error) over 6 h for spiny-cheek crayfish males, spiny-cheek crayfish females, and marbled crayfish from aquarium and naturalized populations.

Aggression syndrome

We tested for correlations between activity and aggression. Therefore, we used the means of individual aggression scores and ratios of time spent outside or inside the shelter for all individuals where we had at least three observations for aggression and activity. We calculated separate linear regressions for spiny-cheek crayfish, aquarium marbled crayfish, and naturalized marbled crayfish. Individuals of both sexes of spiny-cheek crayfish have been combined in the analysis since we did not find differences in activity and aggression (see above). Naturalized marbled crayfish and marbled crayfish from aquaria have been tested separately; as they differed in activity (see above).

One aquarium marbled crayfish had only two aggression scores because an interspecific mating took place during the third experiment; thus, the observation was excluded. Also, one naturalized marbled crayfish had only two observations for activity because it was cannibalized during molting before the third experiment could be conducted.

We found that in naturalized marbled crayfish, mean adj. AS (aggression) was positively correlated with the ratio of time spent outside or inside a shelter (activity; Figure 3.4). A similar trend was observed for aquarium-reared marbled crayfish, whereas no such correlation was found for spiny-cheek crayfish.

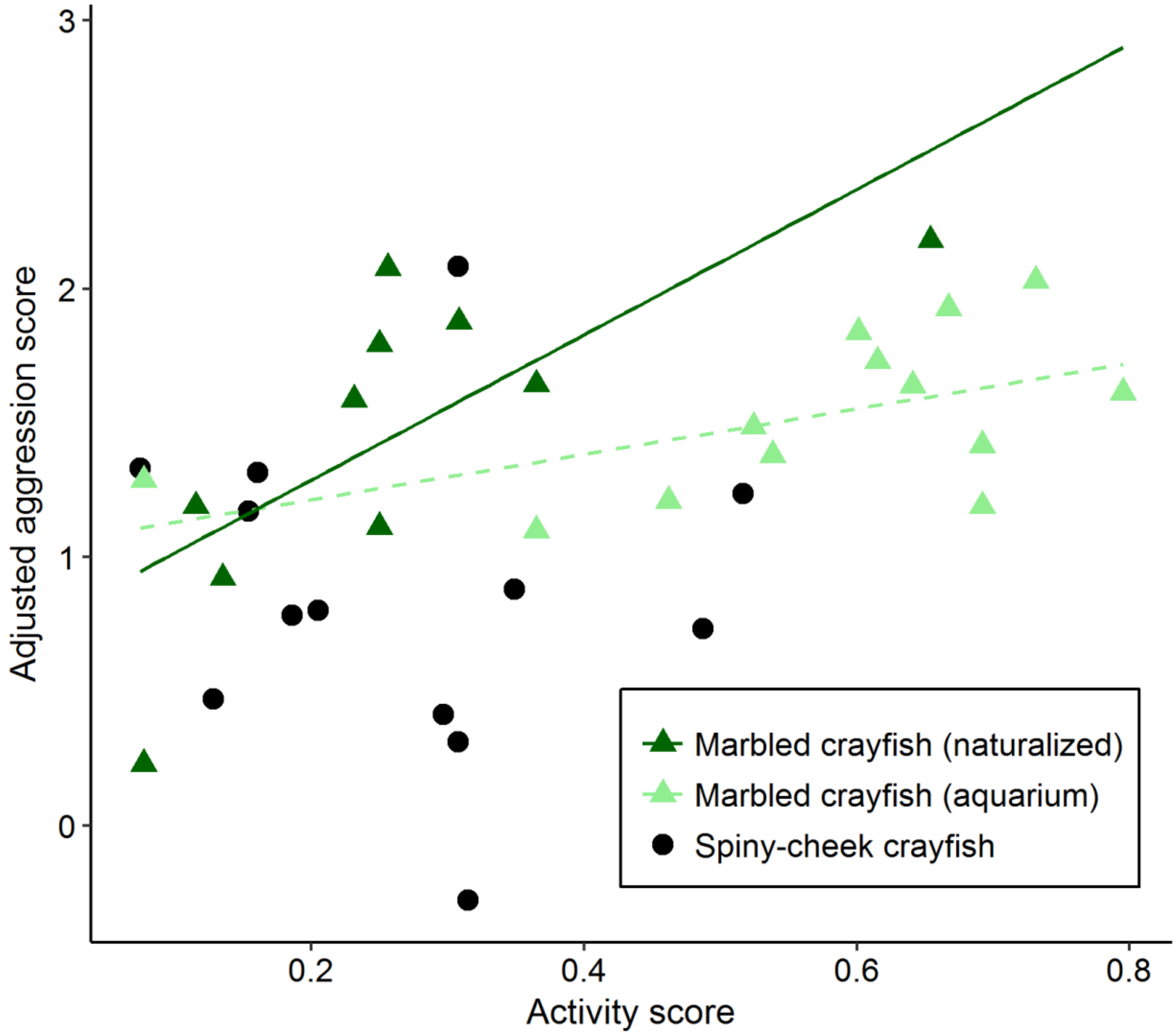

Figure 3.4 – Correlations between mean activity score (ratio of time spent outside/inside the shelter; ACT) and mean adjusted aggression score (adj. AS) of spiny-cheek and marbled crayfish individuals across experiments. Regression lines: naturalized marbled crayfish, Adj. AS ~ 0.75 + 2.7·ACT (linear regression, $t = 3.09$, $P = 0.015$, adj. $R^2 = 0.49$); aquarium marbled crayfish, Adj. AS ~ 1.05 + 0.85·ACT (linear regression, $t = 2.10$, $P = 0.06$, adj. $R^2 = 0.22$). No line is shown for spiny-cheek crayfish, as no trend was observed, Adj. AS: ~ 0.97 – 0.39·ACT; linear regression: $t = -0.295$, $P = 0.77$, *adj.* $R^2 = 0.08$).

Threat response

Crayfish groups significantly differed in their threat response (Pearson's Chi-squared test with simulated P-value, based on 100 000 replicates: $\chi^2 = 54.91$, $P < 0.001$, Figure 3.5, Table 3.4). The CL was not related to threat response in any of the groups (spiny-cheek crayfish (male): $r_S = -0.046$, $P = 0.87$; spiny-cheek crayfish (female): $r_S = 0.149$, $P = 0.60$; marbled crayfish (aquarium): $r_S = -0.321$, $P = 0.29$; marbled crayfish (naturalized): $r_S = 0.217$, $P = 0.40$. Remarkably, marbled crayfish frequently stopped and ducked in response to the approaching threat (37% or 47% for aquarium or naturalized marbled crayfish, respectively), whereas spiny-cheek crayfish did not show such behavior. Male spiny-cheek crayfish mainly responded with aggression (93% of all trials), whereas females mostly displayed flight behavior (93%). If not “freezing” in response to a threat, marbled crayfish most often fled from the threat (62% or 37% for aquarium or naturalized marbled crayfish, respectively). Aquarium marbled crayfish did not fight, whereas naturalized marbled crayfish showed fight behavior in about 15% of the trials.

Table 3.4 – Chi-square statistics (χ^2) of all pairwise comparisons between groups of crayfish and among all groups tested for their threat response.

Comparison	χ^2	P
spiny-cheek (female) *vs.* spiny-cheek (male)	19.29	<0.001
marbled crayfish (naturalized) *vs.* marbled crayfish (aquarium)	3.20	0.21
spiny-cheek crayfish *vs.* marbled crayfish (species)	21.96	<0.001
marbled crayfish (aquarium) *vs.* all naturalized crayfish (origin)	7.20	0.055
all female crayfish *vs.* male spiny-cheek crayfish (sex)	35.81	<0.001
comparison between all groups	50.44	<0.001

Notes: P-values are corrected for multiple testing using the Bonferroni-Holm method.

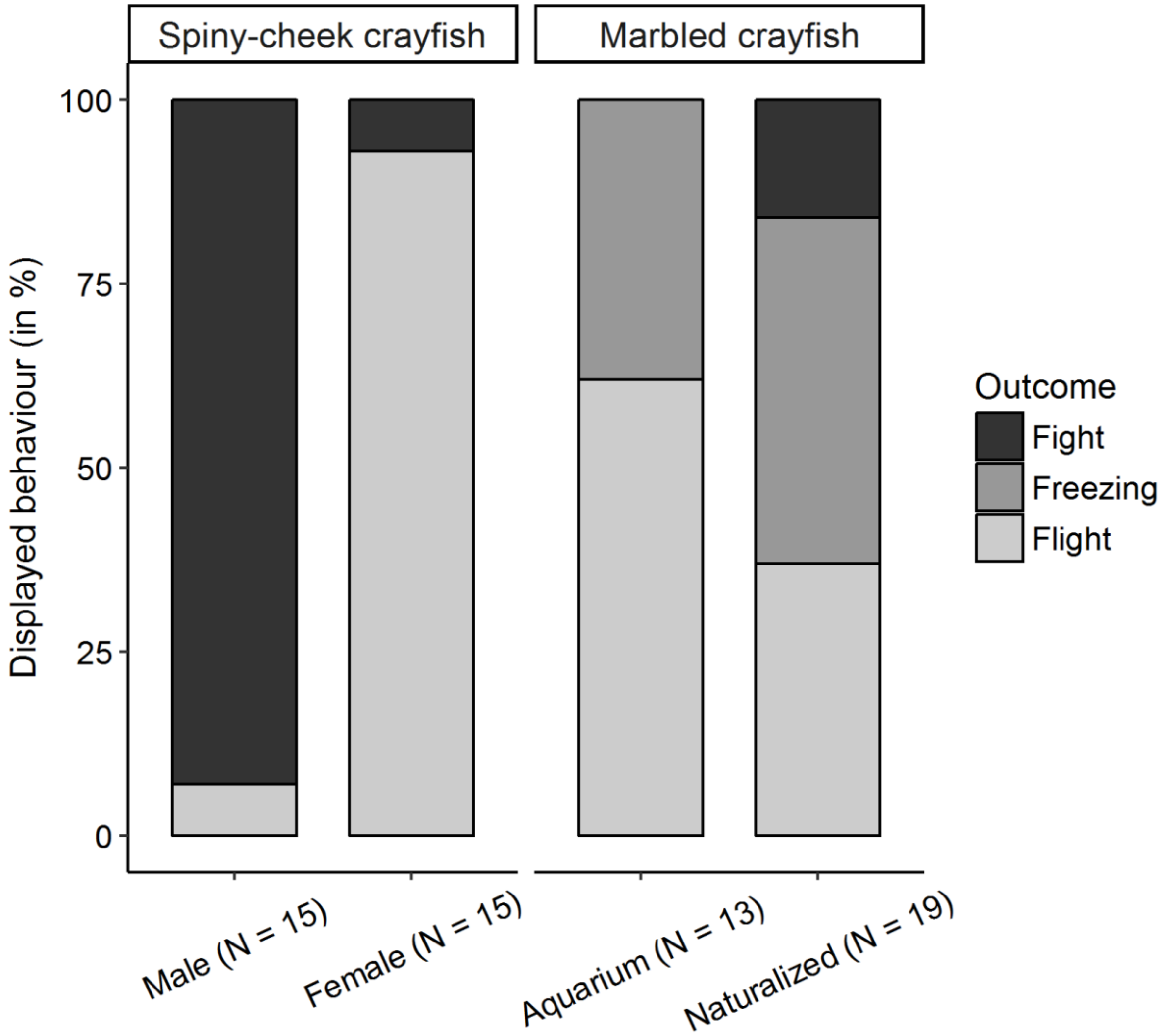

Figure 3.5 – The percentage of displayed behaviors in response to a simulated threat for spiny-cheek crayfish of either sex and marbled crayfish from aquarium and naturalized populations. The behaviors displayed encompass aggressive behavior (dark grey bars), freezing (grey bars) or flight behavior (light grey bars).

Discussion

Differences between species and implications

Our results illustrate some of the key behavioral characteristics in invasion success of crayfish. In the over-invasion scenario we investigated, the recently invading marbled crayfish were able to dominate resident spiny-cheek crayfish of either sex in agonistic encounters even if their opponents were larger and had larger claws.

Aggressiveness has been one of the main behavioral traits associated with species displacement in crayfish (Capelli and Munjal 1982, Usio et al. 2001). So far, interspecific aggression has only been tested for juveniles of marbled crayfish interacting with red swamp crayfish (*Procambarus clarkii*), which were similarly aggressive (Jimenez and Faulkes 2011). As we demonstrated here, the lack of sexual dimorphism does not constrain the ability of marbled crayfish to win agonistic encounters against another species. Aggressive dominance also translates to superiority in competition over shelters, an important resource for crayfish that relieves them from predation pressure (Gherardi and Daniels 2004, Moore 2007). Thus, we assume that preferred resources of spiny-cheek crayfish like shelters would be frequently occupied by invading marbled crayfish where both species co-occur.

In former invasions of North American species across Europe, interspecific competition between crayfish was mostly no relevant determinant of invasion success because the crayfish plague (*Aphanomyces astaci*, Leptolegniaceae) often completely eradicated native competitors before or shortly after introduction of non-native crayfish (Gherardi and Holdich 1999). The die-off of potential competitors and its high tolerance toward poor habitat quality probably had a major effect on the former success of spiny-cheek crayfish. There is surprisingly little work on the competitive ability of spiny-cheek crayfish in contrast to other major invasive crayfish in Europe like the red swamp crayfish or the signal crayfish (*Pacifastacus leniusculus*). In the few available studies on spiny-cheek crayfish aggression, they were usually inferior in agonistic encounters (either in their native range against an invader or against another invader in their introduced range (Klocker and Strayer 2004, Hudina et al. 2011). It should be noted that the average aggression level of the tested spiny-cheek crayfish might be even lower since they were caught with traps, which can select for aggressive individuals (Ogle and Kret 2008).

In the invasion scenarios we are facing today, crayfish plague-resistant species over-invade other plague-resistant species, and the traits and interactions with the community will become paramount for distribution and impacts of crayfish (Russell et al. 2014, James et al. 2016). In general, species distributions and impacts in novel communities and ecosystems can probably be better understood when considering the time of introduction of species rather than simply dividing species into native and non-native ones. The latter, dichotomous classification is often based on a reference year, for example, 1492 which is sometimes rounded to 1500 (DAISIE 2009): Species present before this year are considered native, and species introduced thereafter are considered non-native. A finer approach considering the time of introduction and the eco-evolutionary experience of introduced and resident species (Saul and Jeschke 2015) seems to be a promising way forward.

Behavioral syndromes

We also explored the flexibility and the correlations among behaviors (i.e., behavioral syndromes). We observed two traits in particular that are beneficial during the introduction and spread of species: Higher aggression jointly with higher activity was observed in marbled as compared to spiny-cheek crayfish. Positive correlations of aggressiveness and activity have been referred to as so-called aggression syndromes in invasive species (Sih et al. 2004, Pintor et al. 2009). Our results suggest that marbled crayfish exhibit such an aggression syndrome which can lead to more agonistic encounters, but may also be positively related to attacks on prey, that is, increased foraging rate (Sih et al. 2004, Sih and Bell 2008, Pintor et al. 2009). Both would facilitate species displacement through either interspecific aggression or competition for resources.

Thus far, marbled crayfish have not outcompeted spiny-cheek crayfish in water bodies where both species co-occur (Chucholl and Pfeiffer 2010). Trade-offs associated with the aggression syndrome, for example, higher intraspecific aggression, might limit the success of marbled crayfish. Elevated intraspecific aggression levels might, for example, constrain marbled crayfish densities. We did not include intraspecific aggression in our study design, but from our observations in communal tanks, we suspect intraspecific aggression to be low. High genetic relatedness has been shown to lower intraspecific aggression in insects (Carazo et al. 2014, Jandt et al. 2014), but marbled crayfish also form dominance hierarchies (Luna et al. 2009).

Marbled crayfish might also suffer higher predation rates despite similar activity levels because their antipredator behavior (i.e., threat response) is not appropriate or their morphology makes them easier to attack. After handling both species for years, we have the impression that spiny-cheek crayfish have a thinner carapace and they have, as their name implies, spines in contrast to marbled crayfish. To our knowledge, data on exoskeleton thickness are not available in the literature for either species. We also noted that, if lifted up, spiny-cheek crayfish pull their legs together beneath the carapace and the abdomen to form a spiny ball that is difficult to swallow for gape-limited predators like fish. The importance of the aggression syndrome for population dynamics and invasion success should therefore be examined in relation to predators foraging on marbled crayfish (Pintor et al. 2009).

Species displacement in crayfish can take decades, as a long-term study on a Finnish lake has demonstrated (Westman et al. 2002). Higher reproduction rates, activity, and aggressive behavior were suspected to promote the displacement of noble crayfish (*Astacus astacus*) by plague-free signal crayfish in the Finnish lake, but the mechanisms of displacement have remained unclear.

Individual differences in behavior are often linked to variation in life-history parameters and morphology. Biro et al. (2014) found that individual differences in life-history and behavior of common yabby (*Cherax destructor*) express very early in life, and variation might arise primarily from genetic or permanent environmental effects. The limited genetic diversity of marbled crayfish, however, should not allow for significant effects on variability in behavior. Permanent environmental effects such as maternal effects, epigenetic effects, and other effects that influence development already before hatching can be the cause for this variation (Dochtermann et al. 2015). For example, clonal Amazon mollies (*Poecilia formosa*) consistently showed individual variation in behavior among isogenic individuals reared in isolation, and social experience during ontogeny had no effect on individual behavioral variation (Bierbach et al. 2017). In marbled crayfish, developmental variation probably explains much of the variation in coloration, growth, lifespan, reproduction, number of sense organs, and behavior, even when they are reared under identical conditions (Vogt et al. 2008). The emergence of personality and its genetic basis are yet barely understood, and studies on isogenic marbled crayfish might help deepen our understanding.

Will these behaviors promote marbled crayfish invasions?

We presented evidence that marbled crayfish are more aggressive and active competitors than spiny-cheek crayfish. Risk assessments confirm that marbled crayfish have many traits promoting high invasiveness (Twardochleb et al. 2013, Chucholl and Wendler 2017). For example, marbled crayfish cope well with low water temperatures despite their origin in warm-water aquaria (Veselý et al. 2015). High aggression and activity together with high potential population growth rates make marbled crayfish exemplary for a fast pace-of-life species (Réale et al. 2010). Marbled crayfish have a higher reproductive potential than most other crayfish, as they lay more clutches and are not bound to mating seasons due to parthenogenesis (Scholtz et al. 2003, Souty-Grosset et al. 2006). By parthenogenetic reproduction, marbled crayfish overcome many challenges that invasive species face after introduction. For example, small founder populations of marbled crayfish should not be impaired by failing to recognize conspecifics or mate choice (Chapple et al. 2012). A single marbled crayfish is sufficient to establish a population. However, parthenogenetic reproduction also reduces adaptability to cope with parasites or changes in the environment.

Predation by native predators, for example, might limit the spread of marbled crayfish. The response to threat or boldness that we observed in marbled crayfish differs from many other crayfish species. We expected marbled crayfish to respond inappropriately to a threat because organisms from the pet trade should be naïve to threats. Fight-or-flight behavior is most often observed in crayfish as appropriate responses to predation threats (Stein and Magnuson 1976). However, marbled crayfish ducked or seemed to freeze before the approaching hand. We tried to minimize contacts with the crayfish during cleaning or feeding and never approached them upfront, but their aquarium legacy might have made them more used to handling. A comparable antipredator behavior was found in New

Zealand big-handed crabs (*Heterozius rotundifrons*) that remained immobile when an enemy approached them (Hazlett and McLay 2005). Marbled crayfish have the eponymous marbled pattern and might rely more on their camouflage, like it was reported for invasive green crabs (*Carcinus maenas*; Lohrer and Whitlatch 2002). The camouflage made marbled crayfish less conspicuous than spiny-cheek crayfish when we caught them in the lakes. We also observed freezing when we approached marbled crayfish in the lakes in a brisk and steady movement, but they still tail-flipped when the movement was more sudden.

Also, chemical stimuli might have been more important for marbled crayfish to elicit tail flips. For example, northern-clearwater crayfish (*Orconectes propinquus*) showed a stronger tail-flip behavior when chemical and tactile cues were presented simultaneously (Bouwma and Hazlett 2001). We can only speculate whether natural enemies like birds or fish are faced in an effective way. Active predators that can detect the crayfish might prey more heavily on marbled crayfish than passive predators that rely on movement of their prey. Studying predator–prey interactions with natural enemies would help to shed light on these questions and could explain population dynamics in invaded lakes.

In the direct agonistic interactions, marbled crayfish sometimes did not react to the threat display of spiny-cheek crayfish and simply ignored them. Ignoring behavior of competitors or predators is rarely included in behavioral studies on crayfish (Bergman and Moore 2003). However, ignoring was found to be pronounced in marbled crayfish. It might be related to problems in sensing signals of the opponent. Chemical communication via the urine plays an important role in intraspecific recognition and social dominance in crayfish (Breithaupt 2011); agonistic interactions last longer when chemical cues are absent (Zulandt Schneider et al. 2001). Both species are part of the same family (Cambaridae), but they are relatively distantly related (Martin et al. 2010). We speculate that marbled crayfish cannot recognize signals of the opponent and engage more strongly in agonistic interactions.

Aquarium vs. naturalized populations of marbled crayfish

We compared a naturalized and an aquarium population of marbled crayfish to look for changes in behavior. In contrast to our predictions, no differences were found in aggressiveness, and small differences in boldness toward a threat. As marbled crayfish are generally more aggressive than spiny-cheek crayfish, they do not have to elevate aggressiveness in sympatry to better compete in agonistic encounters. By contrast, resident spiny-cheek crayfish that live in sympatry with marbled crayfish might have adapted their aggressiveness. For example, native populations of virile crayfish (*Orconectes virilis*) have been shown to be more aggressive when they had prior experience with invading rusty crayfish (*Orconectes rusticus*; Hayes et al. 2009). Additional trials with sympatric spiny-cheek crayfish, which we did not test, might show more elevated aggression levels in these populations. Hayes et al. (2009) asked whether behavioral flexibility or evolution of genotypes in naïve and experienced populations underlie this difference. Marbled crayfish are genetically uniform due to parthenogenesis (see Martin et al. 2007), and behavioral differences should therefore be mostly linked to behavioral flexibility or learning, respectively. However, the correlation of activity and aggressiveness was not decoupled by adapting a lower activity in naturalized marbled crayfish, but instead persisted on a different level. Some individuals seem to be generally more aggressive and active than others, but are still flexible enough to adapt their activity to different environments.

Naturalized marbled crayfish had a lower activity level than aquarium animals and mimicked the activity levels of spiny-cheek crayfish. Rearing conditions in early juvenile stages might have had an influence on their activity. However, the marbled crayfish from the aquarium were reared under similar, stable laboratory conditions. In the critical phase after introduction of a new species, flexibility in behavior is crucial for survival and helps to overcome the problem of small propagule size (Sagata and Lester 2009). Invaders often lack experience in ecological interactions with competitors, prey, and predators (Saul and Jeschke 2015). Invasive signal crayfish, for example, reduced shelter use and increased their foraging activity despite the presence of predator cues (Hirvonen et al. 2007). Behavioral flexibility can counteract potentially maladaptive responses (Wright et al. 2010). The lower activity in naturalized marbled crayfish could be a response to predation. For example, European eels (*Anguilla anguilla*) reduced foraging activity in invasive red swamp crayfish (Aquiloni et al. 2010). In a recent study on rusty crayfish, Reisinger et al. (2017) found that prior experience had a strong effect on activity (i.e., time spent walking or feeding) in the presence of predatory smallmouth bass, but not when predators were absent (*Micropterus dolomieu*). However, they also found that crayfish raised with predatory fish exhibited reduced activity levels in general. We found activity to be lower in experienced individuals even in the absence of predators, which can be attributed to a high capacity of flexible behavior and a notable memory capacity. Invasive crayfish and crabs are behaviorally flexible and able to learn and memorize new predation cues quickly (Hazlett et al. 2002, Roudez et al. 2008). Leaving the shelter to forage is very risky in an environment with predators. Naturalized individuals that have experienced predation seem to have adapted their activity and memorized predation threat also under safe laboratory conditions.

Conclusions

Ecological consequences of over-invasions, specifically the interactions of invaders with other invaders in the community, are largely unknown (Russell et al. 2014). The recent success of marbled crayfish in establishing new populations might be influenced by their superiority in agonistic encounters and their behavioral flexibility. Marbled crayfish seem to be very adaptive and have the potential to competitively exclude or coexist with the most common invasive crayfish in Central Europe when competing for limited resources. Furthermore, experience with natural conditions can reduce activity of invasive crayfish. Marbled crayfish that originated in the aquarium trade showed that they adapt their behavior to the new environment. This trade-off between foraging and defense might, however, limit the impact of marbled crayfish. Behavioral syndromes in marbled crayfish can occur despite genetic uniformity and thus should stem from permanent environmental effects. Our results from the laboratory explain important behavioral mechanisms behind crayfish over-invasions and reveal large behavioral variability in an isogenic crayfish. To predict invasion success and assess ecological risks in nature, the species' reproductive biology, feeding behavior, and predator– prey relationships in the community should be considered. Marbled crayfish (and spiny-cheek crayfish) have been listed in the new EU regulation on invasive alien species (No 1143/2014). This regulation lays the foundation for the prevention of further spread and future introductions of non-native crayfish.

Acknowledgements

We thank Michael Pfeiffer and Christoph Chucholl for sharing their knowledge on crayfish and helping us in the field. We further thank Peer Martin for the provisioning of marbled crayfish. We also thank Thomas Mehner and the participants of the workshop “Scientific Writing” at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) for helpful discussions on an early stage of the manuscript. Finally, we thank two anonymous reviewers for their contributions to improve the manuscript. Financial support was provided by the Deutsche Forschungsgemeinschaft (DFG; JE 288/9-1, JE 288/9-2) and IGB.

Literature cited

Abrahamsson, S. 1965. A method of marking crayfish *Astacus astacus* Linné in population studies. Oikos **17**:228–231.

Aquiloni, L., S. Brusconi, E. Cecchinelli, E. Tricarico, G. Mazza, A. Paglianti, and F. Gherardi. 2010. Biological control of invasive populations of crayfish: the European eel (*Anguilla anguilla*) as a predator of *Procambarus clarkii*. Biological Invasions **12**:3817–3824.

Atema, J., and R. Voigt. 1995. Behavior and sensory biology. Pages 313–348 *in* J. R. Factor, editor. The Biology of the Lobster. Academic Press, San Diego, USA.

Bartón, K. 2013. MuMIn: Multi-Model Inference (ver. 1.9) [R package]. Retrieved from https://CRAN.R-project.org/package=MuMIn.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1–48.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. The Biological Bulletin **205**:26–35.

Bierbach, D., K. L. Laskowski, and M. Wolf. 2017. Behavioural individuality in clonal fish arises despite near-identical rearing conditions. Nature Communications **8**:15361.

Biro, P. A., B. Adriaenssens, and P. Sampson. 2014. Individual and sex-specific differences in intrinsic growth rate covary with consistent individual differences in behaviour. Journal of Animal Ecology **83**:1186–1195.

Bouwma, P., and B. A. Hazlett. 2001. Integration of multiple predator cues by the crayfish *Orconectes propinquus*. Animal Behaviour **61**:771–776.

Breithaupt, T. 2011. Chemical communication in crayfish. Pages 257–276 *in* T. Breithaupt and M. Thiel, editors. Chemical communication in crustaceans. Springer, New York/Dordrecht/Heidelberg/London.

Bubb, D. H., T. J. Thom, and M. C. Lucas. 2006. Movement, dispersal and refuge use of co-occurring introduced and native crayfish. Freshwater Biology **51**:1359–1368.

Capelli, G., and B. L. Munjal. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus *Orconectes*. Journal of Crustacean Biology **2**:486–492.

Carazo, P., C. K. Tan, F. Allen, S. Wigby, and T. Pizzari. 2014. Within-group male relatedness reduces harm to females in *Drosophila*. Nature **505**:672–675.

Chapple, D. G., S. M. Simmonds, and B. B. Wong. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? Trends in Ecology & Evolution **27**:57–64.

Chucholl, C. 2013. Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. Biological Invasions **15**:125–141.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Chucholl, C., and M. Pfeiffer. 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., H. B. Stich, and G. Maier. 2008. Aggressive interactions and competition for shelter between a recently introduced and an established invasive crayfish: *Orconectes immunis* vs. *O. limosus*. Fundamental and Applied Limnology / Archiv für Hydrobiologie **172**:27–36.

Chucholl, C., and F. Wendler. 2017. Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. Biological Invasions **19**:197–208.

DAISIE. 2009. Handbook of alien species in Europe. Springer, Dordrecht, The Netherlands.

Dochtermann, N. A., T. Schwab, and A. Sih. 2015. The contribution of additive genetic variation to personality variation: heritability of personality. Proceedings of the Royal Society B: Biological Sciences **282**:20142201.

Dornelas, M., N. J. Gotelli, B. McGill, H. Shimadzu, F. Moyes, C. Sievers, and A. E. Magurran. 2014. Assemblage time series reveal biodiversity change but not systematic loss. Science **344**:296–299.

Gallardo, B., M. Clavero, M. I. Sánchez, and M. Vilà. 2016. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology **22**:151–163.

Garvey, J. E., and R. A. Stein. 1993. Evaluating how chela size influences the invasion potential of an introduced crayfish (*Orconectes rusticus*). American Midland Naturalist **129**:172–181.

Garvey, J. E., R. A. Stein, and H. M. Thomas. 1994. Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. Ecology **75**:532–547.

Gherardi, F., L. Aquiloni, and E. Tricarico. 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: an imperfect map is better than no map. Current Zoology **58**:567–579.

Gherardi, F., and A. Cioni. 2004. Agonism and interference competition in freshwater decapods. Behaviour **141**:1297–1324.

Gherardi, F., and W. H. Daniels. 2004. Agonism and shelter competition between invasive and indigenous crayfish species. Canadian Journal of Zoology **82**:1923–1932.

Gherardi, F., and D. M. Holdich. 1999. Crayfish in Europe as alien species. CRC Press, Boca Raton, USA.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, and F. Lyko. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nature Ecology & Evolution **2**:567–573.

Hanshew, B. A., and T. S. Garcia. 2012. Invasion of the shelter snatchers: behavioural plasticity in invasive red swamp crayfish, *Procambarus clarkii*. Freshwater Biology **57**:2285–2296.

Hayes, N. M., K. J. Butkas, J. D. Olden, and M. J. V. Zanden. 2009. Behavioural and growth differences between experienced and naïve populations of a native crayfish in the presence of invasive rusty crayfish. Freshwater Biology **54**:1876–1887.

Hazlett, B. A. 1994. Crayfish feeding responses to zebra mussels depend on microorganisms and learning. Journal of Chemical Ecology **20**:2623–2630.

Hazlett, B. A., P. Acquistapace, and F. Gherardi. 2002. Differences in memory capabilities in invasive and native crayfish. Journal of Crustacean Biology **22**:439–448.

Hazlett, B. A., A. Burba, F. Gherardi, and P. Acquistapace. 2003. Invasive species of crayfish use a broader range of predation-risk cues than native species. Biological Invasions **5**:223–228.

Hazlett, B. A., and C. McLay. 2005. Responses to predation risk: alternative strategies in the crab *Heterozius rotundifrons*. Animal Behaviour **69**:967–972.

Hewitt, C. L., and G. R. Huxel. 2002. Invasion success and community resistance in single and multiple species invasion models: Do the models support the conclusions? Biological Invasions **4**:263–271.

Hirvonen, H., S. Holopainen, N. Lempiäinen, M. Selin, and J. Tulonen. 2007. Sniffing the trade-off: effects of eel odours on nocturnal foraging activity of native and introduced crayfish juveniles. Marine and Freshwater Behaviour and Physiology **40**:213–218.

Holdich, D. M., and J. Black. 2007. The spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817)[Crustacea: Decapoda: Cambaridae], digs into the UK. Aquatic Invasions **2**:1–15.

Holdich, D. M., J. D. Reynolds, C. Souty-Grosset, and P. J. Sibley. 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.

Hudina, S., N. Galić, I. Roessink, and K. Hock. 2011. Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. Biological Invasions **13**:1791–1803.

James, J., J. Thomas, A. Ellis, K. Young, J. England, and J. Cable. 2016. Over-invasion in a freshwater ecosystem: newly introduced virile crayfish (*Orconectes virilis*) outcompete established invasive signal crayfish (*Pacifastacus leniusculus*). Marine and Freshwater Behaviour and Physiology **49**:9–18.

Jandt, J. M., S. Bengston, N. Pinter-Wollman, J. N. Pruitt, N. E. Raine, A. Dornhaus, and A. Sih. 2014. Behavioural syndromes and social insects: personality at multiple levels. Biological Reviews **89**:48–67.

Jimenez, S. A., and Z. Faulkes. 2011. Can the parthenogenetic marbled crayfish Marmorkrebs compete with other crayfish species in fights? Journal of Ethology **29**:115–120.

Karavanich, C., and J. Atema. 1998. Individual recognition and memory in lobster dominance. Animal Behaviour **56**:1553–1560.

Kellison, G., D. Eggleston, and J. Burke. 2000. Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*). Canadian Journal of Fisheries and Aquatic Sciences **57**:1870–1877.

Klocker, C. A., and D. L. Strayer. 2004. Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeastern Naturalist **11**:167–178.

Kouba, A., A. Petrusek, and P. Kozák. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems **413**:05.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution **20**:223–228.

Lodge, D. M., A. Deines, F. Gherardi, D. C. Yeo, T. Arcella, A. K. Baldridge, M. A. Barnes, W. L. Chadderton, J. L. Feder, and C. A. Gantz. 2012. Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. Annual Review of Ecology, Evolution, and Systematics **43**:449–472.

Lohrer, A. M., and R. B. Whitlatch. 2002. Interactions among aliens: apparent replacement of one exotic species by another. Ecology **83**:719–732.

Luna, A. J. F., J. I. Hurtado-Zavala, T. Reischig, and R. Heinrich. 2009. Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. Journal of Biological Rhythms **24**:64–72.

Lyko, F. 2017. The marbled crayfish (Decapoda: Cambaridae) represents an independent new species. Zootaxa **4363**:544–552.

Marten, M., C. Werth, and D. Marten. 2004. The Marbled crayfish (Cambaridae, Decapoda) in Germany - another neozoan in the Rhein basin. Lauterbornia **50**:17–23.

Martin, C. W. 2014. Naïve prey exhibit reduced antipredator behavior and survivorship. PeerJ **2**:e665.

Martin, P., N. J. Dorn, T. Kawai, C. van der Heiden, and G. Scholtz. 2010. The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). Contributions to Zoology **79**:107–118.

Martin, P., K. Kohlmann, and G. Scholtz. 2007. The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. Naturwissenschaften **94**:843–846.

Moore, P. A. 2007. Agonistic behavior in freshwater crayfish: the influence of intrinsic and extrinsic factors on aggressive behavior and dominance. Pages 90–112 *in* J. E. Duffy and M. Thiel, editors. Evolutionary ecology of social and sexual systems: Crustacea as model organisms. Oxford University Press, New York, USA.

Musil, M., M. Buřič, T. Policar, A. Kouba, and P. Kozák. 2010. Comparison of diurnal and nocturnal activity between noble crayfish (*Astacus astacus*) and spinycheek crayfish (*Orconectes limosus*). Freshwater Crayfish **17**:189–193.

Ogle, D. H., and L. Kret. 2008. Experimental evidence that captured rusty crayfish (*Orconectes rusticus*) exclude uncaptured rusty crayfish from entering traps. Journal of Freshwater Ecology **23**:123–129.

Penk, M., W.-C. Saul, J. T. A. Dick, I. Donohue, M. E. Alexander, S. Linzmaier, and J. M. Jeschke. 2017. A trophic interaction framework for identifying the invasive capacity of novel organisms. Methods in Ecology and Evolution **8**:1786–1794

Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos **117**:1629–1636.

Pintor, L. M., A. Sih, and J. L. Kerby. 2009. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology **90**:581–587.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/.

Réale, D., D. Garant, M. M. Humphries, P. Bergeron, V. Careau, and P.-O. Montiglio. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philosophical Transactions of the Royal Society B: Biological Sciences **365**:4051–4063.

Reisinger, L. S., A. K. Elgin, K. M. Towle, D. J. Chan, and D. M. Lodge. 2017. The influence of evolution and plasticity on the behavior of an invasive crayfish. Biological Invasions **19**:815–830.

Roudez, R. J., T. Glover, and J. S. Weis. 2008. Learning in an invasive and a native predatory crab. Biological Invasions **10**:1191–1196.

Russell, J. C., N. S. Sataruddin, and A. D. Heard. 2014. Over-invasion by functionally equivalent invasive species. Ecology **95**:2268–2276.

Sagata, K., and P. J. Lester. 2009. Behavioural plasticity associated with propagule size, resources, and the invasion success of the Argentine ant *Linepithema humile*. Journal of Applied Ecology **46**:19–27.

Saul, W. C., and J. M. Jeschke. 2015. Eco-evolutionary experience in novel species interactions. Ecology Letters **18**:236–245.

Scholtz, G., A. Braband, L. Tolley, A. Reimann, B. Mittmann, C. Lukhaup, F. Steuerwald, and G. Vogt. 2003. Ecology: Parthenogenesis in an outsider crayfish. Nature **421**:806–806.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, and M. Arianoutsou. 2017. No saturation in the accumulation of alien species worldwide. Nature Communications **8**:14435.

Seitz, R., K. Vilpoux, U. Hopp, S. Harzsch, and G. Maier. 2005. Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. Journal of Experimental Zoology Part A: Comparative Experimental Biology **303**:393–405.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution **19**:372–378.

Sih, A., and A. M. Bell. 2008. Insights for behavioral ecology from behavioral syndromes. Advances in the Study of Behavior **38**:227–281.

Sih, A., J. Cote, M. Evans, S. Fogarty, and J. Pruitt. 2012. Ecological implications of behavioural syndromes. Ecology Letters **15**:278–289.

Souty-Grosset, C., D. M. Holdich, P. Y. Noël, J. D. Reynolds, and P. Haffner. 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France.

Stein, R. A., and J. J. Magnuson. 1976. Behavioral response of crayfish to a fish predator. Ecology **57**:751–761.

Twardochleb, L. A., J. D. Olden, and E. R. Larson. 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science **32**:1367–1382.

Usio, N., M. Konishi, and S. Nakano. 2001. Species displacement between an introduced and a ‘vulnerable’crayfish: the role of aggressive interactions and shelter competition. Biological Invasions **3**:179–185.

van Kleunen, M., W. Dawson, D. Schlaepfer, J. M. Jeschke, and M. Fischer. 2010. Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecology Letters **13**:947–958.

Veselý, L., M. Buřič, and A. Kouba. 2015. Hardy exotics species in temperate zone: can “warm water” crayfish invaders establish regardless of low temperatures? Scientific Reports **5**:16340.

Vogt, G., M. Huber, M. Thiemann, G. van den Boogaart, O. J. Schmitz, and C. D. Schubart. 2008. Production of different phenotypes from the same genotype in the same environment by developmental variation. Journal of Experimental Biology **211**:510–523.

Vorburger, C., and G. Ribi. 1999. Aggression and competition for shelter between a native and an introduced crayfish in Europe. Freshwater Biology **42**:111–119.

Weis, J. S. 2010. The role of behavior in the success of invasive crustaceans. Marine and Freshwater Behaviour and Physiology **43**:83–98.

Westman, K., R. Savolainen, and M. Julkunen. 2002. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: a 30-year study. Ecography **25**:53–73.

Wong, B., and U. Candolin. 2015. Behavioral responses to changing environments. Behavioral Ecology **26**:665–673.

Wright, T. F., J. Eberhard, E. Hobson, M. L. Avery, and M. Russello. 2010. Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. Ethology Ecology & Evolution **22**:393–404.

Yokota, T., R. Masuda, N. Arai, H. Mitamura, Y. Mitsunaga, H. Takeuchi, and T. Tsuzaki. 2007. Hatchery-reared fish have less consistent behavioral pattern compared to wild individuals, exemplified by red tilefish studied using video observation and acoustic telemetry tracking. Hydrobiologia **582**:109–120.

Zulandt Schneider, R. A., R. Huber, and P. A. Moore. 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour **138**:137–153.

Appendix C

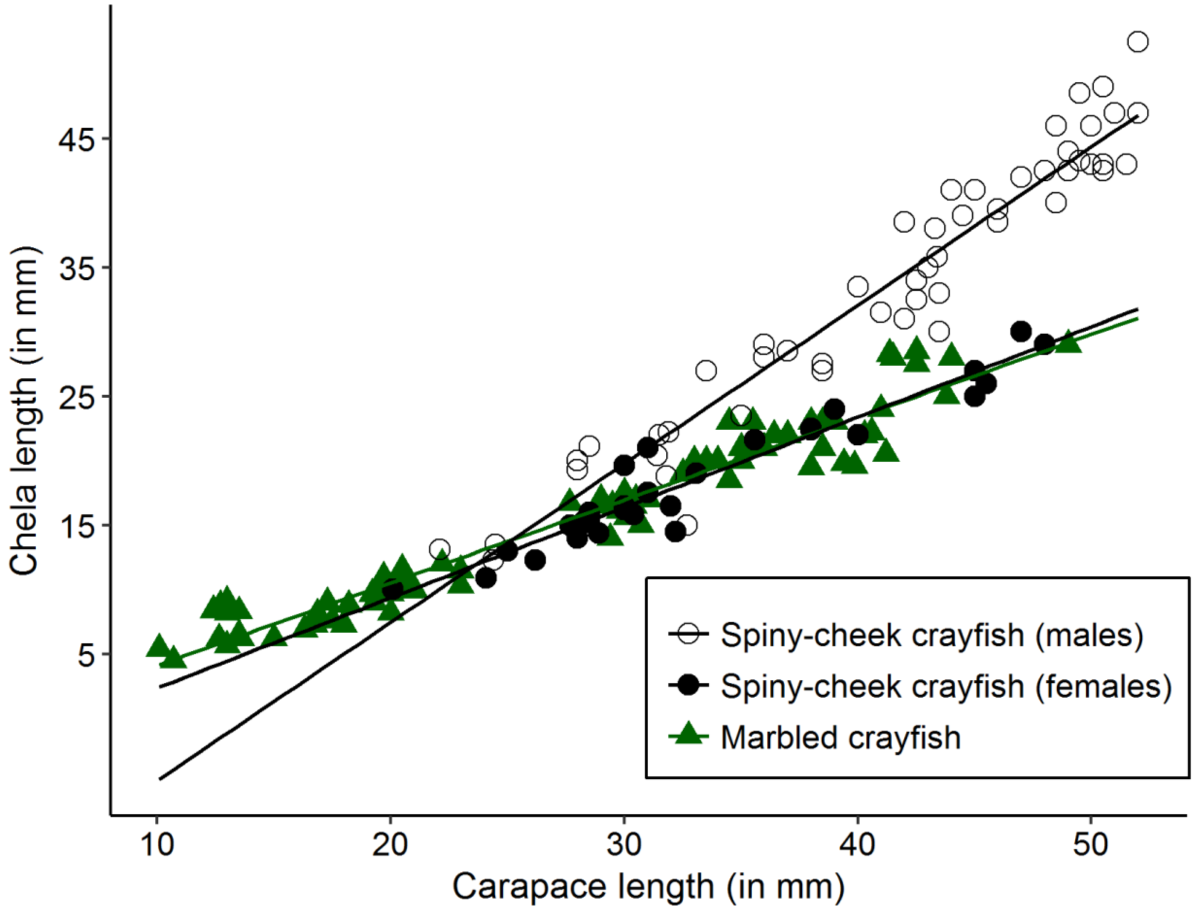

Figure C.1 – Chela length (in mm) plotted against carapace length (in mm) for spiny-cheek crayfish males (open circles; *N* = 52), spiny-cheek crayfish females (solid circles; *N* =28) and all-female marbled crayfish (dark green triangles; *N* = 81). Regression lines: spiny cheek crayfish females: Chela length ~ −4.66 + 0.7·CL (t = 18.26, $P < 0.001$, adj. $R^2 = 0.92$); spiny-cheek crayfish males: Chela length ~ −17.15 + 1.23·CL (t = 26.45, $P < 0.001$, adj. $R^2 = 0.93$); marbled crayfish: Chela length ~ −2.33 + 0.64·CL (t = 35.46, $P < 0.001$, adj. $R^2 = 0.94$).

Table C.1 – Linear mixed-effects models of adjusted aggression score (adj. AS) analysis. All models include the individual as random factor. Indicated positive or negative effects of variables relate to the values of these variables given in brackets (m = male, SC = spiny-cheek crayfish, aq = aquarium origin); these are compared to female marbled crayfish from the field as reference. The table lists all models with decreasing Akaike's model weight (AIC).

Model	**delta-**	**AIC**
− species(SC) + size	0	0.22057
species(SC) + size − species(SC):size	1.291	0.11567
− species(SC) + size + origin(aq)	1.686	0.09494
− species(SC) + size − sex(m)	1.978	0.08204
− species(SC) + size + sex(m) − sex(m):size	2.497	0.06329
species(SC) + size + origin(aq) − species(SC):size	2.851	0.05302
− species(SC)	3.018	0.04878
species(SC) + size − sex(m) − species(SC):size	3.291	0.04255
− species(SC) + size + origin(aq) − sex(m)	3.662	0.03535
− species(SC) + size − origin(aq) + origin(aq):size	3.672	0.03517
− species(SC) + size + origin(aq) + sex(m) − sex(m):size	4.059	0.02898
− species(SC) + size + sex(m) + species(SC):size − sex(m):size	4.497	0.02328
species(SC) + size + origin(aq) − species(SC):size − origin(aq):size	4.788	0.02013
species(SC) + size + origin(aq) − sex(m) − species(SC):size	4.851	0.01951
− species(SC) + origin(aq)	4.983	0.01826
− species(SC) + sex(m)	5.007	0.01804
− species(SC) + size − origin(aq) − sex(m) + origin(aq):size	5.65	0.01308
− species(SC) + size + origin(aq) + sex(m) − sex(m):size − origin(aq):size	6.007	0.01094
− species(SC) + size + origin(aq) + sex(m) − species(SC):size − sex(m):size	6.056	0.01068
species(SC) + size + origin(aq) − sex(m) − species(SC):size −	6.787	0.00741
− species(SC) + sex(m) + origin(aq)	6.973	0.00675
size + origin(aq) − sex(m)	7.178	0.00609
size + origin(aq) + sex(m) − sex(m):size	7.231	0.00593
− species(SC) + size + origin(aq) + sex(m) − species(SC):size − sex(m):size	7.992	0.00406
size − sex(m)	8.26	0.00355
size + sex(m) − size:sex(m)	8.606	0.00298
sex(m) + size + origin(aq) − sex(m):size − origin(aq):size	9.058	0.00238
− sex(m) + size + origin(aq) − origin(aq):size	9.176	0.00224
size + origin(aq)	9.491	0.00192
size + origin(aq) + size:origin(aq)	11.49	0.00071
origin(aq) − sex(m)	11.772	0.00061
− sex(m)	11.821	0.0006
origin(aq)	13.462	0.00026
size	14.54	0.00015
null model	16.717	0.00005

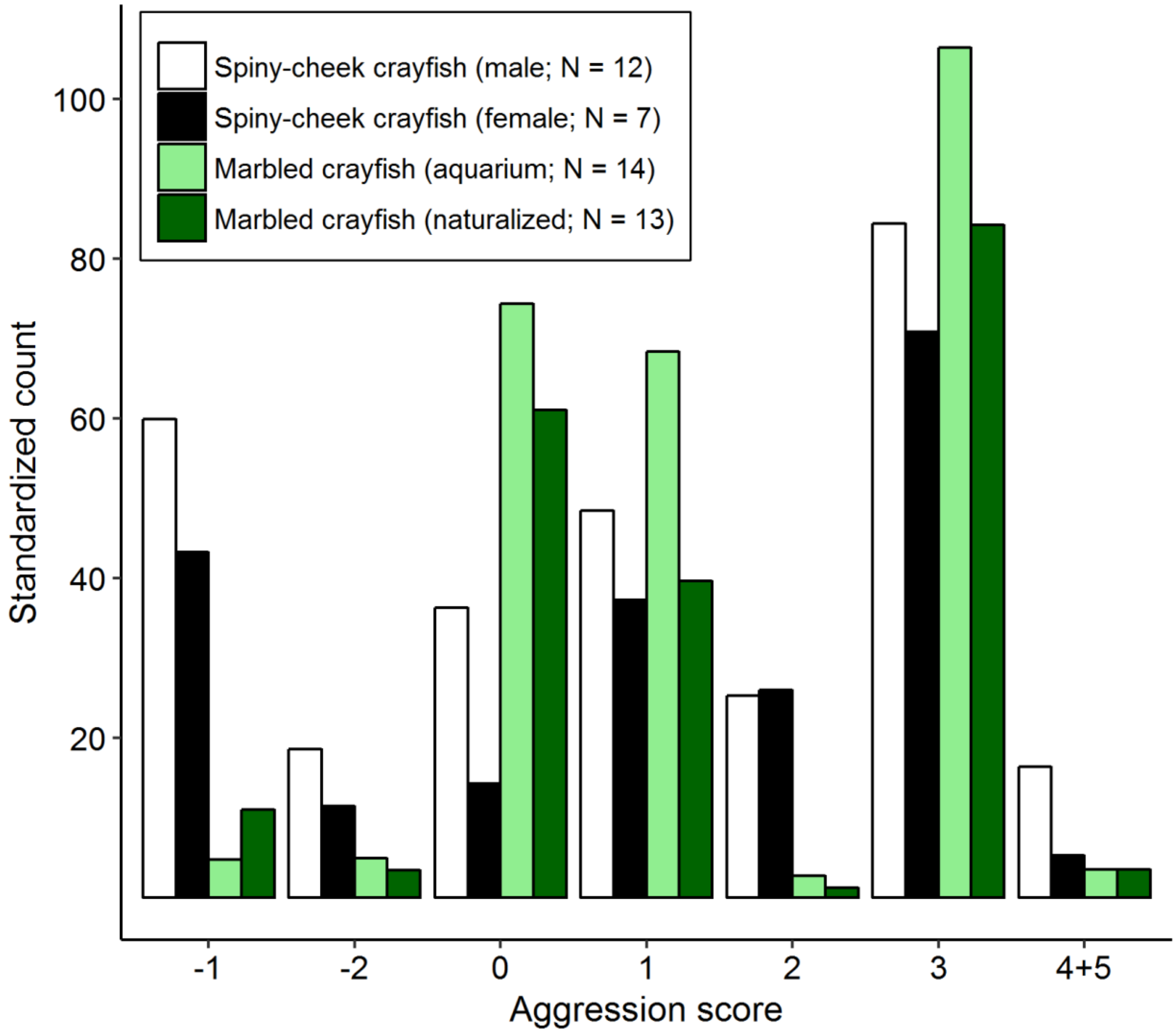

Figure C.2 – Standardized counts (by sample size) of the different scores corresponding to different behaviors in agonistic encounters. The counts are given as the total number of recorded behaviors divided by the number of trials (N) for spiny-cheek crayfish males (open bars), spiny-cheek crayfish females (black bars) and marbled crayfish from aquaria (light green bars) and naturalized populations (dark green bars).

Table C.2 – Generalized linear mixed-effects model results of activity analysis. All models include the individual as random factor. Indicated positive or negative effects of variables relate to the values of these variables given in brackets (m = male, SC = spiny-cheek crayfish, aq = aquarium origin); these are compared to female marbled crayfish from the field as reference. The table lists all models with decreasing Akaike's model weight (AIC).

Model	**delta-AIC**	**AIC weight**
origin(aq) – size	0.000	0.16063
origin(aq)	0.403	0.13132
origin(aq) – size – origin(aq):size	1.427	0.07870
sex(m) + origin(aq) – size – sex(m):size	1.465	0.07722
sex(m) + origin(aq) – size – sex(m):size – origin(aq):size	1.779	0.06600
sex(m) + origin(aq) – size	1.890	0.06243
– species(SC) + origin(aq) – size	1.985	0.05954
sex(m) + origin(aq)	2.276	0.05148
– species(SC) + origin(aq)	2.400	0.04838
– species(SC) + sex(m) + origin(aq) – size – sex(m):size	3.218	0.03214
sex(m) + origin(aq) – size – origin(aq):size	3.312	0.03066
– species(SC) + origin(aq) – size – origin(aq):size	3.418	0.02908
– species(SC) + sex(m) + origin(aq) – size – sex(m):size –	3.588	0.02671
– species(SC) + sex(m) + origin(aq) – size	3.614	0.02637
species(SC) + origin(aq) – size – species(SC):size	3.837	0.02359
– species(SC) + sex(m) + origin(aq)	4.074	0.02095
– species(SC) + sex(m) + origin(aq) – size + species(SC):size –	4.450	0.01736
species(SC) + origin(aq) – size – species(SC):size – origin(aq):size	4.742	0.01500
– species(SC) + sex(m) + origin(aq) – size – origin(aq):size	5.066	0.01276
– species(SC) + sex(m) + origin(aq) – size – species(SC):size –	5.382	0.01089
species(SC) + sex(m) + origin(aq) – size – species(SC):size	5.423	0.01067
species(SC) + sex(m) + origin(aq) – size – species(SC):size –	6.304	0.00687
– species(SC) – size	12.357	0.00033
– species(SC) + sex(m) – size	14.099	0.00014
– size	14.239	0.00013
– species(SC) – size + species(SC):size	14.263	0.00013
– species(SC) + sex(m) – size + species(SC):size – sex(m):size	14.522	0.00011
– species(SC) + sex(m) – size – sex(m):size	14.678	0.00010
– sex – size	14.870	0.00009
sex(m) – size – sex(m):size	15.472	0.00007
– species(SC) + sex(m) – size + species(SC):size	16.025	0.00005
– species(SC)	16.397	0.00004
– species(SC) + sex(m)	18.213	0.00002
null model	19.004	0.00001
sex(m)	19.166	0.00001

CHAPTER 4

TROPHIC ECOLOGY OF INVASIVE MARBLED AND SPINY-CHEEK CRAYFISH POPULATIONS

Submitted as:

Linzmaier, Stefan M.; Camille Musseau; Sven Matern & Jonathan M. Jeschke: *Trophic ecology of invasive marbled and spiny-cheek crayfish populations*.

Abstract

North American cambarid crayfish have been highly successful in establishing and spreading across Europe and are now over-invading earlier arrivals in many water bodies. Parthenogenetic marbled crayfish (*Procambarus virginalis*), which originated from aquarium stocks, are relatively recent invaders and have established in lakes previously invaded by spiny-cheek crayfish (*Faxonius limosus*). However, the feeding ecology of marbled crayfish and consequential impacts on the non-native species' coexistence are largely unexplored. By combining laboratory experiments with stable isotope analyses of field samples, we were able to (i) determine food preferences of both species under controlled conditions and (ii) explore their trophic niches in three lakes where both species co-occur. In the prey-choice laboratory experiments, the two species showed similar prey preferences and consumption rates. Consistently, the stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) highlighted the intermediate trophic position of both species. Marbled crayfish and spiny-cheek crayfish occupied a wide range of trophic positions corresponding to a very generalist diet. However, marbled crayfish were more relying on arthropod prey than spiny-cheek crayfish which fed more on mollusks. This is the first work providing evidence for trophic plasticity of marbled crayfish in lake food webs. Our results suggest that the addition of marbled crayfish increases grazing pressure on macrophytes and macrophyte-dependent organisms and the allochthonous detritus decomposition in ecosystems already invaded by spiny-cheek crayfish. Since both species are listed on the EU Regulation on invasive alien species, further assessments of potentially endangered prey organisms are needed.

Keywords

prey-choice; stable isotope analysis; over-invasions; MixSIAR; functional equivalence; trophic niche

Introduction

As the number of invasions increases worldwide, many formerly successful invasive species compete with new non-native species in so-called over-invasion scenarios (Russell et al. 2014, Seebens et al. 2017). These new waves of species invasions can have unpredictable, yet wide-ranging impacts on food webs. Invasive crayfish species in Europe are an illustrative example for aquatic over-invasions, as they can induce profound changes in aquatic systems and compete for food and shelter with other crayfish species (Gherardi 2007). Nowadays, invasive crayfish rarely compete with native ones in Europe, as the latter have disappeared from many water bodies due to pollution, habitat destruction or crayfish plague (*Aphanomyces astaci*; Holdich 2002).

Instead, formerly successful invaders are now facing competition with new non-native species which are introduced via different pathways (Chucholl 2013b, Chucholl and Wendler 2017). For example, spiny-cheek crayfish (*Faxonius limosus*) were introduced for aquaculture and are now co-occurring with marbled crayfish (*Procambarus virginalis*) coming from the aquarium pet trade (Chucholl et al. 2012). Co-occurrences of two or more non-native crayfish species are becoming increasingly common in Europe, but the interactions between these species and those with their prey have rarely been studied (Hudina et al. 2011, James et al. 2016, Linzmaier et al. 2018). Every established population will have to adapt its trophic niche yet again if the invader has a similar functional role (Tilman 2004). Established crayfish species have been shown to shift or constrict trophic niches if new non-native crayfish exhibit similar niches (Jackson et al. 2016, Larson et al. 2017). Although omnivorous crayfish are usually very flexible in their diet, some species can be more specialized than others, and their invasion impact and response to competition may differ (Johnston et al. 2011, Stites et al. 2017). Potential invaders would thus benefit from a larger trophic niche and could cause more substantial changes in the food web.

Marbled crayfish are one of several new non-native crayfish invading fresh waters (Kouba et al. 2014). This species is unique among decapods, as it is able to reproduce through parthenogenesis and was probably created in aquaria (Scholtz et al. 2003, Gutekunst et al. 2018). Marbled crayfish were described in a lake for the first time in Germany in 2003 (Marten et al. 2004). Its taxonomic status, morphology and development have been extensively studied, but the ecology of the species has rarely been looked at (Gutekunst et al. 2018, Vogt et al. 2018). To our knowledge, there is only one study on the trophic ecology of marbled crayfish (Lipták et al. 2019) and some studies on its wild progenitor, the slough crayfish (*Procambarus fallax*) which is native to the south-eastern USA (Sargeant et al. 2010, VanArman 2011), both species have been associated with low trophic levels. Whether invasive marbled crayfish utilizes similar resources as slough crayfish is currently unclear.

Most known water bodies invaded by marbled crayfish are small lentic habitats, such as gravel pit lakes, close to larger cities (Chucholl et al. 2012). In Central Europe, these habitats have mostly been invaded by spiny-cheek crayfish (Kouba et al. 2014). The number of naturalized marbled crayfish populations increases in Europe (especially Germany; Chucholl 2015), and the European Union included it in the "List of Invasive Alien Species of Union Concern" (EU Regulation 1143/2014). Thus, the question of its ecological impact becomes ever more pressing if management plans should be implemented.

Generally, crayfish are omnivorous generalists and often situated at the center of aquatic food webs occupying intermediate trophic positions (TPs; secondary consumers; Usio and Townsend 2002, Roth et al. 2006). The individual TP of crayfish is, however, related to body size, and usually increases as they grow (Roth et al. 2006). Yet, some studies species show a reverse pattern of decreasing TPs with body size (Taylor and Soucek 2010). Thus, invaded food webs are affected by crayfish on many levels, as crayfish can utilize a wide range of prey items and prey sizes (Souty-Grosset et al. 2006). As prey, they also support native predators' diets for several species of birds (Poulin et al. 2007), reptiles (Ottonello et al. 2005), mammals (Fischer et al. 2009) and fish (Haertel Borer et al. 2005).

However, non-native crayfish can destructively affect food webs. Their grazing activity can reduce aquatic vegetation, leading to strong habitat loss for many species like birds, amphibians and freshwater macroinvertebrates (Gherardi and Acquistapace 2007, van der Wal et al. 2013). Through predation, they can threaten amphibians (Kats and Ferrer 2003) and dragonfly larvae (Siesa et al. 2014), mollusks (Chucholl 2013a) and other prey organisms (Bubb et al. 2009). Finally, some species can crucially affect ecosystem functions due to their high rate of litter decomposition (Dunoyer et al. 2014, Doherty-Bone et al. 2018). Many of these impacts have been attributed to particularly detrimental invaders like red swamp crayfish (*Procambarus clarkii*; Nentwig et al. 2018). But are different invasive crayfish species equally harmful or are they functionally equivalent (Hubbell 2005)? Evidence that crayfish invaders take up similar or different roles in food webs compared to established species is equivocal (Ercoli et al. 2014, Jackson et al. 2014, Larson et al. 2017), and species-specific data on diet and TP are presently lacking for many crayfish species.

In this study, we aimed at exploring the trophic interactions of marbled and spiny-cheek crayfish by combining an experimental approach with field work in invaded lakes: (1) experiments were conducted in the laboratory to determine prey choice under controlled conditions for both species, and (2) stable isotope data from field samples were analyzed to investigate both trophic niche breadth and diet composition of the two species, including the question if marbled crayfish affect sympatric spiny-cheek crayfish. We hypothesized that marbled crayfish occupy lower TPs than spiny-cheek crayfish, and feed mainly on macrophytes and allochthonous detritus similar to its progenitor in its natural environment. Furthermore, we expected trophic niche segregation between sympatric populations of marbled crayfish and spiny-cheek crayfish. We also expected that individual TP depend on crayfish body size.

Methods

Study sites and sampling

In total, five lakes were sampled in Germany, spiny-cheek crayfish have established populations in these five lakes and marbled crayfish recently established in three of them, in sympatry with the spiny-cheek crayfish. In June 2016, we captured spiny-cheek crayfish and marbled crayfish living in sympatry in lake Moosweiher, and spiny-cheek crayfish living in allopatry in lake Silbersee; both gravel pit lakes are located in Baden-Württemberg, Germany. In May 2017, we captured spiny-cheek crayfish and marbled crayfish living in sympatry in the natural lake Krumme Lanke, Berlin, Germany. Crayfish were hand-collected in the littoral at different accessible sampling spots with head lamps after sunset. In lakes Moosweiher and Silbersee, we also used traps baited with dogfood (type "PIRAT", 610 × 315 × 250 mm, mesh width 40 × 10 mm, Rapurosvo, Parainen, Finland). Shallow to moderately deep spots were favored due to their higher accessibility. We measured the crayfish to the nearest millimeter with a sliding caliper and sexed them afterwards. Crayfish used in prey-choice trials were transported to the laboratory in boxes with macrophytes. Crayfish and all other samples used for stable isotope analyses were put on ice during field sampling and frozen in the lab at −20 °C. Crayfish length was measured as carapace length (CL) from the tip of the rostrum to the posterior edge of the carapace.

We collected allochthonous detritus (decaying leaves) and macrophytes in the littoral at least in four locations within each lake. The most common invertebrates were collected either with a surber net (500 µm mesh size) or by hand from different substrates (macrophytes, stones, woody debris). We caught fish by electrofishing and pelagic multi-mesh gillnet (Germany: 30 m x 1.5 m; twelve panels each 2.5 m long with mesh-sizes 5, 6.25, 8, 10, 12.5, 16, 19.5, 24, 29, 35, 43 and 55 mm; Lundgrens Fiskredskapsfabrik, Stockholm, Sweden). For each lake, we measured total phosphorous (µg/l) from a water sample at 1 m depth and determined the Secchi depth (m). The lakes' characteristics are given in Table 4.1.

Lakes Moosweiher, Silbersee and Krumme Lanke are small, mesotrophic and dimictic lakes (see Chucholl and Pfeiffer 2010, Vogt et al. 2018 for more detailed descriptions of lake Moosweiher). Alder (*Alnus glutinosa*), oak (*Quercus robur*), pine (*Pinus sylvestris*), and beach (*Fagus sylvatica*) trees grow along the shorelines of lakes Moosweiher and Krumme Lanke interspersed by open bathing areas. Lake Silbersee has wider bathing areas and less canopy cover. The littoral of both lakes Moosweiher and Krumme Lanke is mainly covered with macrophytes (e.g. *Potamogeton sp.* and *Elodea canadensis*) and coarse woody debris on sediments of coarse gravel and some soft-bottomed areas. In lake Silbersee, the littoral consisted mainly of macrophytes and open, gravel sediments. In all lakes, we found Eurasian perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), pike (*Esox lucius*) and eel (*Anguilla anguilla*). Lake Moosweiher and Silbersee were also invaded by very abundant pumpkinseed sunfish (*Lepomis gibbosus*). Lakes Moosweiher and Krumme Lanke were inhabited by European catfish (*Silurus glanis*).

Table 4.1 – Location, physical and chemical characteristics of the sampled lakes invaded by marbled (M) and spiny-cheek (S) crayfish.

Lake	**Species**	**Lat. (dec.°)**	**Long. (dec.°)**	**Altitude (m)**	**Surface Area (ha)**	**Z_{max} (m)**	**Secchi (m)**	**TP (µg/l)**
Moosweiher	M + S	48.030679	7.804152	216	7.6	8.0	7.6	17
Silbersee	S	48.061923	7.817354	202	4.0	14.0	6.1	14
Krumme Lanke	M + S	52.452050	13.232418	38	15.4	6.6	3.6	12
Meitzer See	M + S	52.569557	9.788003	36	19.6	23.5	4.5	3
Steinwedeler Teich	S	52.400005	10.000238	55	11.0	9.1	3.0	7

Animal maintenance

The crayfish were transferred to a climate chamber (17 °C, 14 h light: 10 h dark) and housed in single-individual tanks (300 × 200 × 200 mm). Tanks were equipped with air-driven sponge filters, gravel and PVC-pipes for shelter. Half a ring of commercial crayfish food (Crabs Natural, sera, Heinsberg, Germany) was fed to each crayfish daily. Water was exchanged once a week (ca. 75% fresh tap water).

The protocol and procedures employed were ethically reviewed and approved by the Landesamt für Gesundheit und Soziales (LAGeSo), Berlin, Germany. All experiments were performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Prey-choice experiments

Prey choice was compared between marbled crayfish and spiny-cheek crayfish according to Haddaway et al. (2012). All individuals were used in two experiments where they could choose between mobile and non-mobile prey. For mobile prey, we tested n = 10 marbled crayfish (mean ± SD, CL = 33 mm ± 6) and n = 14 spiny-cheek crayfish (CL = 36 mm ± 4); for non-mobile prey, we tested n = 13 marbled crayfish (CL = 33 mm ± 5) and n = 18 spiny-cheek crayfish (CL = 34 mm ± 4). Species body size distributions were similar for both trials (Kolmogorov-Smirnov test, mobile prey: D = 0.457, p = 0.175; non-mobile prey: D = 0.399, p = 0.155). In each trial, four prey sources (either mobile or non-mobile) were presented to an individual crayfish. The four mobile prey items used in the experiments were: amphipods, *Dikerogammarus villosus*; hard-shelled snails, *Bithynia tentaculata*; soft-shelled snails *Physidae*; bloodworms, Chironomidae larvae. The four non-mobile preys were: macrophytes, *Potamogeton* sp.; decaying oak leaves, *Quercus robur*; dead roach, *Rutilus rutilus*; small freshwater mussels, *Dreissena* sp. All prey organisms were collected on the premises of our institute (oak leaves) and the adjoining lake Müggelsee (roach, amphipods, snails, mussels and macrophytes), except for bloodworms, which were bought alive in a local pet store.

For each trial, one individual crayfish was transferred to the experimental tank (400 × 400 × 200 mm) containing sand as a substrate, a PVC-pipe as shelter and tap water up to 150 mm height. Then, 0.3 g (wet weight) of each food item was spread on the sandy areas of the tank and the crayfish were left for 24 h. The next day, the crayfish were removed, and food remains were collected and weighed again. The food items were dabbed with tissues prior to weighing in order to remove superficial water.

Differences between species in their prey-choice preferences (i.e. proportions of consumed prey) were tested using beta-regression models. Beta-regressions are commonly used for dealing with values distributed within the standard unit interval (0,1) and following a beta distribution, like proportions (Cribari-Neto and Zeileis 2010). Since, beta-regressions cannot handle true zeros and ones, these were transformed by adding or subtracting 0.001. We used the R package *betareg* and the *betareg* function (link = "logit"; Cribari-Neto and Zeileis 2010). Additionally, we tested whether prey choice differs between species by including the interaction term with prey type. The precision parameter (Φ) indicates the goodness of fit of the model: the larger it is, the smaller is the variance of the response variable.

Stable isotope samples

We caught and processed marbled crayfish (n = 14) and spiny-cheek crayfish (n = 10) in lake Moosweiher, spiny-cheek crayfish (n = 20) in lake Silbersee, and marbled crayfish (n = 24) and spiny-cheek crayfish (n = 16) in lake Krumme Lanke for stable isotope analysis. We prepared the crayfish muscle tissue of the abdomen. For fish samples, we used muscle tissue from the lateral area in front of the dorsal fin; and for benthic invertebrates, we used whole organisms which were pooled to achieve 2 – 6 samples for each group, depending on the availability of the species in our samples; macrophytes and decaying leaves were washed with distilled water and pooled to 3 – 5 samples. All samples were dried for 24 h at 60 °C in aluminum trays. The samples were homogenized with mortar and pestle, and then weighed at 1 mg for each sample of animal tissue, 4 mg for macrophytes, and 5 mg for detritus. Samples were wrapped in tin cups and analyzed for carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) and elemental content (%C and %N) by the University of California, Davis Stable Isotope Facility, USA, using a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The resulting delta values refer to Vienna PeeDee Belemnite carbon and air nitrogen as calibration standards. Long-term standard deviations provided by UC Davis are 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. We report stable isotope values in standard delta notation: $\delta^{H}X = [(R_{sample} / R_{standard}) - 1] \times 10^3$, where X is the element, H is the relative isotopic mass of the heavier isotope, and R is the ratio of the heavy-to-light isotope in the sample and standard. We then lipid-corrected $\delta^{13}C$-values of macrophytes, allochthonous detritus and invertebrates mathematically according to Post et al. (2007).

We included additional stable isotope data from a newly reported marbled crayfish population in the gravel pit lake Meitzer See, Lower Saxony, Germany. The sample of lake Meitzer See included marbled crayfish (n = 5) and spiny-cheek crayfish (n = 2). Samples processing followed similar procedures as described above (please see Trudeau 2018 for details). In addition, we included data of a reference gravel pit lake inhabiting only spiny-cheek crayfish (n = 7; lake Steinwedeler Teich). The samples from these two lakes were collected in September and October of 2016.

Isotopic niches and mixing models

Trophic position of populations

We used Bayesian models from package *tRophicPosition* in R (version 3.5.1.) to calculate the median and mode of TPs for each crayfish population with stable isotope data ($\delta^{13}C$ and $\delta^{15}N$). The model applies Markov Chain Monte Carlo Simulations for stable isotope data to estimate TP (see Quezada-Romegialli et al. 2018). We used the full two baselines model that incorporates the factor α proposed by Post (2002), accounting for differences between littoral and pelagic food webs within lakes (Vadeboncoeur et al. 2002). We also calculated comparisons of the posterior samples of TPs and α (from Post 2002) between lakes (Table D.1).

We used littoral (snails: *Bithynia tentaculata*, *Lymnea* sp.) and pelagic (mussels: *Dreissena* sp., *Corbicula fluminea*, *Unio* sp.) primary consumers as baseline organisms for the Bayesian models. Filter-feeding mussels and herbivorous snails are longer-lived primary consumers that are often recommended to use as baseline organisms, as they integrate the highly variable isotopic signals from primary producers from pelagic and littoral food webs over longer time scales (Post 2002). The estimated TP of the baseline organisms was set to $\lambda = 2$.

Calculations of TPs are known to be sensitive to trophic discrimination factors (TDFs) which can in turn vary between species and food types (Caut et al. 2009), thus the choice of TDF is critical. However, estimating TPs of omnivores like crayfish cannot be based upon TDF derived from experiments with single food items. Omnivores like crayfish pose a problem, thus we here decided to use the TDF proposed by Post (2002) that are commonly used in stable isotope ecology ($\Delta^{15}N = 3.4 \pm 0.98$ ‰, $\Delta^{13}C = 0.39 \pm 1.3$ ‰). In addition, we calculated TPs with TDF from McCutchan et al. (2003) and non-Bayesian TPs (Post 2002) to check for sensitivity (Table D.2).

The crayfish sampled for stable isotope analyses were tested for size differences between populations (lakes) using Kolmogorov-Smirnov tests. The null hypothesis was that the samples were drawn from the same distribution. We excluded the spiny-cheek crayfish sample from lake Meitzer See due to small sample size (*n* = 2). We then used the implemented pairwise comparison of posterior TP estimates from the Bayesian model of the *tRophicPosition* package in R (Quezada-Romegialli et al. 2018).

Trophic niche breadth

All stable isotope values were baseline-corrected because primary resources can be highly variable between sites (Vander Zanden and Rasmussen 1999). The nitrogen isotopes were expressed as TP according to Minagawa and Wada (1984). Since the Bayesian framework from package *tRophicPosition* has no individual model implemented, we calculated TP for each individual separately (i) following Post (2002) as:

$$\mathrm{TP_i} = \lambda + (\delta^{15}\mathrm{N_i} - [\,\delta^{15}\mathrm{N_{littoral}} \times \alpha + \delta^{15}\mathrm{N_{pelagic}} \times (1-\alpha)])/\Delta^{15}\mathrm{N} \qquad \text{Eq. 1}$$

Here, $\delta^{15}N_i$ is the measured $\delta^{15}N$-value of the individual crayfish for which TP should be calculated. $\delta^{15}N_{littoral}$ and $\delta^{15}N_{pelagic}$ are the measured $\delta^{15}N$-values of the baseline organisms chosen to represent littoral and pelagic food webs of each lake as described above. The λ represents the estimated TP of baseline primary consumers ($\lambda = 2$). The TDF for the calculations was set to $\Delta^{15}N = 3.4‰$. The contribution of littoral food webs (α) to the consumer signature was estimated using carbon isotopes ($\delta^{13}C$; see Post 2002).

Further, we corrected $\delta^{13}C$ values of the crayfish for differences in basal resources following Olsson et al. (2009):

$$\delta^{13}\mathrm{Ccorr_i} = \frac{\delta^{13}\mathrm{C_i} - \delta^{13}\mathrm{C_{meaninv}}}{\delta^{13}\mathrm{C_{maxinv}} - \delta^{13}\mathrm{C_{mininv}}} \qquad \text{Eq. 2}$$

The corrected carbon isotope value ($\delta^{13}Ccorr_i$) for each consumer *i* was calculated from the individual carbon isotopic ratio ($\delta^{13}C_i$), the mean carbon isotopic ratio of all sampled invertebrates ($\delta^{13}C_{meaninv}$), which represent potential prey items, and the carbon range of the invertebrates CR_{inv} used to calculate the baseline of TPs (i.e. the primary consumers).

We then calculated sample-size corrected standard ellipse areas (SEAc) in the isotopic space of individual TPs and $\delta^{13}Ccorr_i$ with the package *SIBER* in R for each population. We compared trophic niches of marbled crayfish and spiny-cheek crayfish within and among lakes in the biplots and calculated SEAc overlap for the crayfish populations in sympatry (lakes Moosweiher and Krumme Lanke) and the allopatric spiny-cheek crayfish reference populations of lake Silbersee with spiny-cheek crayfish from lake Moosweiher (Jackson et al. 2011). Also, we looked at individual TP in relation to CL of individual crayfish with linear regression models.

Stable isotope mixing models

Diet of crayfish was estimated using Bayesian inference. Bayesian mixing models are commonly used to estimate the proportion of different food resources in consumers' diets based on stable isotope analysis (Phillips et al. 2014). Models were run for both marbled and spiny-cheek crayfish in the R package 'Mixing Models for Stable Isotope Analysis in R' (*MixSIAR*; Parnell et al. 2013, Stock et al. 2018). The potential prey was grouped into six different food categories (sources) based on sampled communities in each lake: (1) detritus (decomposing leaves, $n = 5$); (2) macrophytes ($n = 3 – 5$); (3) benthic invertebrates including gammarids, Heptageniidae, Chironomids, Odonata and *Asellus aquaticus* ($n = 3 – 26$); (4) mussels including *Dreissena* sp., Asian clam *Corbicula fluminea* and *Unio* sp. ($n = 4 – 9$); (5) gastropods including *Bithynia tentaculata*, *Lymnaea* sp. and *Planorbarius corneus* ($n = 2 – 7$); and (6) fish (*Perca fluviatilis*, as the species was dominant in the different lakes). Since no TDF measurements on marbled crayfish exist, we incorporated the TDFs from laboratory-controlled experimental measurements of *O. rusticus* published in Glon et al. (2016) into our mixing models and allocated them to comparable prey types. For plant-based food items (i.e. macrophytes and detritus) we used $\Delta^{15}N = 3.35‰ \pm 2.77$ and $\Delta^{13}C = 1.57‰ \pm 2.08$, and for animal food items (i.e. fish and all invertebrates) we used $\Delta^{15}N = 1.20‰ \pm 2.50$ and $\Delta^{13}C = 0.80‰ \pm 1.05$.

Initially, for all MixSIAR models, the Markov Chain Monte Carlo (MCMC) parameters were set as follow: chain length = 1 000 000, burn = 500 000, thin = 500, chains = 3. These parameters correspond to the "very long" run provided by the run_model function of the *MixSIAR* package. Convergence of each model was evaluated by using the Gelman-Rubin and Geweke diagnostic tests (see package R '*MixSIAR*'). Since diagnostic tests were not satisfying for marbled crayfish models, we used the "extreme" run (chain length = 3 000 000, burn = 1 500 000, thin = 500, chains = 3). MixSIAR offers a statistical framework in which knowledge on trophic ecology of consumers can be included via informative prior distributions (Stock et al. 2018). We used average standardized and transformed proportions from the prey-choice experiments with informative priors as follow: 0.01, 0.358, 1, 0.853, 0.583 and 0.883 for detritus, macrophytes, fish, arthropods, snails and mussels, respectively. Informative priors were used *a posteriori* for combining food items and explore diet of both crayfish species (Stock et al. 2018). The lake identity was added as a random factor in the models. Proportions of food resources were additively combined to obtain the total plant-based tissue proportions (macrophytes and detritus), invertebrate proportions (arthropods, mussels and snails) and fish proportions. The proportions of resources assimilated by crayfish reported are the median of the Bayesian simulations.

Results

Prey choice

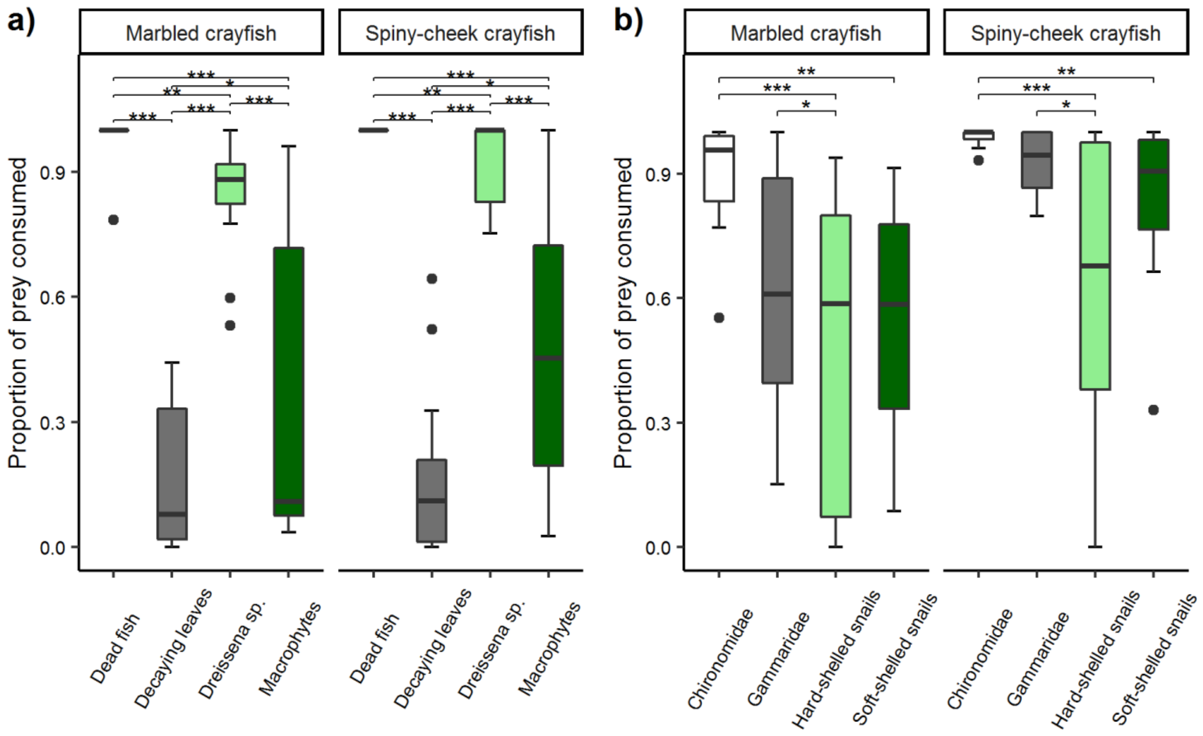

Figure 4.1 – Non-mobile (a) and mobile (b) prey consumed by marbled and spiny-cheek crayfish in the prey-choice experiments. Boxplots show medians with lower and upper quartiles, whiskers indicate the largest and smallest value still within the 1.5 interquartile range, outliers are denoted as black dots. Significant differences between prey types are denoted by asterisks.

There was no difference in the proportion of consumed prey between spiny-cheek crayfish and marbled crayfish for most prey items besides *Dreissena* mussels (non-mobile) and soft-shelled snails (mobile). Of the non-mobile prey items, dead fish were significantly preferred over the other prey items followed by *Dreissena* mussels, macrophytes and decaying leaves (N = 124; $\Phi = 2.22 \pm 0.29$, $p < 0.001$; Figure 4.1). We found higher proportions of consumed *Dreissena* mussels (non-mobile) and soft-shelled snails (mobile) for spiny-cheek crayfish. The most preferred mobile prey items for both species were chironomids and gammarids, whereas both soft- and hard-shelled snails were consumed less (N = 96; $\Phi = 1.32 \pm 0.18$, $p < 0.001$). We did not find species-specific prey preferences.

Trophic positions and niche space

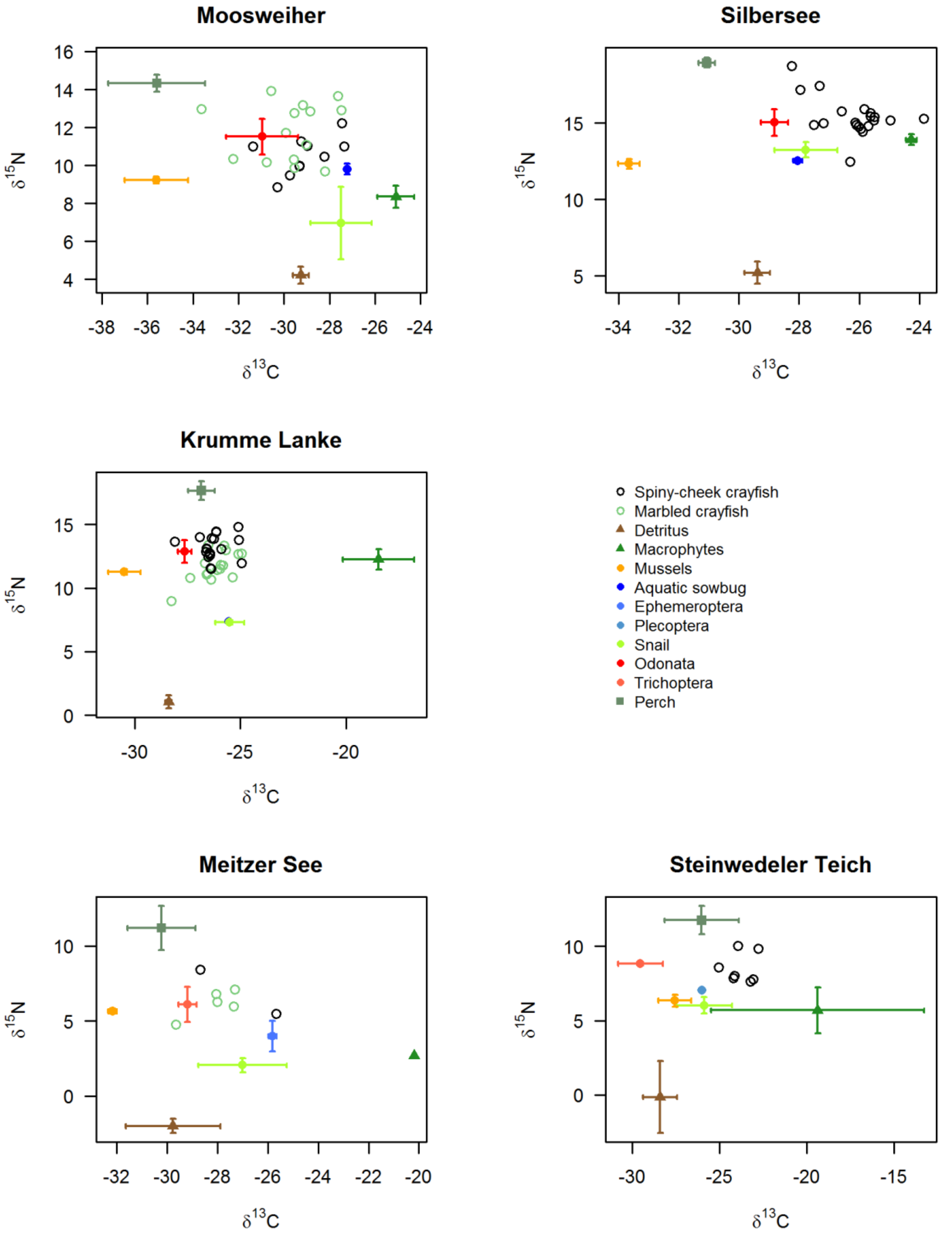

Figure 4.2 – Isotopic biplots ($\delta^{13}C$ and $\delta^{15}N$) of individual crayfish (open circles) in the studied lakes. Potential prey organisms are depicted as means of stable isotope values (± SD). Benthic invertebrates are represented as circles, macrophytes and detritus as triangles, and fish (perch) as squares.

Both crayfish species took up central positions in lake food webs (Figure 4.2). TPs for marbled crayfish ranged from 2.91 in lakes Krumme Lanke and Meitzer to 3.20 in lake Moosweiher (Figure 4.3, Table 4.2), and TPs of spiny-cheek crayfish ranged from 2.66 in lake Silbersee and 3.33 in lake Krumme Lanke. All crayfish populations relied on littoral resources with α values ranging from 0.50 to 0.96 (Table 4.2). Comparisons of the posterior samples of TP and α between lakes are given in Table D.1. The core niche of marbled crayfish and spiny-cheek crayfish overlapped by 16.9% in lake Moosweiher and 10.5% in lake Krumme Lanke.

Table 4.2 – Summary of niche statistic (sample-size corrected Standard Ellipse Area (SEAc)), posterior TP estimates and posterior α estimates (median and [95% credibility interval]) for marbled crayfish (M) and spiny-cheek crayfish (S) populations.

Lake	**SEAc**		**Trophic position**		**Littoral reliance (α)**	
	M	S	M	S	M	S
Moosweiher	0.24	0.10	3.20 [2.61, 3.81]	2.87 [2.30, 3.51]	0.68 [0.50, 0.88]	0.76 [0.59, 0.94]
Silbersee	-	0.13	-	2.66 [2.42, 2.93]	-	0.96 [0.82, 1.00]
Krumme Lanke	0.11	0.12	2.91 [2.59, 3.25]	3.33 [3.04, 3.65]	0.79 [0.61, 0.96]	0.75 [0.58, 0.93]
Meitzer See	0.09	N/A	2.91 [2.11, 6.61]	3.88 [2.12, 9.23]	0.59 [0.12, 0.96]	0.50 [0.04, 0.97]
Steinwedeler T.	-	0.18	-	2.72 [2.22, 4.10]	-	0.71 [0.11, 0.99]

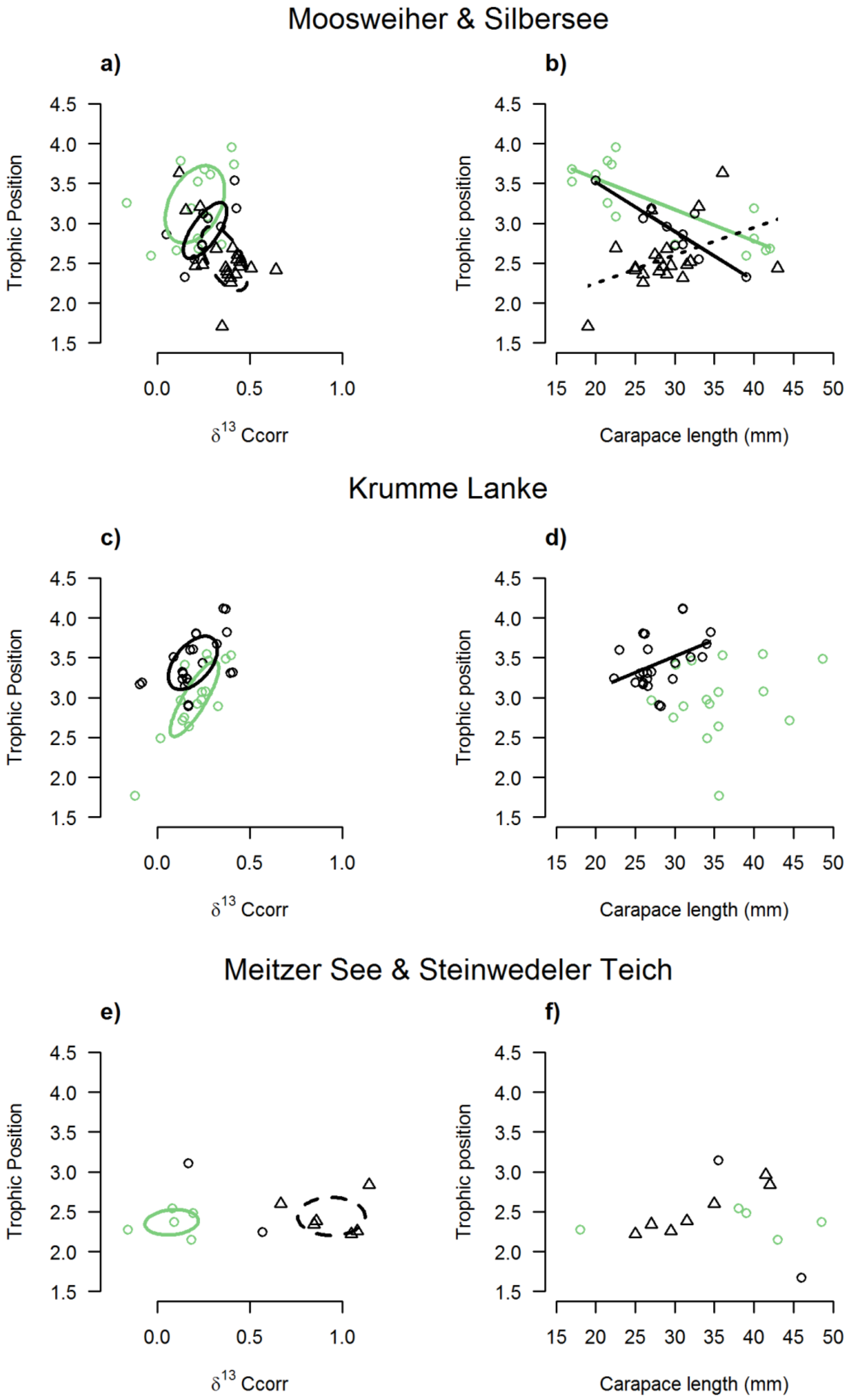

Figure 4.3 – The left column depicts the trophic niche of marbled crayfish and spiny-cheek crayfish. Marbled crayfish are displayed as green circles, while spiny-cheek crayfish co-occurring with marbled crayfish in lake Moosweiher (a), lake Krumme Lanke (c) and lake Meitzer See (e) are displayed as black circles. The allopatric spiny-cheek crayfish populations in the reference water bodies lake Silbersee (a), lake Steinwedeler Teich (e) are shown as black triangles. Trophic niche breadth is represented as Standard Ellipses Areas (SEAc). The right column (b, d, and f) shows the relationship of trophic position and carapace length (in mm) of individual marbled and spiny-cheek crayfish of the corresponding lakes.

Except marbled crayfish from lake Moosweiher, which were significantly smaller than other populations (Kolmogorov-Smirnov test, $D = 0.571$, $p < 0.05$), crayfish did not significantly differ from each other in their size. There was a significantly negative relationship between size and TP in marbled crayfish ($R^2 = 0.63$, $p < 0.001$; Figure 4.3) and spiny-cheek crayfish ($R^2 = 0.76$, $p < 0.001$) from lake Moosweiher. In this lake, very small marbled crayfish (< 23 mm) had distinctively high TPs (Figure D.1). In lake Silbersee, spiny-cheek crayfish exhibited a positive trend for the size-TP relationship ($R^2 = 0.15$, $p = 0.053$). In lake Krumme Lanke we did not find a relationship of crayfish size with TP in marbled crayfish ($R^2 = –0.06$, $p = 0.874$), but size and TP were positively correlated for spiny-cheek crayfish ($R^2 = 0.13$, $p < 0.05$). There was no significant relationship for the marbled crayfish from lake Meitzer See ($R^2 = –0.31$, $p = 0.845$), and a weak positive trend for spiny-cheek crayfish from Steinwedeler Teich ($R^2 = 0.30$, $p = 0.074$).

Diet estimates

The estimated contribution of different food items to spiny-cheek and marbled crayfish diets highlight that both species are generalist consumers (Table 4.3). In general – without considering the variability between lakes – combined proportion of invertebrates was a 0.62 (95% CI: 0.27–0.88), combined proportion of plants-based tissues was 0.21 (95% CI: 0.04 – 0.29) and proportion of dead fish was 0.16 (95% CI: 0.01 – 0.24) in the diet of marbled crayfish. For spiny-cheek crayfish, invertebrates represented 0.54 (95% CI: 0.26 – 0.77) of the diet, plants 0.31 (95% CI: 0.11 – 0.54) and dead fish 0.14 (95% CI: 0.02 – 0.20). Exploring lake by lake diet composition of combined food resources, we found consistency within and between species.

The diet differences between lakes showed that spiny-cheek crayfish ingested more macrophyte tissues than marbled crayfish while the proportion of allochtonous detritus was higher for marbled crayfish. Estimated proportions of snails and mussels show that both crayfish species rely similarly on these food resources (Table 4.3). However, the prey type most consumed by marbled crayfish was arthropods. This is particularly true in lakes Krumme Lanke and Moosweiher, while the spiny-crayfish in these lakes mostly consumed mussels.

Table 4.3 – Bayesian mixing model estimated proportions (median, 95% CI) of arthropods, mussels, detritus, fish, macrophytes and snails in diets of spiny-cheek crayfish and marbled crayfish in the five sampled lakes.

Lake	Food item	Spiny-cheek crayfish	Marbled crayfish
Global	arthropods	0.139 (0.009 – 0.438)	0.207 (0.015 – 0.547)
	mussels	0.181 (0.032 – 0.422)	0.171 (0.023 – 0.427)
	detritus	0.088 (0.005 – 0.306)	0.126 (0.008 – 0.366)
	fish	0.141 (0.023 – 0.362)	0.166 (0.013 – 0.396)
	macrophytes	0.194 (0.054 – 0.402)	0.060 (0.003 – 0.264)
	snails	0.163 (0.010 – 0.458)	0.177 (0.013 – 0.451)
Moosweiher	arthropods	0.119 (0.005 – 0.503)	0.329 (0.008 – 0.879)
	mussels	0.171 (0.007 – 0.406)	0.099 (0.002 – 0.326)
	detritus	0.124 (0.002 – 0.468)	0.089 (0.001 – 0.337)
	fish	0.097 (0.005 – 0.281)	0.144 (0.001 – 0.415)
	macrophytes	0.172 (0.009 – 0.407)	0.049 (0.001 – 0.317)
	snails	0.189 (0.005 – 0.529)	0.141 (0.002 – 0.463)
Silbersee	arthropods	0.102 (0.003 – 0.578)	–
	mussels	0.086 (0.003 – 0.253)	–
	detritus	0.064 (0.062 – 0.218)	–
	fish	0.058 (0.002 – 0.175)	–
	macrophytes	0.389 (0.166 – 0.552)	–
	snails	0.155 (0.004 – 0.674)	–
Krumme Lanke	arthropods	0.096 (0.004 – 0.389)	0.183 (0.006 – 0.548)
	mussels	0.335 (0.036 – 0.595)	0.218 (0.015 – 0.463)
	detritus	0.062 (0.002 – 0.273)	0.131 (0.003 – 0.364)
	fish	0.234 (0.023 – 0.500)	0.161 (0.006 – 0.386)
	macrophytes	0.098 (0.008 – 0.228)	0.045 (0.001 – 0.153)
	snails	0.101 (0.004 – 0.331)	0.193 (0.005 – 0.475)
Meitzer See	arthropods	0.152 (0.005 – 0.373)	0.168 (0.005 – 0.550)
	mussels	0.154 (0.004 – 0.286)	0.206 (0.011 – 0.572)
	detritus	0.056 (0.001 – 0.124)	0.122 (0.002 – 0.372)
	fish	0.149 (0.003 – 0.263)	0.164 (0.003 – 0.376)
	macrophytes	0.144 (0.006 – 0.222)	0.056 (0.001 – 0.207)
	snails	0.107 (0.002 – 0.216)	0.187 (0.004 – 0.482)
Steinwedeler Teich	arthropods	0.122 (0.004 – 0.522)	–
	mussels	0.176 (0.006 – 0.555)	–
	detritus	0.064 (0.001 – 0.272)	–
	fish	0.136 (0.007 – 0.345)	–
	macrophytes	0.175 (0.033 – 0.347)	–
	snails	0.184 (0.005 – 0.659)	–

Discussion

We reported rarely observed comparisons of sympatric populations of invasive spiny-cheek crayfish in natural systems after an over-invasion by marbled crayfish, allopatric populations of the over-invaded spiny-cheek crayfish and compared both with laboratory feeding experiments. We found that both species did not differ in their prey choice under controlled conditions where they preferentially fed on animal prey. The stable isotope analyses showed that both species were highly plastic in their trophic niche, occupied different positions in the isotopic niche space and had a very generalist diet.

Prey selection in controlled environment

Non-mobile prey trials in the laboratory showed that both species prefer animal tissues, which is consistent with previous experimental studies on other species where crayfish preferred protein-rich food (Correia 2003, Chucholl 2012, Haddaway et al. 2012). In the mobile prey trials, both species chose chironomids and gammarids, i.e. invertebrates with little defense against crayfish predators. As native populations of marbled crayfish do not exist, the best indicators of its potential trophic niche came from its progenitor species: the slough crayfish. This species can thrive under a wide range of biotic and abiotic conditions (Hendrix and Loftus 2000, Dorn and Volin 2009). In contrast to our assumptions based on published data from free-living slough crayfish, marbled crayfish and spiny-cheek crayfish showed low interest in plant tissues. The prey choice in the laboratory reflects simple optimal foraging scenarios where easily accessible, energy-rich food is preferred (Charnov 1976). Under natural conditions, however, variations in both resource availability and predation risk additionally shape the diet of consumers (Anholt and Werner 1995), and thus the patterns observed in the laboratory might not hold true in the field.

Sympatric crayfish act as key species in food webs but differ in diets

In the invaded lakes, we found that individual crayfish occupied a large range of trophic positions from 1.7 to 4.1, meaning that they act as primary consumers or detritivores up to predators. This wide span of trophic functions puts both crayfish species in a key position for energy transfer between trophic levels (Alp et al. 2016, Kreps et al. 2016). The population niche breadth we observed was mostly consistent between the lakes and between species except for lake Mooweiher. In contrast to the signal crayfish studied by Larson et al. (2017), TPs of sympatric populations were not generally higher for one species, but niche partitioning among species differed between lakes. Marbled crayfish in lake Krumme Lanke had lower TPs than sympatric spiny-cheek crayfish, whereas in lake Moosweiher marbled crayfish seemed to occupy higher TPs. High trophic variability among populations is commonly observed in crayfish (Ercoli et al. 2014) and some sympatric species pairs even exhibit trophic differences (Larson et al. 2017). The core niches of sympatric marbled and spiny-cheek crayfish, however, only marginally overlapped. Similar results were found by Jackson et al. (2014) for four other crayfish invaders that all separated within isotopic niche space. Overall, we consider our findings for lakes Moosweiher and Krumme Lanke robust, but the sample size for lake Meitzer See was close to the minimum to calculate niche ellipses (Jackson et al. 2011). As both species exhibited niche partitioning and high trophic plasticity, the impact of marbled crayfish invasions depends on the differences in resource use.

Our mixing models showed that spiny-cheek crayfish consumed higher proportions of macrophytes, and marbled crayfish fed more on allochthonous detritus. Even small differences in resource utilization or predation yield important implications on the community and ecosystem functioning (Jackson et al. 2014). On the one hand, macrophytes and macrophyte-dependent organisms might be more affected by spiny-cheek crayfish. Invasive crayfish have been shown to severely reduce macrophyte stands and inhibit their growth (van der Wal et al. 2013, Baldridge and Lodge 2014). On the other hand, marbled crayfish might modify ecosystem functioning by potentially accelerating litter decomposition. Such effects have been found in highly invasive red swamp crayfish and signal crayfish, which both increased litter decomposition rates, compared to native species or spiny-cheek crayfish (Dunoyer et al. 2014, Alp et al. 2016). The density of crayfish or prey organisms in the study lakes also affects these processes (Reynolds et al. 2013). Momot (1995) suggested that high crayfish densities force crayfish to utilize less favorable, but abundant resources like detritus or macrophytes. Accordingly, niche breadth and TPs are negatively correlated to crayfish abundance (Kreps et al. 2016, Jackson et al. 2017).

Carnivory in marbled crayfish

Mesocosm experiments and stable isotope studies showed that slough crayfish can have a large impact on gastropods, but generally are associated with feeding on leaf litter and macrophytes, being detritivores or herbivores (Sargeant et al. 2010, VanArman 2011). In contrast to its progenitor, our mixing models and prey-choice trials indicated that marbled crayfish rely less on macrophytes and detritus, but more on animal prey. However, scavenging on dead fish was of minor importance in the field compared to prey-choice trials, probably because fish carcasses are relatively rare there. But both crayfish studied here were comparatively predatory with up to 67% of invertebrates in their diet. For comparison, northern clearwater crayfish (*Orconectes propinquus*) and rusty crayfish (*Orconectes rusticus*) consumed about 42% of animal material, from which fish comprised approximately 12% (Taylor and Soucek 2010).

Invertebrate prey was generally preferred in the populations we studied. High degrees of invertivory can be problematic when taxa of conservation concern like dragonflies are negatively affected; but also less conspicuous taxa like leeches, mayflies and caddisflies can be suppressed by invasive crayfish (Mathers et al. 2016). Both species similarly consumed snails and mussels, but marbled crayfish had a stronger preference for arthropods like e.g. dragonflies and waterlice. In conjunction with the elevated grazing of spiny-cheek crayfish on macrophytes, the associated invertebrates could be put under additional pressure when marbled crayfish invade. Especially, native shredders might be affected by marbled crayfish as they prey on them and compete for detritus (Jackson et al. 2014). This impact has, for example, been found in rusty crayfish which reduce shredder biomass and detritus availability (Bobeldyk and Lamberti 2008).

A recent stable-isotope study on a population of naturalized marbled crayfish in Slovakia revealed that algae and detritus provide their main food resources, similar to the aforementioned results on slough crayfish (Lipták et al. 2019). While our results are consistent with Lipták et al. (2019) regarding the low importance of macrophytes and generally a wide range of ingested resources, the populations we studied were much more carnivorous. Such high trophic plasticity is often found in successful invaders and especially crayfish.

High trophic plasticity: role of ontogeny and environment

Both species populations displayed high trophic plasticity either in their trophic position, littoral reliance, niche breadth or diet composition. The largest niche breadth was quantified for the marbled crayfish population living in Moosweiher, the population with the highest proportion of arthropods consumed by marbled crayfish. In this lake, very small individuals were sampled and showed higher trophic positions than the other size classes. Ontogenetic dietary shifts are well known in many crayfish species where smaller crayfish usually prey upon aquatic invertebrates while adults feed on plant tissues (Parkyn et al. 2001, Correia 2003, Stenroth et al. 2006, Taylor and Soucek 2010). These ontogenetic patterns of size and TP can, however, be reversed (e.g. Larson et al. 2017) and are linked to lake productivity (Jackson et al. 2017).

Generally, trophic niches of crayfish are shaped by lake size, trophic state (Post et al. 2000) and the existing habitats (Ruokonen et al. 2014). The gravel pit lakes Meitzer See and Steinwedeler See were more oligotrophic and larger in size than the other sampled lakes, which might explain why crayfish TPs were lower in those systems. The niche differences could also be related to habitat segregation. We found some differences in reliance on littoral food sources in lake Moosweiher (and lake Meitzer See), where α differed by about 9% between species; in lake Krumme Lanke, however, the difference was only 4%. In addition, higher proportions of typical littoral food items like snails and macrophytes were estimated by the mixing model for spiny-cheek crayfish of lakes Moosweiher and Meitzer See. The crayfish species could feed at different depths and access more profundal sources, as isotopic signals of crayfish differ along depth gradients (Ruokonen et al. 2012). Chucholl and Pfeiffer (2010) found that marbled crayfish in lake Moosweiher, for example, preferred shallow areas composed of mainly detritus, mud and wood; and spiny-cheek crayfish preferred more stony substrates. We cannot quantify the distribution of crayfish in lake but can qualitatively confirm the findings from Chucholl and Pfeiffer (2010) based on our samplings at lakes Moosweiher and Krumme Lanke.

High trophic plasticity enables crayfish to modify their trophic niche when a functionally similar species is present (Jackson et al. 2016, Larson et al. 2017). Allopatric and sympatric spiny-cheek crayfish populations had a similar niche breadth, which fits the observations made by Jackson et al. (2014) on other co-occurring crayfish invaders. Yet, niche shifts do not necessarily follow over-invasion. Pacific rats (*Rattus exulans*), for example, retained their trophic position also in the presence of numerically and morphologically superior black rats (*Rattus rattus*; Russell et al. 2015). Data on additional allopatric and sympatric populations could support these observations and better quantify the impact on spiny-cheek trophic niches.

Trophic plasticity of crayfish is further driven by predation pressure (Hill and Lodge 1994), resource availability (Roth et al. 2006) and the availability of shelters, a key resource for crayfish (Martin and Moore 2007). The latter might play a particularly important role in over-invasions of crayfish (Hudina et al. 2011, James et al. 2016). Also, marbled crayfish have been shown to be more aggressive than spiny-cheek crayfish (Linzmaier et al. 2018, Hossain et al. 2019) and might have caused the observed niche differences through interference competition.

Conclusions

Marbled crayfish invasions are symptomatic for a new wave of ornamental crayfish that compete with established invaders in Europe (Kouba et al. 2014, Chucholl and Wendler 2017). The literature on crayfish ecology suggests that most species are functionally equivalent, which is supported by our experimental results on prey choice. Furthermore, stable isotope data suggest that spiny-cheek crayfish and marbled crayfish both represent keystone species in the food webs because of their trophic position and trophic plasticity. However, crayfish have been shown to partition niches when co-occurring with other species (Jackson et al. 2014, Larson et al. 2017). Our study supports these findings for marbled crayfish over-invading lakes inhabited by spiny-cheek crayfish, where they seem to partition their diet to some extent.

Niche partitioning probably explains their co-existence as long as food or shelter are not limiting in the invaded systems. So far, marbled crayfish are known to coexist in Europe with other invasive species (spiny-cheek crayfish and red swamp crayfish (Chucholl et al. 2012)) and in Madagascar with native crayfish species (Andriantsoa et al. 2019). Our results suggest that the addition of marbled crayfish brings new functions in ecosystems already invaded by spiny-cheek crayfish. However, the latter seemingly adapt to this new competitor. To our knowledge, marbled crayfish were recorded in the studied systems of lakes Moosweiher and Krumme Lanke in 2003 and 2009, respectively, and have yet maintained healthy stocks of both species.

The complex feeding ecology of crayfish can have varying and unexpected ecosystem consequences during invasions, leading to trophic cascades (Jackson et al. 2014). Marbled crayfish invasions can lead to increased consumption of allochthonous detritus and a wide range of invertebrates which entail several direct and indirect consequences for ecosystem functions. Our study provides information to assess important aspects of marbled crayfish and crayfish isotope ecology, which is needed in the face of ongoing management plans like the European Union’s “List of Invasive Alien Species of Union Concern”.

Acknowledgements

We thank Michael Pfeiffer and Christoph Chucholl for sharing their knowledge on crayfish and helping us in the field. We further thank Larissa Goebel for taking care of the crayfish and assisting us during the study. Finally, we thank Sofia Schütt, Robert Nikolaus, Alban Sagouis and Jorrit Lucas for their help during sampling and Ashley Trudeau for her support in the lab. Furthermore, we would like to thank Robert Arlinghaus for providing additional data. SL and JJ were financially supported by IGB and the Deutsche Forschungsgemeinschaft (DFG; JE 288/9-1, JE 288/9-2). CM was funded by the German Federal Ministry of Education and Research BMBF within the Collaborative Project ‘Bridging in Biodiversity Science - BIBS’ (funding number 01LC1501A-H). SM was jointly financed by the German Federal Ministry of Education and Research (BMBF) and the German Federal Agency for Nature Conservation (BfN) with funds granted by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU; grant number: 01LC1320A; www.baggersee-forschung.de).

Literature cited

Alp, M., J. Cucherousset, M. Buoro, and A. Lecerf. 2016. Phenological response of a key ecosystem function to biological invasion. Ecology Letters **19**:519–527.

Andriantsoa, R., S. Tönges, J. Panteleit, K. Theissinger, V. C. Carneiro, J. Rasamy, and F. Lyko. 2019. Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. BMC Ecology **19**:8.

Anholt, B. R., and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology **76**:2230–2234.

Baldridge, A. K., and D. M. Lodge. 2014. Long-term studies of crayfish-invaded lakes reveal limited potential for macrophyte recovery from the seed bank. Freshwater Science **33**:788–797.

Bobeldyk, A. M., and G. A. Lamberti. 2008. A decade after invasion: evaluating the continuing effects of rusty crayfish on a Michigan river. Journal of Great Lakes Research **34**:265–275.

Bubb, D. H., O. J. O'Malley, A. C. Gooderham, and M. C. Lucas. 2009. Relative impacts of native and non-native crayfish on shelter use by an indigenous benthic fish. Aquatic Conservation: Marine and Freshwater Ecosystems **19**:448–455.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology **46**:443–453.

Charnov, E. L. 1976. Optimal foraging, the marginal value theorem. Theoretical Population Biology **9**:129–136.

Chucholl, C. 2012. Understanding invasion success: life-history traits and feeding habits of the alien crayfish *Orconectes immunis* (Decapoda, Astacida, Cambaridae). Knowledge and Management of Aquatic Ecosystems **404**:04.

Chucholl, C. 2013a. Feeding ecology and ecological impact of an alien ‘warm-water’omnivore in cold lakes. Limnologica **43**:219–229.

Chucholl, C. 2013b. Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. Biological Invasions **15**:125–141.

Chucholl, C. 2015. Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion pathway. Pages 83–114 *in* T. Kawai, Z. Faulkes, and G. Scholtz, editors. Freshwater crayfish: a global overview. CRC Press, Boca Raton, USA.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Chucholl, C., and M. Pfeiffer. 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., and F. Wendler. 2017. Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. Biological Invasions **19**:197–208.

Correia, A. M. 2003. Food choice by the introduced crayfish *Procambarus clarkii*. Annales Zoologici Fennici **40**:517–528.

Cribari-Neto, F., and A. Zeileis. 2010. Beta Regression in R. Journal of Statistical Software **34**:24.

Doherty-Bone, T. M., A. M. Dunn, C. Liddell, and L. E. Brown. 2018. Transformation of detritus by a European native and two invasive alien freshwater decapods. Biological Invasions **20**:1799–1808.

Dorn, N. J., and J. C. Volin. 2009. Resistance of crayfish (*Procambarus* spp.) populations to wetland drying depends on species and substrate. Journal of the North American Benthological Society **28**:766–777.

Dunoyer, L., L. Dijoux, L. Bollache, and C. Lagrue. 2014. Effects of crayfish on leaf litter breakdown and shredder prey: are native and introduced species functionally redundant? Biological Invasions **16**:1545–1555.

Ercoli, F., T. J. Ruokonen, H. Hämäläinen, and R. I. Jones. 2014. Does the introduced signal crayfish occupy an equivalent trophic niche to the lost native noble crayfish in boreal lakes? Biological Invasions **16**:2025–2036.

Fischer, D., P. Pavluvčík, F. Sedláček, and M. Šálek. 2009. Predation of the alien American mink, *Mustela vison* on native crayfish in middle-sized streams in central and western Bohemia. Folia Zoologica **58**:45–56.

Gherardi, F. 2007. Understanding the impact of invasive crayfish. Pages 507–542 *in* F. Gherardi, editor. Biological invaders in inland waters: Profiles, distribution, and threats. Springer, Dordrecht, The Netherlands.

Gherardi, F., and P. Acquistapace. 2007. Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. Freshwater Biology **52**:1249–1259.

Glon, M., E. Larson, and K. Pangle. 2016. Comparison of ^{13}C and ^{15}N discrimination factors and turnover rates between congeneric crayfish *Orconectes rusticus* and *O. virilis* (Decapoda, Cambaridae). Hydrobiologia **768**:51–61.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, and F. Lyko. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nature Ecology & Evolution **2**:567–573.

Haddaway, N. R., R. H. Wilcox, R. E. Heptonstall, H. M. Griffiths, R. J. Mortimer, M. Christmas, and A. M. Dunn. 2012. Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. Plos One **7**:e32229.

Haertel Borer, S. S., D. Zak, R. Eckmann, U. Baade, and F. Hölker. 2005. Population density of the crayfish, *Orconectes limosus*, in relation to fish and macroinvertebrate densities in a small mesotrophic lake–implications for the lake's food web. International Review of Hydrobiology **90**:523–533.

Hendrix, A. N., and W. F. Loftus. 2000. Distribution and relative abundance of the crayfishes *Procambarus alleni* (Faxon) and *P. fallax* (Hagen) in southern Florida. Wetlands **20**:194–199.

Hill, A. M., and D. M. Lodge. 1994. Diel changes in resource demand: competition and predation in species replacement among crayfishes. Ecology **75**:2118–2126.

Holdich, D. M. 2002. Crayfish in Europe–an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. Pages 15–34 *in* Management & Conservation of Crayfish. Environment Agency, Bristol/, Nottingham, UK.

Hossain, M. S., J. Kubec, A. Kouba, P. Kozák, and M. Buřič. 2019. Still waters run deep: marbled crayfish dominates over red swamp crayfish in agonistic interactions. Aquatic Ecology **53**:97–107.

Hubbell, S. P. 2005. Neutral theory in community ecology and the hypothesis of functional equivalence. Functional Ecology **19**:166–172.

Hudina, S., N. Galić, I. Roessink, and K. Hock. 2011. Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. Biological Invasions **13**:1791–1803.

Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology **80**:595–602.

Jackson, M. C., C. Evangelista, T. Zhao, A. Lecerf, J. R. Britton, and J. Cucherousset. 2017. Between-lake variation in the trophic ecology of an invasive crayfish. Freshwater Biology **62**:1501–1510.

Jackson, M. C., J. Grey, K. Miller, J. R. Britton, and I. Donohue. 2016. Dietary niche constriction when invaders meet natives: evidence from freshwater decapods. Journal of Animal Ecology **85**:1098–1107.

Jackson, M. C., T. Jones, M. Milligan, D. Sheath, J. Taylor, A. Ellis, J. England, and J. Grey. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. Freshwater Biology **59**:1123–1135.

James, J., J. Thomas, A. Ellis, K. Young, J. England, and J. Cable. 2016. Over-invasion in a freshwater ecosystem: newly introduced virile crayfish (*Orconectes virilis*) outcompete established invasive signal crayfish (*Pacifastacus leniusculus*). Marine and Freshwater Behaviour and Physiology **49**:9–18.

Johnston, K., B. J. Robson, and P. G. Fairweather. 2011. Trophic positions of omnivores are not always flexible: evidence from four species of freshwater crayfish. Austral Ecology **36**:269–279.

Kats, L. B., and R. P. Ferrer. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. Diversity and Distributions **9**:99–110.

Kouba, A., A. Petrusek, and P. Kozák. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems **413**:05.

Kreps, T. A., E. R. Larson, and D. M. Lodge. 2016. Do invasive rusty crayfish (*Orconectes rusticus*) decouple littoral and pelagic energy flows in lake food webs? Freshwater Science **35**:103–113.

Larson, E. R., L. A. Twardochleb, and J. D. Olden. 2017. Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. Limnology **18**:275–286.

Linzmaier, S. M., L. S. Goebel, F. Ruland, and J. M. Jeschke. 2018. Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. Ecosphere **9**:e02385.

Lipták, B., L. Veselý, F. Ercoli, M. Bláha, M. Buřič, T. J. Ruokonen, and A. Kouba. 2019. Trophic role of marbled crayfish in a lentic freshwater ecosystem. Aquatic Invasions **14**:299–309.

Marten, M., C. Werth, and D. Marten. 2004. The Marbled crayfish (Cambaridae, Decapoda) in Germany - another neozoan in the Rhein basin. Lauterbornia **50**:17–23.

Martin, A. L., and P. A. Moore. 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. Ethology **113**:1192–1201.

Mathers, K. L., R. P. Chadd, M. J. Dunbar, C. A. Extence, J. Reeds, S. P. Rice, and P. J. Wood. 2016. The long-term effects of invasive signal crayfish (*Pacifastacus leniusculus*) on instream macroinvertebrate communities. Science of the Total Environment **556**:207–218.

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**:378–390.

Minagawa, M., and E. Wada. 1984. Stepwise enrichment of δ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochimica Et Cosmochimica Acta **48**:1135–1140.

Momot, W. T. 1995. Redefining the role of crayfish in aquatic ecosystems. Reviews in Fisheries Science **3**:33–63.

Nentwig, W., S. Bacher, S. Kumschick, P. Pyšek, and M. Vilà. 2018. More than “100 worst” alien species in Europe. Biological Invasions **20**:1611–1621.

Olsson, K., P. Stenroth, P. Nystrom, and W. Graneli. 2009. Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? Freshwater Biology **54**:1731–1740.

Ottonello, D., S. Salvidio, and E. Rosecchi. 2005. Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). Amphibia-Reptilia **26**:562–565.

Parkyn, S. M., K. J. Collier, and B. J. Hicks. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? Freshwater Biology **46**:641–652.

Parnell, A. C., D. L. Phillips, S. Bearhop, B. X. Semmens, E. J. Ward, J. W. Moore, A. L. Jackson, J. Grey, D. J. Kelly, and R. Inger. 2013. Bayesian stable isotope mixing models. Environmetrics **24**:387–399.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology **92**:823–835.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**:703–718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia **152**:179–189.

Post, D. M., M. L. Pace, and N. G. Hairston. 2000. Ecosystem size determines food-chain length in lakes. Nature **405**:1047–1049.

Poulin, B., G. Lefebvre, and A. Crivelli. 2007. The invasive red swamp crayfish as a predictor of Eurasian bittern density in the Camargue, France. Journal of Zoology **273**:98–105.

Quezada-Romegialli, C., A. L. Jackson, B. Hayden, K. K. Kahilainen, C. Lopes, and C. Harrod. 2018. tRophicPosition, an R package for the Bayesian estimation of trophic position from consumer stable isotope ratios. Methods in Ecology and Evolution **9**:1592–1599.

Reynolds, J., C. Souty-Grosset, and A. Richardson. 2013. Ecological roles of crayfish in freshwater and terrestrial habitats. Freshwater Crayfish **19**:197–218.

Roth, B. M., C. L. Hein, and M. J. Vander Zanden. 2006. Using bioenergetics and stable isotopes to assess the trophic role of rusty crayfish (*Orconectes rusticus*) in lake littoral zones. Canadian Journal of Fisheries and Aquatic Sciences **63**:335–344.

Ruokonen, T., M. Kiljunen, J. Karjalainen, and H. Hämäläinen. 2012. Invasive crayfish increase habitat connectivity: a case study in a large boreal lake. Knowledge and Management of Aquatic Ecosystems **407**:08.

Ruokonen, T. J., J. Karjalainen, and H. Hämäläinen. 2014. Effects of an invasive crayfish on the littoral macroinvertebrates of large boreal lakes are habitat specific. Freshwater Biology **59**:12–25.

Russell, J. C., S. Caut, S. H. Anderson, and M. Lee. 2015. Invasive rat interactions and over-invasion on a coral atoll. Biological Conservation **185**:59–65.

Russell, J. C., N. S. Sataruddin, and A. D. Heard. 2014. Over-invasion by functionally equivalent invasive species. Ecology **95**:2268–2276.

Sargeant, B. L., E. E. Gaiser, and J. C. Trexler. 2010. Biotic and abiotic determinants of intermediate-consumer trophic diversity in the Florida everglades. Marine and Freshwater Research **61**:11–22.

Scholtz, G., A. Braband, L. Tolley, A. Reimann, B. Mittmann, C. Lukhaup, F. Steuerwald, and G. Vogt. 2003. Ecology: Parthenogenesis in an outsider crayfish. Nature **421**:806–806.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, and M. Arianoutsou. 2017. No saturation in the accumulation of alien species worldwide. Nature Communications **8**:14435.

Siesa, M. E., E. Padoa-Schioppa, J. Ott, F. De Bernardi, and G. F. Ficetola. 2014. Assessing the consequences of biological invasions on species with complex life cycles: impact of the alien crayfish *Procambarus clarkii* on Odonata. Ecological Indicators **46**:70–77.

Souty-Grosset, C., D. M. Holdich, P. Y. Noël, J. D. Reynolds, and P. Haffner. 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France.

Stenroth, P., N. Holmqvist, P. Nyström, O. Berglund, P. Larsson, and W. Granéli. 2006. Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): the influence of tissue, sample treatment, and season. Canadian Journal of Fisheries and Aquatic Sciences **63**:821–831.

Stites, A. J., C. A. Taylor, and E. J. Kessler. 2017. Trophic ecology of the North American crayfish genus *Barbicambarus* Hobbs, 1969 (Decapoda: Astacoidea: Cambaridae): evidence for a unique relationship between body size and trophic position. Journal of Crustacean Biology **37**:263–271.

Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips, and B. X. Semmens. 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ **6**:e5096.

Taylor, C. A., and D. J. Soucek. 2010. Re-examining the Importance of Fish in the Diets of Stream-dwelling Crayfishes: Implications for Food Web Analyses and Conservation. The American Midland Naturalist **163**:280–293.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proceedings of the National Academy of Sciences **101**:10854–10861.

Trudeau, A. 2018. Impact of lake characteristics on foraging niches, size structure, and abundance of Eurasian perch (*Perca fluviatilis*): a comparative study across German gravel pit lakes. master thesis. Humboldt University of Berlin, Berlin.

Usio, N., and C. R. Townsend. 2002. Functional significance of crayfish in stream food webs: roles of omnivory, substrate heterogeneity and sex. Oikos **98**:512–522.

Vadeboncoeur, Y., M. J. Vander Zanden, and D. M. Lodge. 2002. Putting the lake back together: reintegrating benthic pathways into lake food web models. Bioscience **52**:44–54.

van der Wal, J. E., M. Dorenbosch, A. K. Immers, C. V. Forteza, J. J. Geurts, E. T. Peeters, B. Koese, and E. S. Bakker. 2013. Invasive crayfish threaten the development of submerged macrophytes in lake restoration. Plos One **8**:e78579.

VanArman, P. G. 2011. Role of native crayfish, *Procambarus alleni* (Faxon) and *Procambarus fallax* (Hagen), in everglades food webs: a literature review and conceptual model. Florida Scientist **74**:100–125.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology **80**:1395–1404.

Vogt, G., N. J. Dorn, M. Pfeiffer, C. Lukhaup, B. W. Williams, R. Schulz, and A. Schrimpf. 2018. In-depth investigation of the species problem and taxonomic status of marbled crayfish, the first asexual decapod crustacean. bioRxiv:356170.

Appendix D

Table D.1 – Pairwise comparisons of posterior trophic positions and Post's (2002) α (littoral reliance). Each row gives the probability that species (S = spiny-cheek crayfish, M = marbled crayfish) from that lake have a posterior trophic position/alpha less than or equal to crayfish from the lake in the column.

Trophic position

	Moos. S.	Moos. M.	Silber. S	Krumm. S	Krumm. M	Meitz. M	Stein. S
Moosweiher S	0	0.792	0.236	0.917	0.544	0.757	0.536
Moosweiher M	0.208	0	0.045	0.666	0.183	0.668	0.355
Silbersee S	0.764	0.955	0	0.998	0.893	0.829	0.677
Krumme Lanke S	0.083	0.334	0.002	0	0.034	0.629	0.282
Krumme Lanke M	0.456	0.817	0.107	0.966	0	0.747	0.498
Meitzer See M	0.243	0.332	0.171	0.371	0.253	0	0.293
Steinwedeler Teich S	0.464	0.645	0.323	0.718	0.502	0.707	0

Alpha (α)

	Moos. S.	Moos. M.	Silber. S	Krumm. S	Krumm. M	Meitz. M	Stein. S
Moosweiher S	0	0.261	0.965	0.474	0.611	0.221	0.263
Moosweiher M	0.739	0	0.987	0.716	0.815	0.309	0.373
Silbersee S	0.035	0.013	0	0.032	0.054	0.039	0.033
Krumme Lanke S	0.526	0.284	0.968	0	0.628	0.229	0.262
Krumme Lanke M	0.389	0.185	0.946	0.372	0	0.194	0.216
Meitzer See M	0.779	0.691	0.961	0.771	0.806	0	0.577
Steinwedeler Teich S	0.737	0.627	0.967	0.738	0.784	0.423	0

Table D.2 – Posterior TP estimates and posterior alpha (α) estimates for marbled crayfish (M) and spiny-cheek crayfish (S) populations from Bayesian Models with two different sets of trophic discrimination factors (median and [95% credibility interval]) and parametric trophic position estimates (± standard deviation) according to Post (2002).

Lake	**Trophic position**		**Alpha (α)**	
	M	S	M	S
Trophic positions Bayesian Model TDF = Post 2002				
Moosweiher	3.21 [2.60, 3.83]	2.89 [2.30, 3.52]	0.68 [0.50, 0.88]	0.76 [0.59, 0.94]
Silbersee	-	2.67 [2.43, 2.93]	-	0.96 [0.82, 1.00]
Krumme Lanke	2.91 [2.58, 3.25]	3.33 [3.03, 3.63]	0.79 [0.61, 0.96]	0.75 [0.58, 0.93]
Meitzer See	2.94 [2.11, 7.75]	3.80 [2.12, 9,31]	0.59 [0.12, 0.96]	0.50 [0.04, 0.97]
Steinwedler Teich	-	2.72 [2.22, 4.10]	-	0.71 [0.11, 0.99]
Trophic positions Bayesian Model TDF = Mc Cutchan 2003				
Moosweiher	3.20 [2.64, 3.80]	2.88 [2.30, 3.53]	0.67 [0.49, 0.88]	0.76 [0.58, 0.95]
Silbersee	-	2.67 [2.43, 2.93]	-	0.97 [0.81, 0.99]
Krumme Lanke	2.91 [2.58, 3.24]	3.33 [3.05, 3.65]	0.79 [0.62, 0.96]	0.75 [0.59, 0.93]
Meitzer See	2.90 [2.09, 7.50]	3.90 [2.11, 9,33]	0.58 [0.11, 0.97]	0.50 [0.04, 0.96]
Steinwedler Teich	-	2.73 [2.24, 4.19]	-	0.72 [0.10, 0.99]
Mean of individual trophic positions (parametric; Post 2002)				
Moosweiher	3.24 ± 0.48	2.91 ± 0.35	-	-
Silbersee	-	2.56 ± 0.40	-	-
Krumme Lanke	2.98 ± 0.47	3.44 ± 0.33	-	-
Meitzer See	2.37 ± 0.16	2.41 ± 1.04	-	-
Steinwedler Teich	-	2.52 ± 0.29	-	-

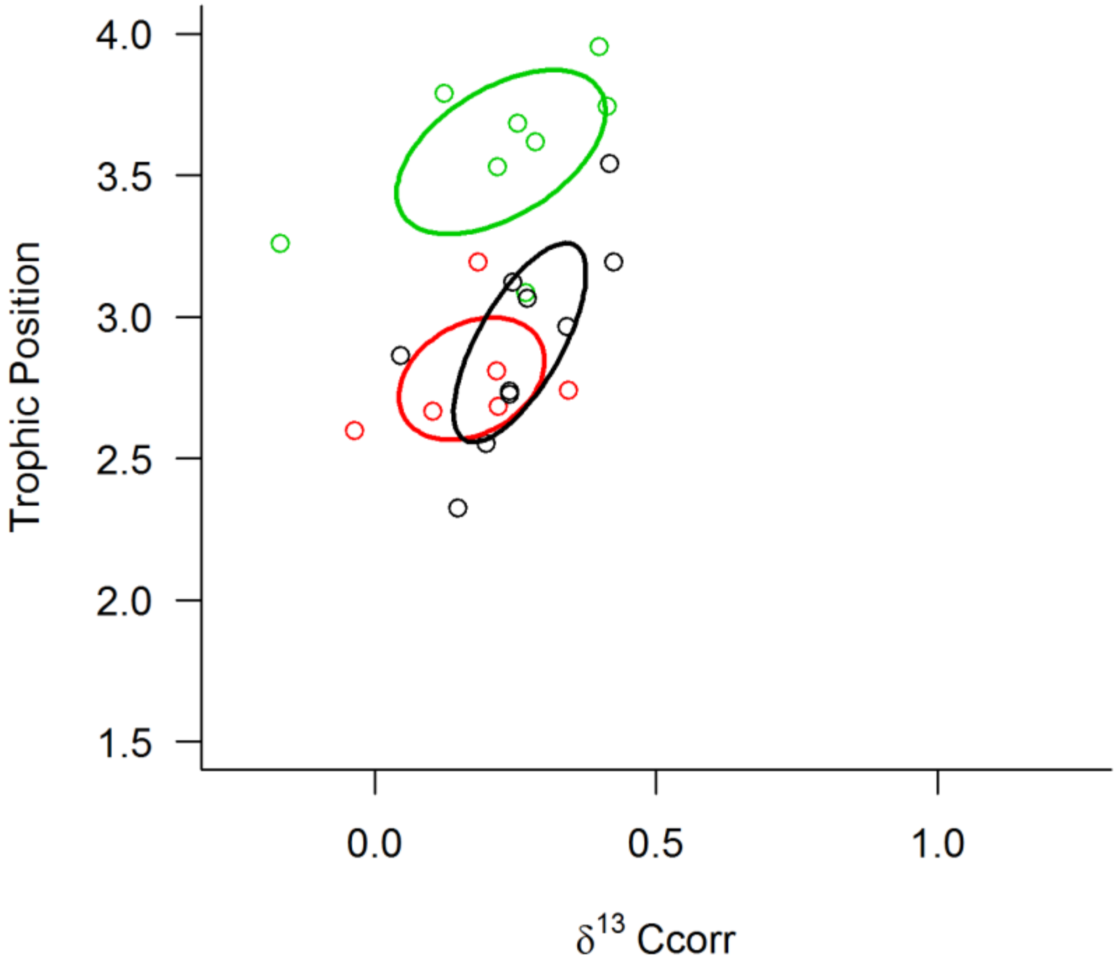

Figure D.1 – Trophic niche of spiny-cheek crayfish (black circles), marbled crayfish < 23 mm (green circles) and marbled crayfish > 30 mm (red circles) in lake Moosweiher.

GENERAL DISCUSSION

Biotic exchanges and the subsequent emergence of novel ecosystems forge new links among species and can bring about fundamental changes (Gallardo et al. 2016, Nentwig et al. 2018). Novel organisms affect the behavior, morphology, physiology and life-history traits of native, non-native and other novel species within the invaded community (Berthon 2015). These interactions can take different forms for the taxa involved, including positive, neutral (commensalism or mutualism) or negative (predation, competition or parasitism) interactions and these interactions can also limit the spread of invasive species (Louthan et al. 2015). Further, the altered interactions can have positive, neutral or negative ramifications on ecosystem services (Hobbs et al. 2009). All of these consequences are often hard to predict prior to invasions. Thus, understanding the mechanisms behind these interactions that may determine the invasion process and ultimately the success or failure of novel organisms, is important for our basic ecological understanding of community assembly and ecosystem management (Boit et al. 2012, Pearson et al. 2018).

In my thesis, I addressed changes in ecological interactions within aquatic communities due to the arrival of a novel crayfish and investigated which species adaptations might result from these changes. In addition, I explored the role of behavioral traits and their variability within species as these interactions take place on the individual level. Together with my co-authors, I therefore implemented an integrative conceptual framework (Chapter 1) to compare traits related to resource exploitation (Chapter 2), and important aspects of behavior (Chapter 3) between similar crayfish species and finally combined laboratory and field data of crayfish trophic function in natural food webs (Chapter 4). We examined the novel marbled crayfish that co-occurs with established populations of previously invasive spiny-cheek crayfish. The empirical and theoretical results and implications are interrelated and advance the mechanistic understanding of the model system (Figure 5.1). My thesis revealed important aspects of the complex interactions of functionally similar invasive species and stands as an example for frequent, but rarely studied, over-invasions in freshwater crustaceans. The main results can be summarized as follows:

- The framework presented in Chapter 1 established a methodology to quantify changes in interaction strength caused by the arrival of novel organisms. This was achieved by measuring trophic traits of the interaction partners including alternative prey, congeners and predators, and integrating existing data of their biology and food-web structure. This framework was further illustrated by introducing the marbled crayfish into a conceptual food web.
- A subset of the interactions derived from the conceptual framework in Chapter 1 was empirically tested in Chapter 2. The long-established spiny-cheek crayfish showed greater interaction strength than marbled crayfish in resource exploitation of mussel prey, but marbled crayfish reached higher long-term consumption rates. Also, consumptive effects differed

between sexes and reproductive forms of crayfish. The mechanistic basis of these interactions can be precisely quantified and demonstrated that mathematical models adequately predict interaction outcomes. Overall, no general relationships of favorable behavioral traits in feeding or species invasion were established. However, individual behavior was correlated with lower handling times for aggressive marbled crayfish and spiny-cheek crayfish with an aggressive threat response.

- The detailed assay on behavioral traits between the novel marbled crayfish and the functionally similar spiny-cheek crayfish in Chapter 3 showed that marbled crayfish were more aggressive in interspecific interactions with spiny-cheek crayfish. Activity and aggression of marbled crayfish were correlated in an aggression syndrome. Additionally, marbled crayfish captured in lakes showed decreased activity compared to aquarium-reared individuals. Threat responses differed between sexes of spiny-cheek crayfish, which showed either high aggression (males) or flight behavior (females), and between spiny-cheek crayfish and marbled crayfish, the latter often “freezing” in response to a simulated threat. Overall, the differences between the species were most pronounced in aggression and threat response, while they were highly flexible for individuals of each species.
- The components of natural food webs analyzed in Chapter 4 showed that although the marbled crayfish and its congener occupied central roles in the invaded food web, these also showed functional differences in their trophic niches. This was surprising, given that both species are generalists with a highly flexible trophic niche. Trophic interactions of multiple prey items with both crayfish in the laboratory revealed high preference for animal tissue. However, marbled crayfish utilized proportionally more detritus and spiny-cheek crayfish fed more on macrophytes in the studied lakes. Also, marbled crayfish preyed more on arthropods than spiny-cheek crayfish, which fed more on mollusks.

In this section, I discuss the significance of these main findings for the ecology, evolution and management of novel species with an emphasis on non-indigenous crayfish (NICs). In addition, I outline some limitations of the thesis and provide recommendations for future research.

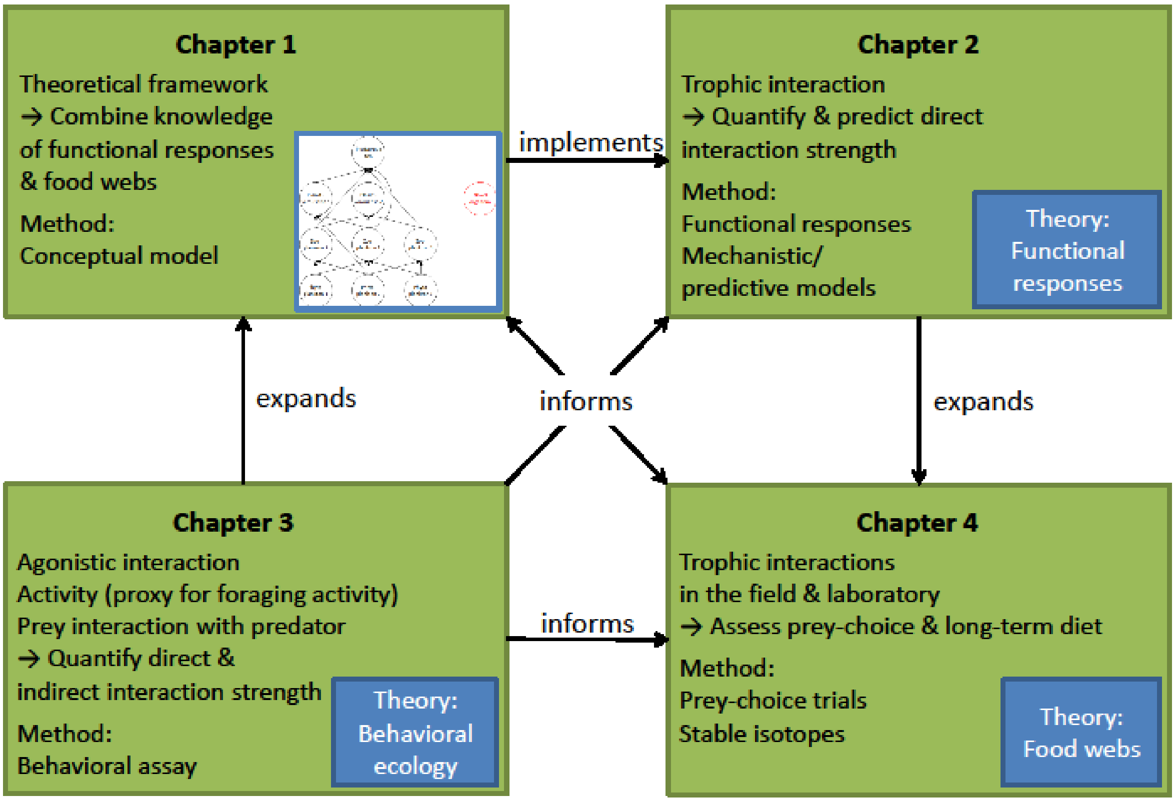

Figure 4 – Conceptual overview of the thesis chapters and content.

Novel species traits and interactions

In Chapter 1, a framework was established to quantify the change in interaction strength caused by the arrival of novel organisms and applied in a case study on NICs. The underlying concept of phenomenologically observing functional responses is one of the most fundamental ecological concepts (Holling 1959), and it has been rediscovered in invasion ecology in the last decade (Dick et al. 2013). Most of the comparative functional responses, however, focused on a single predator-prey interaction (e.g. Rosewarne et al. 2016, Xu et al. 2016). In contrast, the framework presented here adds vital biological context by including competitors, different prey items and predators in the assessment.

Another important issue was addressed within the exemplary case of marbled crayfish, by looking at systems with similar co-occurring invaders. Risk assessments and invasion biology, in general, have always studied the interactions of invaders with resident species to gain insights into ecological and evolutionary processes (Ricciardi et al. 2013). While understanding invader-native interactions is very important for conservation biology, over-invasion scenarios, where functionally similar invasive species co-occur or replace each other, should be more frequently studied due to their increasing importance in novel ecosystems (Hobbs et al. 2014). Native communities, which are hard to define in the first place, are being increasingly replaced by novel communities, especially in urban environments (Gaertner et al. 2017). Interactions with ICs are comparatively rare when new NICs enter aquatic systems in Europe, as these systems have for the most part already been previously invaded by NICs (Kouba et al. 2014). Thus, over-invasion scenarios can explain many of the changes we see in novel ecosystems (Rauschert and Shea 2017). Interactions between multiple invaders and the risks of invader displacement were discussed and tested with the crayfish model.

Investigating the mechanisms that enable functionally and phylogenetically similar invasive species to coexist can yield important insights for species or ecosystem management (Russell et al. 2014). For example, many islands are invaded by several rat species that resulted in one species becoming dominant (Jones et al. 2008, Russell et al. 2015). Therefore, managers should consider apparent differences in the competitive ability among the invasive species. It may even be possible to utilize such trait differences to manage species invasions. For example, invasive mice could be eradicated, by deliberately over-invading populations with genetically engineered mice that have a fitness advantage over the established population but spread deleterious genes (Backus and Gross 2016). Yet, the competitive advantage of an over-invader does not necessarily translate into higher impacts on the community. For example, Russell et al. (2014) point out that “common wasps (*Vespula vulgaris*) over-invaded German wasps (*V. germanica*) in New Zealand, but have less impact on the bee-keeping industry” (Clapperton et al. 1989). Thus, these processes are far from being understood, and trait differences could be functionally linked to these observations to mechanistically explain interaction outcomes.

Interactions among invaders are sometimes very strong and can be difficult to predict (Simberloff and Von Holle 1999). The effects of multiple invasions on the ecosystem can be adding to, attenuating or amplifying existing invader effects (Johnson et al. 2009). For example, the quagga mussel (*Dreissena bugensis*) had a smaller detrimental effect on native mussels of the family Unionidae than the zebra mussel (*Dreissena polymorpha*) they replaced in the Great Lakes region (Burlakova et al. 2014). Furthermore, invasive mammals facilitate the spread of non-native trees in New Zealand by spreading their mycorrhizal symbionts (Wood et al. 2015). The framework in Chapter 1 integrates multiple invader effects, as it systematically compares a subset of trophic links connected to the two invasive model crayfish and their (invasive) prey. Building upon this framework, the functional responses in Chapter 2 as well as the stable isotope data from Chapter 4 quantify these predator-prey interactions.

Predicting interaction strength

The thesis contributes to the ongoing efforts of the European Union to prevent and manage invasive species introductions (EU Regulation 1143/2014). Inferring biotic interactions from proxies has been proposed as means to disentangle the complexity behind (novel) ecosystems (Morales-Castilla et al. 2015). For example, risk assessments based on predator-prey interaction strength measured as functional responses provide potential applications for conservation management (Dick et al. 2014, Dick et al. 2017). The framework presented in Chapter 1 can help advance the usage of functional responses for managers to identify important components of novel ecosystems that demand monitoring or intervention. Robust, repeatable and comparable risk assessments, like the one presented here, are very much needed, as the prevention of new introductions is the most effective way to avoid negative consequences (Leung et al. 2002). Furthermore, Chapter 2 applies phenomenological observations derived from the framework and added the prospect of viable predictions of invader effects based on mechanistic models.

The phenomenological data derived from functional responses in Chapter 2 suggest that co-occurring marbled and spiny-cheek crayfish might add to the predation pressure on *Dreissena* mussel populations, as spiny-cheek crayfish had high consumption rates and marbled crayfish had more voracious feeding habits. When these results are viewed in concert with crayfish dietary preferences from Chapter 4, marbled crayfish might also amplify feeding pressure on other prey groups when both species partition their niches, especially on arthropods that are highly relevant to conservation efforts, such as dragonflies. These differences can modify interactions between intermediate consumers, and changes at the base of food webs are also conceivable. For example, rusty crayfish (*Orconectes rusticus*) displace other crayfish by agonistic interactions and reduce macrophytes as well as associated invertebrates (Lodge et al. 2000). Chapter 3 showed that spiny-cheek crayfish utilize macrophytes to a higher degree, while marbled crayfish show a greater reliance on detritus and exhibited highly aggressive behavior towards the congener. Through agonistic interactions, marbled crayfish might displace spiny-cheek crayfish from higher-quality rock or wood shelters and thus force them to seek shelter in macrophytes, which also serve as a food resource. These observations could result in a more fundamental change within water bodies invaded by both species.

Evolutionary or behavioral changes of prey organisms can dramatically alter functional responses (Saul and Jeschke 2015). For example, changes in anti-predator behavior have been shown to drive trophic cascades in ecosystems (Schmitz et al. 2004). In contrast to most other comparative studies of functional responses, the mechanistic approach used here to predict interaction outcomes connected the different phases of the predation process and functional feeding responses. Precise quantification of predation parameters makes it possible to resolve differences between or changes within predator and prey traits (Jeschke et al. 2002), like swarming effects (Jeschke and Tollrian 2005). Also, variation in functional responses due to habitat differences (Toscano and Griffen 2013) could be better explained by mechanistic models. Moreover, the mechanistic approach could prove particularly useful in phenotypically plastic organisms that adapt their traits to resource availability (Agrawal 2001, Davidson et al. 2011). The results from Chapter 2 and prior work on other predator-prey systems (Jeschke and Tollrian 2005, Jeschke and Hohberg 2008) demonstrated how individual differences in predation parameters shape functional feeding responses and that these models can predict real consumption rates from independently derived parameters.

The highly-detailed quantification of trophic traits in Chapter 2 came at the cost of a limited set of trophic links being investigated in the process as opposed to the idealized approach from Chapter 1. Alternative prey or potential predators were largely omitted in the mathematical modeling and phenomenological functional responses of both crayfish species. Follow-up studies should include multiple interactions with potential prey items of novel organisms to get more robust predictions of impact (Smout et al. 2010). However, prey choice and stable isotope studies on invasive marbled crayfish populations (Chapter 4) estimated many of these interactions a posteriori. The food-web interactions derived from stable isotopes outlined the interactive network of marbled crayfish and their congener under natural conditions. Here, the mixing models showed that spiny-cheek crayfish species indeed preferred mollusks as prey, like the *Dreissena* mussels tested in functional response experiments, while marbled crayfish tended more towards arthropods. The functional response and parametrization experiments also revealed a higher functional response of spiny-cheek crayfish feeding on mollusks compared to marbled crayfish. Thus, marbled crayfish might have higher functional responses for arthropod prey compared to spiny-cheek crayfish. Follow-up experiments with arthropods as prey could inform about the potential impacts on this species group.

The stable isotope data also demonstrated that co-occurring populations of spiny-cheek crayfish and marbled crayfish in the field exhibited similar intermediate trophic positions. However, the two crayfish species showed functional differences in their trophic niche. This pattern is in accordance with other studies: NICs that did not differ in niche size when living in sympatry with natives or other NICs partition their niches (Jackson et al. 2014, Larson et al. 2017). This niche differentiation can, for example, be explained by the agonistic interactions classified in Chapter 3. In most systems, resources should not limit crayfish invaders due to their polyphagous diet (Butler and Stein 1985). Black rats (*Rattus rattus*) and Polynesian rats (*R. exulans*), for example, exhibit the same trophic positions on invaded islands, and black rats limit their Polynesian rat populations mainly through interference competition (Russell et al. 2015). Also, highly invasive fire ants (*Solenopsis invicta*) and equally invasive Argentine ants (*Linepithema humile*) showed that they are both omnivorous, but limited by interference competition in their native range (LeBrun et al. 2007). The data presented here indicate a similar case, where polyphagous NICs with different aggression potential act on a similar trophic level, but preferentially feed on certain prey items, like arthropods in case of marbled crayfish.

The density-dependent interactions with these prey items can have varying consequences for the prey community. The phenomenological functional responses showed exclusively type II responses which are usually considered destabilizing for predator-prey relationships (Oaten and Murdoch 1975). Missing differences in the type of functional response could thus have important consequences for predicting the outcome of predator-prey interactions. For example, type III responses offer prey a low-density refuge and overall acts stabilizing in predator-prey interactions (Dick et al.

2014, Barrios-O'Neill et al. 2015). A finer resolution at low prey densities could be achieved by adding more replicates which could still reveal type III responses. However, marbled crayfish predatory impact on mussels was not particularly high for mussels, as they were lower than spiny-cheek crayfish, which co-occur with *Dreissena* mussels for decades. And functional feeding traits of the NICs are probably not distinctive enough to cause fundamental changes to the community (e.g. trophic cascades) by the predation of *Dreissena* mussels (Havel et al. 2015).

Even if no differences are apparent in functional responses, over-invasion of functionally similar species can rapidly take place when the compared species differ in other traits that we did not take into account. Quagga mussels, for example, do not significantly differ in filtration rates and functional responses from zebra mussels, but they can replace the competitor by lower respiration rates, higher assimilation efficiency and spawning earlier in the year (Baldwin et al. 2002, Stoeckmann 2003). Marbled crayfish had a lower consumption rate compared to the resident species. This has also been found in other successful novel species. Asian shore crabs (*Hemigrapsus sanguineus*), for example, had a lower per capita effect on native mussel prey than the established, invasive European green crabs (*Carcinus maenas*) by intra-guild predation and lower levels of cannibalism (Lohrer and Whitlatch 2002). When the consumption rates of both species outlined in Chapter 2 are compared to the reproductive life-history traits (c.f. Byers 2000, Baldwin et al. 2002), marbled crayfish seem to more effectively convert resources to body tissues and reproductive units. In the laboratory, marbled crayfish produce clutches of 50 to 200 eggs every four to eight weeks[6], while spiny-cheek crayfish from natural European populations produce only one clutch of about 400 eggs per year (Vogt et al. 2004, Souty-Grosset et al. 2006, Vogt 2010). In the long-term, such differences in energy conversion rates and reproduction could shift invasion outcomes. Thus, metabolic processes related to resource conversion and reproduction would complete the picture of trophic relationships compared by functional responses.

The thesis focused on different interactions among the two invasive crayfish and between the crayfish with selected prey organisms, including behavior, dietary preferences and foraging behavior. Therefore, measures of interaction strength were derived at different levels, but some additional aspects should also be considered. The compiled data are important for risk assessments, but would ideally be combined with numerical responses of the respective organisms (Dick et al. 2017). Such a combined approach would lead to a thorough understanding of the invasion process and provide more solid risk assessments. Comparisons could be expanded by integrating simultaneous changes of the environment, like temperature changes from ongoing climate change (Gilman et al. 2010), as functional response and temperature increase are strongly interrelated (Alexander et al. 2015). Finally,

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[6] In lakes, much higher numbers of eggs could be expected. A naturalized marbled crayfish caught in lake Moosweiher carried 724 eggs (Chucholl, C., and M. Pfeiffer. 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).

interactions of novel organisms with competitors and prey also lead to a number of indirect effects. For example, non-consumptive predator-driven mortality of dragonfly larvae preying on carp fry was lowered significantly when marbled crayfish were present (Veselý et al. 2017). The authors argue that marbled crayfish are either scavenging on fry immobilized by dragonfly larvae or that both consumers modify their behavior in the presence of each other. Such interactions are very hard to predict a priori, but if they are identified should be considered in comparative studies and risk assessments. Existing frameworks should thus be further refined by identifying important autecological and synecological traits that alter impact. Such concepts are very much needed to identify ecologically or economically harmful organisms in a world with a rising number of translocated species.

Invader behavior in novel communities

Comparative functional response studies have proven to reliably identify potentially harmful invaders or negative interactive outcomes for certain prey items (Dick et al. 2017). However, studies on community change by novel organisms and risk assessments often focus on consumptive interactions or effects, but neglect behavioral mechanisms like interference competition, activity or the response to predators, all of which are linked to invasion success (Chapple et al. 2012). The results on resource exploitation (Chapter 2) showed superiority in interaction strength of the established spiny-cheek crayfish over marbled crayfish in resource exploitation of *Dreissena* mussels, a key organism of many freshwater systems, but, the agonistic interactions among species as outlined in Chapter 3 favored marbled crayfish. The thesis examined these rarely-investigated links between behavioral traits and trophic traits, as well as their role in the invasion processes by integrating the results on behavior with foraging measurements on an individual basis. Thus, a more detailed understanding of the mechanics and trophic trait differences in the predator-prey interaction was achieved.

The interplay of resource consumption and direct agonistic interactions can have important ecological and evolutionary consequences (Peiman and Robinson 2010). Also, behavioral studies ideally complement mechanistic studies on feeding behavior and integrate the response of individual novel organisms to the new environment (Cooke et al. 2013). The higher resource consumption observed in spiny-cheek crayfish, however, might not facilitate biotic resistance when they are driven from shelters through agonistic interactions. The outcomes of aggression trials in crayfish are difficult to link to invasion success in the field as ecosystem contexts matter (Garvey et al. 1994, Reynolds 2011). Food resources are often not the limiting polyphagous crayfish, but competition among crayfish can be fierce for shelters (Bergman and Moore 2003, Martin and Moore 2007). Thus, systems lacking sheltering habitats like woody debris and larger rocks should favor the more aggressive marbled crayfish. Therefore, if they are important to the target organism’s ecology, structural, abiotic components of potential invasion sites should be considered in risk assessments as well as comparative studies.

Although marbled crayfish have smaller chelae, they proved to be dominant in agonistic interactions against one of the most successful crayfish invaders of Europe. They were further able to adapt their activity level within a natural environment, and the existence of a behavioral syndrome was found for the marbled crayfish. Behavioral syndromes have been proposed to entail increased impact on prey organisms as they increase encounter rates with preferred prey (Pintor et al. 2009). The consumption rate of *Dreissena* mussels was lower for marbled crayfish, but no differences between species were apparent for encounter rates (Chapter 2). Nevertheless, consumption rates might still be disproportionally higher for other prey organisms like arthropods (as described in the previous section). It is likely that the elevated aggression and behavioral syndromes of aggression and activity put marbled crayfish in line with many successful invaders (Sih and Bell 2008, Pintor et al. 2009). Even the most successful crayfish worldwide, the highly aggressive red swamp crayfish, have been shown to be inferior in interspecific fights (Jimenez and Faulkes 2011, Hossain et al. 2019). In a more recent study, however, juvenile marbled crayfish have turned out to be inferior in agonistic interactions with juvenile signal crayfish and common yabby (*Cherax destructor*) (Fořt et al. 2019). The authors attribute these results to the size differences in claw proportions as well as phylogeny of marbled crayfish opponents. The data on agonistic interactions compiled here cannot confirm an important role of claw size in agonistic encounters, but support the dominance over a crayfish from the family Cambaridae. Overall, the behavioral measurements provide new information on marbled crayfish behavior and include important ethological concepts like the behavioral syndrome, i.e. correlated suits of behavioral traits (Sih et al. 2004), which stress the potential risk of behavioral dominance of this novel species.

As outlined in Chapter 1, the behavioral and trophic traits of functionally-similar intermediate polyphagous consumers have to be seen in the light of other interaction partners (Russell et al. 2014). Native and invasive predators can have a large effect on behavior, habitat selection, and food consumption of crayfish that shift the competitive advantage among competing species (Stein and Magnuson 1976, Werner and Anholt 1996). Additionally, interference among predators or multiple predator effects can alter predator-prey interactions (Sih et al. 1998). Barrios-O'Neill et al. (2014) showed that prey consumption of invasive mysids (*Hemimysis anomala*) and a higher-order fish predator (*Gasterosteus aculeatus*) were additive, but this was not the case for the native mysid (*Mysis salemaai*). Per-capita effects of marbled crayfish and spiny-cheek crayfish might be altered when foraging is observed in sympatry or the presence of other predators. Boldness towards predators is also frequently part of aggression syndromes which are thought to result in high food-uptake rates (Kortet and Hedrick 2007, Pintor et al. 2008). The data from Chapters 2 and 3 support the link between aggression and food uptake for marbled crayfish, and between aggressive threat response and food uptake for spiny-cheek crayfish. However, marbled crayfish exhibited a more passive threat-response behavior. They often did not show signs of either flight or fight, which suggests a limited food uptake under predation. Response to predation differed strongly between sexes of spiny-cheek crayfish, but functional responses also differed between sexes and even reproductive forms of crayfish. The link of anti-predator behavior and trophic traits could therefore not be fully established; intrinsic sexual differences in behavior might better explain resource use of the behavioral types. Predation effects of real predators on both crayfish species should be studied in more detail to advance the mechanistic understanding of the functional response.

Differences in the defenses of novel organisms against predators alter interaction outcomes (Saul and Jeschke 2015). For example, brown trout (*Salmo trutta*) preferentially prey on native *Gammarus pulex* compared to invasive *Gammarus roeseli* due to its spines (Bollache et al. 2006). The biological meaning of the restrained threat response of marbled crayfish or the aggressive threat response of male spiny-cheek crayfish shown in Chapter 3 could not be assessed. The response to predators differed between species, but cannot be ascribed to be effective per se as it depends on the type of predator (Thawley and Langkilde 2017). Maladaptation against predators (e.g. Ward-Fear et al. 2009) is conceivable in organisms from aquaria and might favor the established spiny-cheek crayfish stocks. Some predators might benefit disproportionately from novel marbled crayfish (Tablado et al. 2010) and therefore also change the predation pressure on the congener. A further implementation of our conceptual framework that includes predators within my model scenario could provide a more accurate explanation of food-web changes. This could be achieved by laboratory experiments between crayfish and predatory fish (Reynolds 2011) or indirect measurements of predation success in the field, combining abundance estimates in the lake with diet data from predator stomachs or stable isotopes.

Predator avoidance could also explain some of the field data on trophic niches from Chapter 4, where niche partitioning between both NICs could be shown. The study shows that species invasions are highly dependent on the ecological context and the outcome of resource exploitation, and that interference competition can differ greatly between invaded regions. If predators of crayfish differentially prey on the two species, one of the species might be disproportionately forced to decrease foraging effort (Hirvonen et al. 2007) or forage on less profitable prey (Nilsson et al. 2000). Such predation effects could be included by calculating mixing models of potential predators or by examining their stomach content and relate these results to abundance estimates of crayfish. The niche partitioning between the two crayfish can also be related to habitat partitioning. In the case of lake Moosweiher, some data exist that marbled crayfish and spiny-cheek crayfish partition their habitat where they co-occur (Chucholl and Pfeiffer 2010). Future research should therefore quantify species distribution in natural environments to better explain observed trophic niches.

Evolution of novel organisms

The presented trophic and behavioral traits can be highly plastic and will be shaped by evolution (Berg and Ellers 2010). High phenotypic plasticity has often been related to invasion success (Agrawal 2001). Sympatric NICs have been shown to vary in predation pressure on specific prey groups under natural conditions (Meira et al. 2019), and several behaviors like agonistic behavior and anti-predator behavior have been found to be plastic in crustaceans (Gherardi et al. 2012 and references therein). Functional responses and behaviors of individual crayfish turned out to be highly flexible. Since high phenotypic plasticity is independent of heterozygosity (Scheiner 1993), it could be expected even in isogenic marbled crayfish and contribute to their successful establishment in Europe. High plasticity in functional responses should lead to an enlarged realized niche and therefore a reduced possibility for coexistence with each other (Berg and Ellers 2010). However, marbled crayfish and spiny-cheek crayfish showed little overlap in their trophic niches in lakes where they live in sympatry (Chapter 4). The reasons for these niche differences have yet to be established, but they might be found in response to the overall differences in agonistic behavior established in Chapter 3 or as a consequence of predator-induced mortality (Stein and Magnuson 1976, Werner and Anholt 1996). Invasion biology and risk assessment have to take into account the fast evolutionary adaptation in the wake of new species introductions.

Equalizing mechanisms that minimize the average fitness between species, such as predation and stabilizing mechanisms like resource partitioning or competitive dominance between consumers, can enable species coexistence (Chesson 2000). A meta-analysis showed that most interactions among invasive paired malacostraca (decapods and amphipods) are either neutral or negative (Jackson 2015). The occurrence of multiple, functionally similar invaders can lead to shifts in dominance (Ortega and Pearson 2005), but coexistence of functionally similar invaders is possible if intraspecific competition is greater than interspecific competition (Chesson 2000). For example, growth and reproduction of two invasive snail species, the New Zealand mud snail (*Potamopyrgus antipodarum*) and European physa (*Physella acuta*), were influenced more by the density of conspecifics than the density of the congeners. There is some evidence that marbled crayfish have low levels of intraspecific aggression (Luna et al. 2009, Fořt et al. 2019) and thus might be less territorial than other species, but in-depth examination is lacking. Furthermore, competition models based on Lotka–Volterra equations have shown that also similar invasive species with comparable intra- and interspecific competition levels can coexist (Rauschert and Shea 2017). Therefore, intraspecific competition should be compared for invaders, as invasions often result in the co-existence of invaders and not displacement. Combining the data on trophic and behavioral traits presented in this thesis with existing knowledge of established populations (e.g. Chucholl et al. 2012) point to a mean neutral effect of marbled crayfish on the performance of spiny-cheek crayfish the species (sensu Jackson 2015), enabling both species to coexist.

Novel species and interacting organisms evolve and adapt to the new environment (Prentis et al. 2008, Davidson et al. 2011). Adaptive responses of marbled crayfish to the new environment were investigated by comparing functional responses (Chapter 2) and behavioral traits (Chapter 3) of marbled crayfish from aquaria and lakes. Berthon (2015) developed a scheme to assess the evolutionary responses of native to invasive species dependent on the interaction strength of the invader and the evolutionary capacity of the established (native) species. Berthon's (2015) scheme suggests that spiny-cheek crayfish should be pre-adapted to later invading crayfish species and thus have a high capacity for evolution. The trophic interactions or competition between both crayfish are estimated to be medium to high and thus (co-)evolution of both species might be conceivable. As the evolutionary capacity of marbled crayfish is limited by parthenogenetic reproduction, the evolutionary impact of the invader should decrease over time, lead to stable spiny-cheek crayfish phenotypes (Berthon 2015). Thus, evolutionary responses of both species entail important information for the long-term success of both species.

High individual variation in trophic traits and behavior was observed even among isogenic individuals. Invasive species have often shown to successfully adapt to new environments and thrive despite genetic bottlenecks caused by the introduction (Sax and Brown 2000). High behavioral flexibility, for example, can mediate this disadvantage and has often been associated with invasion success (Sol et al. 2002, Wright et al. 2010). Recent studies have resolved the importance of such variation within one species on the food web (Des Roches et al. 2018), and their consequences for species invasions have proven to be significant (Evangelista et al. 2019). The limited genetic diversity could make marbled crayfish an interesting model organism for personality research. Although adaptability is thought to be impaired by asexual reproduction, epigenetic phenotype variation can positively affect the adaptation of isogenic, clonal invaders in marbled crayfish (Vogt 2017). For example, Vogt et al. (2015) compared marbled crayfish from their laboratory to the naturalized crayfish from lake Moosweiher and found higher methylation in the laboratory animals. The high degree of intraspecific variability in prey consumption of both species supports the notion that different populations can have distinct effects on invaded systems. And this thesis support findings on clonal Amazon molly (*Poecilia formosa*) which suggest that behavioral types develop even in isogenic species in standardized environments (Bierbach et al. 2017). However, the significance of these findings for fitness in the natural environment, especially in invasions, remains to be explored.

Perfect invaders - marbled crayfish in Germany vs. Madagascar

The data presented here and existing knowledge on marbled crayfish show that the species possesses all of the traits that Havel et al. (2015) ascribe to successful aquatic invaders: "asexual breeding, high reproductive output, generalist feeding, and broad environmental tolerance". Yet in Europe, marbled crayfish have, up to now, mostly been found within German gravel pit lakes and in close proximity to urban centers (Chucholl et al. 2012, Chucholl 2015). In contrast, marbled crayfish in Madagascar became quickly invasive and have colonized a number of habitats since 2005 when they were detected for the first time (Jones et al. 2009, Andriantsoa et al. 2019). The number of populations in German lakes increased since it was first recorded in the field in 2003 but at a comparably low rate (Marten et al. 2004, Chucholl et al. 2012, Chucholl 2015). These more or less simultaneous invasions illustrate the importance of ecosystem context in invasions.

The rapid spread around the island of Madagascar was most likely facilitated by human activities because marbled crayfish there are a sought-after food items and thrive well in rice fields, rivers and lakes (Jones et al. 2009, Gutekunst et al. 2018, Andriantsoa et al. 2019). In addition, marbled crayfish have been reported to move over land (Chucholl et al. 2012). On Madagascar, marbled crayfish colonized a variety of habitats including lotic environments and coexist with highly threatened native crayfish species, as their populations seem to be plague-free (Andriantsoa et al. 2019). Unfortunately, this invasion process was hardly studied; changes associated with increasing marbled crayfish numbers have not been scientifically documented because scientific infrastructure and research effort is missing. Notably, Andriantsoa et al. (2019) found that marbled crayfish could mitigate the occurrence of a dangerous disease, human schistosomiasis, by feeding on the intermediate host snail. Studying the interactions between Madagascan marbled crayfish and native crayfish species and other members of their diverse freshwaters communities could inform current risk assessments of this species. Other scenarios of predator-prey interactions could emerge among the increasing number of invaded water bodies in Europe and elsewhere. Thus, monitoring and examining more populations with different ecological contexts could reveal new important links in the food web.

Conclusions

The studies on interactions between novel marbled crayfish with established spin-cheek crayfish and their prey provides new insights for community ecology, behavioral ecology and risk assessment of novel species. The framework presented in Chapter 1 provided a sound and structured approach to assess impact of any existing or future novel organisms. This has been showcased by applying important elements of the framework on the NIC model. Here, I conclude that in Europe, marbled crayfish might not be the *perfect invader* that Jones et al. (2009) coined. Although human-mediated spread and warmer temperatures have been shown to favor the invasion success of marbled crayfish, this thesis explored the hitherto unknown aspects of their behavior and ecology by quantifying the interactions with each other and key components of the community. Prior to my research, almost no data existed on marbled crayfish trophic interactions and their trophic role in the limited number of invaded lakes had not been systematically asserted. This lack of information has impeded management decisions, particularly those relating to EU-regulation No 1143/2014 to minimize potential harm caused by marbled crayfish. My thesis showed that marbled crayfish are inferior in their ability to utilize highly abundant *Dreissena* mussels (Chapter 2), but are more carnivorous than anticipated, threatening other invertebrates and potentially affecting ecosystem processes like leaf litter decomposition (Chapter 4). Both species occupy a central role in invaded food webs but partition their realized niche. Also, marbled crayfish have been portrayed as physiologically inferior pet species with limited genetic potential for variability, but I could show that they exhibit high degrees of behavioral and trophic flexibility and are able to assert itself in competition with spiny-cheek crayfish (Chapter 3). These interactions, with the most common competitor in European waters, are driven by a combination of exploitative and interference competition. Thus, marbled crayfish and spiny-cheek crayfish seem to be neither benefiting nor excluding each other, and my findings point towards coexistence between the species in most natural systems.

Literature cited

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science **294**:321–326.

Alexander, J. M., J. M. Diez, and J. M. Levine. 2015. Novel competitors shape species' responses to climate change. Nature **525**:515–518.

Andriantsoa, R., S. Tönges, J. Panteleit, K. Theissinger, V. C. Carneiro, J. Rasamy, and F. Lyko. 2019. Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. BMC Ecology **19**:8.

Backus, G. A., and K. Gross. 2016. Genetic engineering to eradicate invasive mice on islands: modeling the efficiency and ecological impacts. Ecosphere **7**:e01589.

Baldwin, B. S., M. S. Mayer, J. Dayton, N. Pau, J. Mendilla, M. Sullivan, A. Moore, A. Ma, and E. L. Mills. 2002. Comparative growth and feeding in zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*): implications for North American lakes. Canadian Journal of Fisheries and Aquatic Sciences **59**:680–694.

Barrios-O'Neill, D., J. T. A. Dick, M. C. Emmerson, A. Ricciardi, and H. J. MacIsaac. 2015. Predator-free space, functional responses and biological invasions. Functional Ecology **29**:377–384.

Barrios-O'Neill, D., J. T. A. Dick, M. C. Emmerson, A. Ricciardi, H. J. MacIsaac, M. E. Alexander, and H. C. Bovy. 2014. Fortune favours the bold: a higher predator reduces the impact of a native but not an invasive intermediate predator. Journal of Animal Ecology **83**:693–701.

Berg, M. P., and J. Ellers. 2010. Trait plasticity in species interactions: a driving force of community dynamics. Evolutionary Ecology **24**:617–629.

Bergman, D. A., and P. A. Moore. 2003. Field observations of intraspecific agonistic behavior of two crayfish species, *Orconectes rusticus* and *Orconectes virilis*, in different habitats. The Biological Bulletin **205**:26–35.

Berthon, K. 2015. How do native species respond to invaders? Mechanistic and trait-based perspectives. Biological Invasions **17**:2199–2211.

Bierbach, D., K. L. Laskowski, and M. Wolf. 2017. Behavioural individuality in clonal fish arises despite near-identical rearing conditions. Nature Communications **8**:15361.

Boit, A., N. D. Martinez, R. J. Williams, and U. Gaedke. 2012. Mechanistic theory and modelling of complex food-web dynamics in Lake Constance. Ecology Letters **15**:594–602.

Bollache, L., N. Kaldonski, J.-P. Troussard, C. Lagrue, and T. Rigaud. 2006. Spines and behaviour as defences against fish predators in an invasive freshwater amphipod. Animal Behaviour **72**:627–633.

Burlakova, L. E., B. L. Tulumello, A. Y. Karatayev, R. A. Krebs, D. W. Schloesser, W. L. Paterson, T. A. Griffith, M. W. Scott, T. Crail, and D. T. Zanatta. 2014. Competitive Replacement of Invasive Congeners May Relax Impact on Native Species: Interactions among Zebra, Quagga, and Native Unionid Mussels. Plos One **9**:e114926.

Butler, M. J., and R. A. Stein. 1985. An analysis of the mechanisms governing species replacements in crayfish. Oecologia **66**:168–177.

Byers, J. E. 2000. Competition between Two Estuarine Snails: Implications for Invasions of Exotic Species. Ecology **81**:1225–1239.

Chapple, D. G., S. M. Simmonds, and B. B. Wong. 2012. Can behavioral and personality traits influence the success of unintentional species introductions? Trends in Ecology & Evolution **27**:57–64.

Chesson, P. 2000. Mechanisms of Maintenance of Species Diversity. Annual Review of Ecology and Systematics **31**:343–366.

Chucholl, C. 2015. Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion pathway. Pages 83–114 *in* T. Kawai, Z. Faulkes, and G. Scholtz, editors. Freshwater crayfish: a global overview. CRC Press, Boca Raton, USA.

Chucholl, C., K. Morawetz, and H. Groß. 2012. The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquatic Invasions **7**:511–519.

Chucholl, C., and M. Pfeiffer. 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.

Cooke, S. J., D. T. Blumstein, R. Buchholz, T. Caro, E. Fernández-Juricic, C. E. Franklin, J. Metcalfe, C. M. O'Connor, C. C. St. Clair, and W. J. Sutherland. 2013. Physiology, behavior, and conservation. Physiological and Biochemical Zoology **87**:1–14.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecology Letters **14**:419–431.

Des Roches, S., D. M. Post, N. E. Turley, J. K. Bailey, A. P. Hendry, M. T. Kinnison, J. A. Schweitzer, and E. P. Palkovacs. 2018. The ecological importance of intraspecific variation. Nature Ecology & Evolution **2**:57–64.

Dick, J. T., M. E. Alexander, J. M. Jeschke, A. Ricciardi, H. J. MacIsaac, T. B. Robinson, S. Kumschick, O. L. Weyl, A. M. Dunn, and M. J. Hatcher. 2014. Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. Biological Invasions **16**:735–753.

Dick, J. T., K. Gallagher, S. Avlijas, H. C. Clarke, S. E. Lewis, S. Leung, D. Minchin, J. Caffrey, M. E. Alexander, C. Maguire, C. Harrod, N. Reid, N. R. Haddaway, K. D. Farnsworth, M. Penk, and A. Ricciardi. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions **15**:837–846.

Dick, J. T., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, and N. G. Taylor. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology **54**:1259–1267.

Evangelista, C., J. Cucherousset, and A. Lecerf. 2019. Contrasting ecological impacts of geographically close invasive populations. Oecologia **189**:529–536.

Fořt, M., M. S. Hossain, A. Kouba, M. Buřič, and P. Kozák. 2019. Agonistic interactions and dominance establishment in three crayfish species non-native to Europe. Limnologica **74**:73–79.

Gaertner, M., J. R. U. Wilson, M. W. Cadotte, J. S. MacIvor, R. D. Zenni, and D. M. Richardson. 2017. Non-native species in urban environments: patterns, processes, impacts and challenges. Biological Invasions **19**:3461–3469.

Gallardo, B., M. Clavero, M. I. Sánchez, and M. Vilà. 2016. Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology **22**:151–163.

Garvey, J. E., R. A. Stein, and H. M. Thomas. 1994. Assessing how fish predation and interspecific prey competition influence a crayfish assemblage. Ecology **75**:532–547.

Gherardi, F., L. Aquiloni, and E. Tricarico. 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: an imperfect map is better than no map. Current Zoology **58**:567–579.

Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt. 2010. A framework for community interactions under climate change. Trends in Ecology & Evolution **25**:325–331.

Gutekunst, J., R. Andriantsoa, C. Falckenhayn, K. Hanna, W. Stein, J. Rasamy, and F. Lyko. 2018. Clonal genome evolution and rapid invasive spread of the marbled crayfish. Nature Ecology & Evolution **2**:567–573.

Havel, J. E., K. E. Kovalenko, S. M. Thomaz, S. Amalfitano, and L. B. Kats. 2015. Aquatic invasive species: challenges for the future. Hydrobiologia **750**:147–170.

Hirvonen, H., S. Holopainen, N. Lempiäinen, M. Selin, and J. Tulonen. 2007. Sniffing the trade-off: effects of eel odours on nocturnal foraging activity of native and introduced crayfish juveniles. Marine and Freshwater Behaviour and Physiology **40**:213–218.

Hobbs, R. J., E. Higgs, C. M. Hall, P. Bridgewater, F. S. Chapin III, E. C. Ellis, J. J. Ewel, L. M. Hallett, J. Harris, K. B. Hulvey, S. T. Jackson, P. L. Kennedy, C. Kueffer, L. Lach, T. C. Lantz, A. E. Lugo, J. Mascaro, S. D. Murphy, C. R. Nelson, M. P. Perring, D. M. Richardson, T. R. Seastedt, R. J. Standish, B. M. Starzomski, K. N. Suding, P. M. Tognetti, L. Yakob, and L. Yung. 2014. Managing the whole landscape: historical, hybrid, and novel ecosystems. Frontiers in Ecology and the Environment **12**:557–564.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution **24**:599–605.

Holling, C. S. 1959. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. The Canadian Entomologist **91**:293–320.

Hossain, M. S., J. Kubec, A. Kouba, P. Kozák, and M. Buřič. 2019. Still waters run deep: marbled crayfish dominates over red swamp crayfish in agonistic interactions. Aquatic Ecology **53**:97–107.

Jackson, M. C. 2015. Interactions among multiple invasive animals. Ecology **96**:2035–2041.

Jackson, M. C., T. Jones, M. Milligan, D. Sheath, J. Taylor, A. Ellis, J. England, and J. Grey. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. Freshwater Biology **59**:1123–1135.

Jeschke, J. M., and K. Hohberg. 2008. Predicting and testing functional responses: an example from a tardigrade–nematode system. Basic and Applied Ecology **9**:145–151.

Jeschke, J. M., M. Kopp, and R. Tollrian. 2002. Predator functional responses: discriminating between handling and digesting prey. Ecological Monographs **72**:95–112.

Jeschke, J. M., and R. Tollrian. 2005. Effects of predator confusion on functional responses. Oikos **111**:547–555.

Jimenez, S. A., and Z. Faulkes. 2011. Can the parthenogenetic marbled crayfish Marmorkrebs compete with other crayfish species in fights? Journal of Ethology **29**:115–120.

Johnson, P. T., J. D. Olden, C. T. Solomon, and M. J. Vander Zanden. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. Oecologia **159**:161–170.

Jones, H. P., B. R. Tershy, E. S. Zavaleta, D. A. Croll, B. S. Keitt, M. E. Finkelstein, and G. R. Howald. 2008. Severity of the effects of invasive rats on seabirds: a global review. Conservation Biology **22**:16–26.

Jones, J. P., J. R. Rasamy, A. Harvey, A. Toon, B. Oidtmann, M. H. Randrianarison, N. Raminosoa, and O. R. Ravoahangimalala. 2009. The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. Biological Invasions **11**:1475–1482.

Kortet, R., and A. Hedrick. 2007. A behavioural syndrome in the field cricket *Gryllus integer*: intrasexual aggression is correlated with activity in a novel environment. Biological Journal of the Linnean Society **91**:475–482.

Kouba, A., A. Petrusek, and P. Kozák. 2014. Continental-wide distribution of crayfish species in Europe: update and maps. Knowledge and Management of Aquatic Ecosystems **413**:05.

Larson, E. R., L. A. Twardochleb, and J. D. Olden. 2017. Comparison of trophic function between the globally invasive crayfishes *Pacifastacus leniusculus* and *Procambarus clarkii*. Limnology **18**:275–286.

LeBrun, E., C. Tillberg, A. Suarez, P. Folgarait, C. Smith, and D. Holway. 2007. An experimental study of competition between fire ants and Argentine ants in their native range. Ecology **88**:63–75.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. Proceedings of the Royal Society of London B: Biological Sciences **269**:2407–2413.

Lodge, D. M., C. A. Taylor, D. M. Holdich, and J. Skurdal. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. Fisheries **25**:7–20.

Lohrer, A. M., and R. B. Whitlatch. 2002. Interactions among aliens: apparent replacement of one exotic species by another. Ecology **83**:719–732.

Louthan, A. M., D. F. Doak, and A. L. Angert. 2015. Where and when do species interactions set range limits? Trends in Ecology & Evolution **30**:780–792.

Luna, A. J. F., J. I. Hurtado-Zavala, T. Reischig, and R. Heinrich. 2009. Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. Journal of Biological Rhythms **24**:64–72.

Marten, M., C. Werth, and D. Marten. 2004. The Marbled crayfish (Cambaridae, Decapoda) in Germany - another neozoan in the Rhein basin. Lauterbornia **50**:17–23.

Martin, A. L., and P. A. Moore. 2007. Field observations of agonism in the crayfish, *Orconectes rusticus*: shelter use in a natural environment. Ethology **113**:1192–1201.

Meira, A., M. Lopes-Lima, S. Varandas, A. Teixeira, F. Arenas, and R. Sousa. 2019. Invasive crayfishes as a threat to freshwater bivalves: Interspecific differences and conservation implications. Science of the Total Environment **649**:938–948.

Morales-Castilla, I., M. G. Matias, D. Gravel, and M. B. Araújo. 2015. Inferring biotic interactions from proxies. Trends in Ecology & Evolution **30**:347–356.

Nentwig, W., S. Bacher, S. Kumschick, P. Pyšek, and M. Vilà. 2018. More than "100 worst" alien species in Europe. Biological Invasions **20**:1611–1621.

Nilsson, P. A., K. Nilsson, and P. Nyström. 2000. Does risk of intraspecific interactions induce shifts in prey-size preference in aquatic predators? Behavioral Ecology and Sociobiology **48**:268–275.

Oaten, A., and W. W. Murdoch. 1975. Functional response and stability in predator-prey systems. The American Naturalist **109**:289–298.

Ortega, Y. K., and D. E. Pearson. 2005. Weak vs. strong invaders of natural plant communities: assessing invasibility and impact. Ecological Applications **15**:651–661.

Pearson, D. E., Y. K. Ortega, Ö. Eren, and J. L. Hierro. 2018. Community assembly theory as a framework for biological invasions. Trends in Ecology & Evolution **33**:313–325.

Peiman, K. S., and B. W. Robinson. 2010. Ecology and Evolution of Resource-Related Heterospecific Aggression. The Quarterly Review of Biology **85**:133–158.

Pintor, L. M., A. Sih, and M. L. Bauer. 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos **117**:1629–1636.

Pintor, L. M., A. Sih, and J. L. Kerby. 2009. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. Ecology **90**:581–587.

Prentis, P. J., J. R. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008. Adaptive evolution in invasive species. Trends in Plant Science **13**:288–294.

Rauschert, E. S. J., and K. Shea. 2017. Competition between similar invasive species: modeling invasional interference across a landscape. Population Ecology **59**:79–88.

Reynolds, J. 2011. A review of ecological interactions between crayfish and fish, indigenous and introduced. Knowledge and Management of Aquatic Ecosystems **401**:10.

Ricciardi, A., M. F. Hoopes, M. P. Marchetti, and J. L. Lockwood. 2013. Progress toward understanding the ecological impacts of nonnative species. Ecological Monographs **83**:263–282.

Rosewarne, P. J., R. J. G. Mortimer, R. J. Newton, C. Grocock, C. D. Wing, and A. M. Dunn. 2016. Feeding behaviour, predatory functional responses and trophic interactions of the invasive Chinese mitten crab (*Eriocheir sinensis*) and signal crayfish (*Pacifastacus leniusculus*). Freshwater Biology **61**:426–443.

Russell, J. C., S. Caut, S. H. Anderson, and M. Lee. 2015. Invasive rat interactions and over-invasion on a coral atoll. Biological Conservation **185**:59–65.

Russell, J. C., N. S. Sataruddin, and A. D. Heard. 2014. Over-invasion by functionally equivalent invasive species. Ecology **95**:2268–2276.

Saul, W. C., and J. M. Jeschke. 2015. Eco-evolutionary experience in novel species interactions. Ecology Letters **18**:236–245.

Sax, D. F., and J. H. Brown. 2000. The paradox of invasion. Global Ecology and Biogeography **9**:363–371.

Scheiner, S. M. 1993. Genetics and Evolution of Phenotypic Plasticity. Annual Review of Ecology and Systematics **24**:35–68.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters **7**:153–163.

Sih, A., A. Bell, and J. C. Johnson. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution **19**:372–378.

Sih, A., and A. M. Bell. 2008. Insights for behavioral ecology from behavioral syndromes. Advances in the Study of Behavior **38**:227–281.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution **13**:350–355.

Simberloff, D., and B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biological Invasions **1**:21–32.

Smout, S., C. Asseburg, J. Matthiopoulos, C. Fernández, S. Redpath, S. Thirgood, and J. Harwood. 2010. The functional response of a generalist predator. Plos One **5**:e10761.

Sol, D., S. Timmermans, and L. Lefebvre. 2002. Behavioural flexibility and invasion success in birds. Animal Behaviour **63**:495–502.

Souty-Grosset, C., D. M. Holdich, P. Y. Noël, J. D. Reynolds, and P. Haffner. 2006. Atlas of crayfish in Europe. Muséum national d'Histoire naturelle, Paris, France.

Stein, R. A., and J. J. Magnuson. 1976. Behavioral response of crayfish to a fish predator. Ecology **57**:751–761.

Stoeckmann, A. 2003. Physiological energetics of Lake Erie dreissenid mussels: a basis for the displacement of *Dreissena polymorpha* by *Dreissena bugensis*. Canadian Journal of Fisheries and Aquatic Sciences **60**:126–134.

Tablado, Z., J. L. Tella, J. A. Sánchez-Zapata, and F. Hiraldo. 2010. The Paradox of the Long-Term Positive Effects of a North American Crayfish on a European Community of Predators. Conservation Biology **24**:1230–1238.

Thawley, C. J., and T. Langkilde. 2017. Attracting unwanted attention: generalization of behavioural adaptation to an invasive predator carries costs. Animal Behaviour **123**:285–291.

Toscano, B. J., and B. D. Griffen. 2013. Predator size interacts with habitat structure to determine the allometric scaling of the functional response. Oikos **122**:454–462.

Veselý, L., D. S. Boukal, M. Buřič, P. Kozák, A. Kouba, and A. Sentis. 2017. Effects of prey density, temperature and predator diversity on nonconsumptive predator-driven mortality in a freshwater food web. Scientific Reports **7**:18075.

Vogt, G. 2010. Suitability of the clonal marbled crayfish for biogerontological research: a review and perspective, with remarks on some further crustaceans. Biogerontology **11**:643–669.

Vogt, G. 2017. Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: insights from clonal, invasive, polyploid, and domesticated animals. Environmental Epigenetics **3**:dvx002.

Vogt, G., C. Falckenhayn, A. Schrimpf, K. Schmid, K. Hanna, J. Panteleit, M. Helm, R. Schulz, and F. Lyko. 2015. The marbled crayfish as a paradigm for saltational speciation by autopolyploidy and parthenogenesis in animals. Biology Open **4**:1583–1594.

Vogt, G., L. Tolley, and G. Scholtz. 2004. Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. Journal of Morphology **261**:286–311.

Ward-Fear, G., G. P. Brown, M. J. Greenlees, and R. Shine. 2009. Maladaptive traits in invasive species: in Australia, cane toads are more vulnerable to predatory ants than are native frogs. Functional Ecology **23**:559–568.

Werner, E. E., and B. R. Anholt. 1996. Predator-Induced Behavioral Indirect Effects: Consequences to Competitive Interactions in Anuran Larvae. Ecology **77**:157–169.

Wood, J. R., I. A. Dickie, H. V. Moeller, D. A. Peltzer, K. I. Bonner, G. Rattray, and J. M. Wilmshurst. 2015. Novel interactions between non-native mammals and fungi facilitate establishment of invasive pines. Journal of Ecology **103**:121–129.

Wright, T. F., J. Eberhard, E. Hobson, M. L. Avery, and M. Russello. 2010. Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. Ethology Ecology & Evolution **22**:393–404.

Xu, M., X. Mu, J. T. Dick, M. Fang, D. Gu, D. Luo, J. Zhang, J. Luo, and Y. Hu. 2016. Comparative functional responses predict the invasiveness and ecological impacts of alien herbivorous snails. Plos One **11**:e0147017.