New species from the Pacific

Establishment and dispersal of two invasive crabs (genus *Hemigrapsus*) in German coastal waters

Dissertation zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel
vorgelegt von

Jonas C. Geburzi

Kiel, 2018

New species from the Pacific

Establishment and dispersal of two invasive crabs (genus *Hemigrapsus*) in German coastal waters

Dissertation
zur Erlangung des Doktorgrads
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts Universität zu Kiel
vorgelegt von

Jonas C. Geburzi

Kiel, 2018

Referent: Prof. Dr. Günther B. Hartl
Koreferent: PD. Dr. Dirk Brandis

Tag der mündlichen Prüfung: 16. April 2018
Zum Druck genehmigt: 16. April 2018

Abstract

Human-mediated introductions of species crossing oceans and continents gain increasing scientific and public attention as a major biological consequence of globalisation. Marine ecosystems are facing particularly high numbers of biological invasions. Gaining knowledge about factors promoting invasion success and how they interact with each other is crucial to assess impacts of invasions, as well as future invasion events and range extensions. The first study in this thesis gives an introductory overview on this topic, highlighting the importance of vectors, species traits, interactions and invasion history as promoting factors.

The scope of this thesis is an investigation of such promoting factors and their effects in a recent marine invasion in Europe, the notable case of the northwestern Pacific crabs *Hemigrapsus takanoi* and *H. sanguineus*. To gain an in-depth understanding of the investigated factors, a comprehensive approach, combining observational, experimental and genetic data, is used.

The second and third study of this thesis are dedicated to the most recent range extension of *H. takanoi* into the Baltic Sea. The second study contains the first Baltic Sea record of *H. takanoi* which was made in summer 2014. As a follow-up, the third study aimed to clarify the ability of *H. takanoi* to reproduce in the Baltic Sea, being a crucial step in an invasion process. The study contains a reconstruction of the full larval cycle of *H. takanoi* as derived from plankton samples, thus confirming successful reproduction. It further contains the first morphological descriptions of this species' larval stages, as well as data on the distribution of the larvae in Kiel Fjord. The observed spatial patterns indicate small-scale migrations to optimise salinity conditions for larval development. These results ultimately show the ability of *H. takanoi* to very rapidly adapt to new environmental conditions.

The fourth and fifth study broaden the scope from a specific invasion event to general factors contributing to the invasion success of *Hemigrapsus spp.* in Europe. Adopting different methods, namely experimental ecology and population genetics, allowed to gain deeper insights into two previously underrepresented aspects of the invasion biology of the two species. The fourth study, concentrating on early life-history traits between native and invasive crabs in the northern Wadden Sea, highlighted two mechanisms promoting a successful establishment despite the presence of a native competitor, the European shore crab (*Carcinus maenas*). Results of a two-year survey revealed a pronounced temporal shift in recruitment of juveniles, reducing competition between native and invasive crabs in a crucial phase of their life cycle. Interaction experiments further revealed interactions by which *Hemigrapsus spp.* enhanced the recruitment success of conspecific juveniles while at the same time impairing recruitment success of their native competitor.

The fifth study consists of a genetic analysis of European *H. takanoi* populations, including the most recently established populations in the Baltic Sea. It revealed a weak, but significant structuring based on genetic differentiation between populations in different regions, indicating that *H. takanoi* was at least twice independently introduced to Europe. It further shows that the recent invasion of the Baltic Sea results from a secondary introduction from the neighbouring North Sea, highlighting the importance of regional vectors for range extensions of invasive species after initial introductions.

Overall, this thesis shows the invasion of *Hemigrapsus spp.* in Europe to be a still dynamic and ongoing process, illustrated by the reported introduction and establishment of *H. takanoi* in the Baltic Sea. It furthermore shows that combinations of different aspects of a species' biology, in this case ecological adaptations and interactions as well as invasion history, jointly contribute to invasion success in marine species.

Zusammenfassung

Durch menschliche Aktivitäten verursachte Einschleppungen von Arten über die Grenzen von Kontinenten und Ozeanen hinweg erhalten zunehmend wissenschaftliche und öffentliche Aufmerksamkeit als biologische Folge der Globalisierung. Insbesondere marine Ökosysteme sind einer großen Zahl biologischer Invasionen ausgesetzt. Um die Auswirkungen und das Ausweitungspotenzial biologischer Invasionen, ist ein gutes Verständnis all jener Faktoren wichtig, die eine erfolgreiche Invasion ausmachen. Diese Erkenntnisse helfen außerdem, mögliche zukünftige Invasionen abzuschätzen. Eine tiefer gehende Einleitung in die Thematik gibt die erste Studie dieser Arbeit. Darin wird insbesondere auf die Bedeutung von Vektoren, Merkmalen und Wechselwirkungen invasiver Arten, sowie der Invasionsgeschichte als förderliche Faktoren erfolgreicher Invasionen eingegangen.

Im Fokus dieser Arbeit liegt die Untersuchung solcher Faktoren und ihrer Effekte am Beispiel einer aktuellen marinen Invasion in Europa, dem bemerkenswerten Fall der nordwestpazifischen Krabben *Hemigrapsus takanoi* und *H. sanguineus*. Mit einem umfassenden methodischen Ansatz, der Beobachtungs-, ökologische und gentische Daten kombiniert, soll ein detailliertes Verständnis der untersuchten Faktoren erreicht werden.

Die zweite und dritte Studie dieser Arbeit widmen sich der jüngsten Habitatausweitung von *H. takanoi* in den Ostseeraum. Die zweite Studie dokumentiert den ersten Fund von *H. takanoi* in der Ostsee im Sommer 2014. Die daran anschließende dritte Studie verdeutlicht die Fähigkeit von *H. takanoi,* sich lokal zu reproduzieren, was einen entscheidenden Schritt im Etablierungsprozess darstellt. Auf der Basis von Planktonproben wird der vollständige Larvalzyklus von *H. takanoi* rekonstruiert, und so die Fähigkeit zu erfolgreicher Reproduktion bestätigt. Die Studie beschreibt darüber hinaus erstmals die Morphologie der Larvenstadien dieser Art, sowie ihre Verbreitung in der Kieler Förde. Weiterhin werden kleinskalige Migrationsbewegungen nachgewiesen, mit denen die Larven optimale Salinitätsbedingungen für ihre Entwicklung erreichen.

Diese Beobachtungen belegen die Fähigkeit von *H. takanoi*, sich innerhalb kürzester Zeit an neue Umweltbedingungen anzupassen.

Die vierte und fünfte Studie bilden einen Übergang von einem spezifischen Invasionsereignis zu allgemeingültigeren Faktoren, die den Invasionserfolg von *Hemigrapsus spp.* in Europa begründen. Die gewählten Methoden der experimentellen Ökologie und der Populationsgenetik gewähren tiefere Einblicke in zwei bisher wenig untersuchte Aspekte der Invasionsbiologie dieser beiden Arten. Die vierte Studie konzentriert sich dabei auf Interaktionen in frühen Lebensstadien der einheimischen und invasiven Krabben im nördlichen Wattenmeer. Sie belegt die Existenz zweier Mechanismen, die die erfolgreiche Ansiedlung trotz direkter Konkurrenz durch die einheimische Europäische Strandkrabbe *Carcinus maenas* ermöglicht. Die Daten einer zweijährigen Feldstudie zeigen eine deutliche zeitliche Verschiebung der Ansiedlungsperioden juveniler Krabben, was die interspezifische Konkurrenz zwischen einheimischen und invasiven Arten in einer kritischen Phase ihres Lebenszyklus verringert. Interaktionsexperimente verdeutlichen weiterhin einen Mechanismus, mit Hilfe dessen *Hemigrapsus spp.* die Ansiedlung juveniler Artgenossen verstärkt, während die Ansiedlung artfremder, einheimischer Individuen vermindert wird.

Die fünfte Studie beinhaltet eine genetische Analyse der europäischen *H. takanoi* Populationen, einschließlich der kürzlich etablierten Populationen in der Ostsee. Es zeigen sich schwache, aber signifikante genetische Unterschiede zwischen den Populationen unterschiedlicher Regionen. Diese Unterschiede deuten auf mindestens zwei unabhängige Einschleppungen nach Europa hin. Sie belegen weiterhin, dass die aktuelle Invasion in den Ostseeraum auf einen Sekundäreintrag aus der benachbarten Nordsee zurückgeht, und verdeutlicht damit die große Bedeutung regionaler Transportvektoren für die Habitatausweitung von invasiven Arten nach deren ursprünglicher Einschleppung.

Insgesamt zeigen die Ergebnisse dieser Arbeit, dass die Ausbreitung und Etablierung von *Hemigrapsus spp.* in Europa ein weiterhin dynamischer, andauernder Prozess ist. Besonders verdeutlichen dies die Erstfunde und die Etablierung einer fortpflanzungsfähigen Population von *H. takanoi* in der Ostsee. Sie zeigt weiterhin, dass die Kombinationen unterschiedlicher artpspezifischer Aspekte, konkret die Anpassung, Interaktion und Invasionsgeschichte, gemeinsam zum Erfolg mariner Neozoen beitragen.

Danksagung

Viele Menschen haben auf unterschiedliche Weise zum Gelingen dieser Arbeit beigetragen. Bei ihnen allen bedanke ich mich sehr herzlich für alle Hilfe, Unterstützung und gemeinsame Zeit in den vergangenen drei Jahren.

Prof. Dr. Günther Hartl, Dr. Dirk Brandis und Dr. Christian Buschbaum, ein riesengroßer Dank für die Bereitschaft, mich gemeinsam zu betreuen, für alle Unterstützung, Inspiration und die "Freiheit der Forschung", die sie mir von der Planung dieses Promotionsprojektes an gewährt haben. Dirk Brandis außerdem ein besonderer Dank dafür, dass er vor über fünf Jahren den "Erstkontakt" zwischen mir und den Krabben hergestellt hat, die mich seitdem wissenschaftlich begleiten – oder andersherum?

Zu drei Betreuern gehören auch drei Arbeitsgruppen. Allen ihren Mitgliedern bin ich sehr dankbar, dass ich überall das Gefühl hatte, voll dazu zu gehören – obwohl ich die meiste Zeit *nicht* da war.

Die *AG Populationsgenetik der CAU Kiel*,
mit einem besonderen Dank an Juan Valqui für die große Hilfe beim Einstieg in die Populationsgenetik und für das Verbreiten entspannt-postiver Grundstimmung in der hindernisreichen Startzeit; außerdem an Romy, Basti und Lukas für die gute Zeit.

Das *Zoologische Museum Kiel*,
wo prinzipiell immer mal Freitag sein konnte, mit allen schrecklich-schönen Folgen. Für die gemeinsame Zeit als Kellerkinder ein großer Dank an Teff und Sarah, mit denen es auch als "Reisegruppe Singapur" und auf der "Butterfahrt nach Barca" großartig war; sowie an Nena – bei dir würde ich mich gerne melden dürfen, sollte ich irgendwann nochmal über Weihnachten ein Paper schreiben müssen. Ein riesengroßes Dankeschön außerdem an Christine Ewers-Saucedo für die Hilfe bei der Auswertung der Genetikdaten; und an Jana, Hannah, Axel und Michael für die regelmäßige Beteili-

gung an den Freitagsausbrüchen. Zum Glück habe ich in Häkelgarnele und Teleskop-Rückenkratzer bleibende Erinnerungsstücke...

Die *AG Gemeinschafts- und Evolutionsökologie der AWI-Wattenmeerstation in List auf Sylt*,
vor allem danke ich Rebekka und Paula für die beste Übernachtungsgelegenheit der Insel und das geteilte Büro. Ich hoffe, mich irgendwann revanchieren zu können. Außerdem Annika für die große Hilfe bei der Feldarbeit neben ihrer Masterarbeit her, und Lisa, Mathias, Kaibil und Andreas K. für alle tat- und wortkräftige Unterstützung bei der Planung, Umsetzung und Auswertung meiner Experimente. Der "Hottest Crustacean Band": Camille, Sabine, Marieke und Andreas P.; außerdem Ana, Annabell, Eike, Martin, Rune, Lasse und Tobi ein großer Dank für zwei – trotz aller Arbeit – tolle Sommer und eine sehr musikalische Weihnachtsfeier.

Ein großes Dankeschön geht jeweils auch nach Bremen, Bayern und Südafrika.

An Simon und Jannes, den weiteren Mitgliedern der Konferenzreisegruppen und *Hemigrapsus*-Experten für den Austausch und die gute Zeit in Singapur und Barcelona.

Und an Christoph Schubart und seine Arbeitsgruppe in Regensburg dafür, dass ich zwei Mal als Gast viel über Crustaceengenetik lernen durfte, im Tausch für einen mehr oder weniger gut vorbereiteten Vortrag; sowie an Christoph und seine Familie für die herzliche Gastfreundschaft bei beiden Besuchen.

Ohne finanzielle Unterstützung wäre diese Doktorarbeit so nicht möglich gewesen.

Deshalb bin in der *Deutschen Bundesstiftung Umwelt (DBU)* sehr dankbar für das Promotionsstipendium, das mir die Durchführung dieser Arbeit ermöglicht hat. Ganz besonders aber auch für die ideelle Förderung, vor allem den Kontakt und Austausch mit allen StipendiatInnen, die ich auf den Seminaren kennenlernen durfte. Ein besonderer Dank geht an Dr. Volker Wachendörfer für die persönliche Betreuung, und an Claudia, Anne und Anne. Nicht nur, aber auch für die Nacht im Labyrinth...

Und schließlich mein großer, großer Dank an die Menschen, die immer da waren, ganz egal, wie die Arbeit gerade lief.

Meinen Eltern für alle Unterstützung und Ermutigung, sowie meiner Familie und allen Freundinnen und Freunden für jeden guten Gedanken und gedrückten Daumen, vor allem in den letzten Wochen.

Sinikka, dafür dass du da warst, da bist und da bleibst.

Und dir, kleinem Menschen auf dem Weg, dafür dass du mir in den letzten fast neun Monaten immer wieder gezeigt hast, was wirklich wichtig ist. Ich freue mich auf dich!

Contents

List of Figures

List of Tables

Part I

General Introduction

Background

Since humans travel the seas, they transfer marine organisms from their native ranges to foreign coasts, across oceans, and even to different continents. For centuries, this used to be the occasional transport and release of rather few individuals attached to, or dwelled into wooden ship's hulls, hidden between rocks used as ballast, or carried along as live supplies on long-distance cruises (e.g. Brawley et al. 2009). During the last 130 years, however, increasing trade and global traffic turned introductions of non-native marine species into a world-wide mass phenomenon, which is now considered a major consequence of global change (Carlton 1999, Ruiz et al. 2000, Occhipinti-Ambrogi 2007, Simberloff et al. 2013).

Today, even medium-sized container vessels and bulk carriers exchange tens of thousands of cubic meters of ballast water between their ports of origin and destination – including the organisms living in this water (Comittee on Ships' Ballast Operations et al. 1996), and offer several thousands of square meters of underwater hull surface – prone to the establishment of species-rich fouling communities (Gollasch 2002). Up to 10'000 different species are estimated to be transferred over biogeographic boundaries in any 24 hour period this way, making ship traffic the most important vector for marine species' introductions (Carlton 1999). Intentional and unintentional introductions of aquaculture organisms and associated organisms by intensified global exchange of cultured seafood organisms, and artificial waterways, shortening ship travel times and opening new migration routes, further contribute to continuously rising numbers of introduction events (Ruiz et al. 2000, Naylor et al. 2001, Galil 2009, Katsanevakis et al. 2013). Out of the numerous species initially introduced, only a fraction manages to establish in their new habitats, and again a fraction of these undergoes strong population growth and spreads further beyond their initial site of introduction. This last group of non-native species, which significantly impacts the recipient system, is consequently termed 'invasive species' (compare e.g. Bax et al. 2003, Colautti & MacIsaac 2004).

Identifying factors that promote a species' successful establishment and spread is crucial to understand what turns an introduced into an invasive species, to assess its potential for further range extensions and its impact on the invaded ecosystem. Furthermore, knowledge of such factors from a variety of non-native species allows to infer more general patterns, which is crucial for risk assessments and management tools concerning species' invasions (Bremner 2008, Williams & Grosholz 2008). From a purely scientific point of view, successful (and failed) invasions offer unique opportunities to study plastic and evolutionary adaptations of species to a new environment, the development of novel interactions between native and introduced species, and functional community ecology in response to biological perturbations (e.g. Sakai et al. 2001, Facon et al. 2006, Zenni & Nuñez 2013, Papacostas et al. 2017).

Among marine non-native species, crustaceans, especially amphipods and decapods, are a particularly prominent group, in terms of species numbers as well as distribution and impacts (Brockerhoff & McLay 2011, Hänfling et al. 2011). They appear as one of the most species-rich groups in many invasion inventories and studies of invasion vectors from different biogeographic regions (Carlton & Geller 1993, Gollasch 2006, Galil 2009, Buschbaum et al. 2012, de Castro et al. 2017). Many biological characteristics that have generally been attributed to high invasion potential can be observed in invasive decapods. These are, for example, their high diversity, tolerance and adaptability towards changing environmental conditions (biotically and abiotically), and an *r*-selected reproduction strategy with short generation times, early maturity and high fecundity (Hänfling et al. 2011). The last point in particular favours high propagule pressure during initial introduction and subsequent establishment, an important factor for successful invasions (Hollebone & Hay 2007, Lockwood et al. 2009). In connection with their planktonic larval phase, lasting up to a month in many species, it furthermore makes decapods prone to ballast water transport (Brockerhoff & McLay 2011, Hänfling et al. 2011).

The true crabs (Decapoda, Brachyura), with currently over 70 recorded non-native representatives globally (Brockerhoff & McLay 2011), bear some of the most important invasive species in coastal marine ecosystems. Crabs often take key roles in benthic communities, as they can reach high abundances and, often being opportunistic omnivores, can affect the population dynamics of numerous species in these communities (examples in Grosholz et al. 2000, Jensen et al. 2002, Griffen & Byers 2009, Forsström et al. 2015). One of the most striking cases is the European shore crab *Carcinus maenas*, today a global invader occurring on all continents (Carlton & Cohen 2003), which for example strongly affected clam and mussel populations in some invaded areas (Walton et al. 2002, Miron et al. 2005). Similarly strong effects on native benthic communities were also described for the Chinese mitten crab *Eriocheir sinensis* in its invaded ranges in Europe and California (Rudnick et al. 2005, Ojaveer et al. 2007), this species as well being infamous for causing losses in fisheries and damaging dykes and river banks by its burrowing activities (Panning 1938, Rudnick et al. 2005, and references therein). Both, *C. maenas* and *E. sinensis* have consequently been listed among the ‘100 of the world’s worst invasive species’ (Lowe et al. 2004). It is for this great potential for successful invasions, widespread dispersal and significant impacts, that novel introductions of crab species regularly raise high awareness and scientific interest.

In this thesis, I investigate the most recent establishment of non-native crab species in Europe, the notable double-invasion of the ‘Brush-clawed shore crab’ *Hemigrapsus takanoi* Asakura & Watanabe, 2005 and the ‘Asian shore crab’ *Hemigrapsus sanguineus* (De Haan, 1835) . Geographically, this work focuses on the Wadden Sea/southeastern North Sea, and the southwestern Baltic Sea, which are the current northern limits of continuous *Hemigrapsus spp.* distribution in Europe. In this area, their case offers a unique opportunity to study a highly dynamic, early-phase invasion process and its underlying promoters and biological mechanisms. The first discovery of *H. takanoi* in the Baltic Sea in 2014 especially highlighted these dynamics and consequently put the main focus of my work on this species.

The invasion record of *H. takanoi* in the Baltic Sea is included in part II of the thesis, together with a follow-up study reconstructing the full larval cycle of *H. takanoi* in Kiel Fjord and indicating rapid larval adaptations to the specific salinity conditions prevailing in this area. Part III of the thesis is dedicated to a broader investigation of factors contributing to the invasion success, consisting of an ecological study of recruitment processes and interactions of *Hemigrapsus spp.* and native *C. maenas* in the Wadden Sea, and a population genetics study on the invasion history and population structure of *H. takanoi* across Europe.

As a more detailed introduction to the general topic, a review of current concepts for understanding marine invasions, as well as their consequences and implications for management is given in the following chapter.

1. How do they do it? – Understanding the success of marine invasive species

Jonas C. Geburzi[a, b] and Morgan L. McCarthy[c, d]

[a] *Kiel University, Zoological Institute and Museum, Hegewischstr. 3, 24105 Kiel, Germany*
[b] *Alfred-Wegener-Institute, Helmholtz-Centre for Polar and Marine Research, Wadden Sea Station, Hafenstr. 43, 25992 List/Sylt, Germany*
[c] *The University of Queensland, School of Biological Sciences, St. Lucia, Qld 4072, Australia*
[d] *Vrije Universiteit Brussel (VUB), Marine Biology, Pleinlaan 2, 1050 Brussels, Belgium*

YOUMARES 8 – Oceans across boundaries: Learning from each other
The 2017 conference for YOUng MARine RESearchers in Kiel.
Environmental and Earth Sciences eBook collection,
Springer International Publishing (in press)

(References for this manuscript are included in the reference section at the end of Part I of the thesis.)

Abstract

From the depths of the oceans to the shallow estuaries and wetlands of our coasts, organisms of the marine environment are teeming with unique adaptations to cope with a multitude of varying environmental conditions. With millions of years and a vast volume of water to call their home, they have become quite adept at developing specialised and unique techniques for sur-

vival and – given increasing human mediated transport – biological invasions. A growing world human population and a global economy drives the transportation of goods across the oceans and with them invasive species via ballast water. In any given 24-hour period, there are 10’000 species being transported across different biogeographic regions. If any of them manage to take hold and establish a range in an exotic habitat, the implications for local ecosystem can be costly. This review on marine invasions highlights trends among successful non-indigenous species (NIS), from vectors of transport to ecological and physiological plasticity. Apart from summarizing patterns of successful invasions, it discusses the implications of how successfully established NIS have an impact on the local environment, economy and human health. Finally, it looks to the future and discusses what questions need to be addressed and what models can tell us about what the outlook on future marine invasions is.

1.1. Introduction

The continuously rising numbers and extending ranges of non-indigenous species (NIS) are today widely seen as a major biological aspect of global change, affecting invaded ecosystems, economy and even human health (Vitousek et al. 1996, Ruiz et al. 2000, Simberloff et al. 2013). Marine species have been anthropogenically introduced into new habitats since humans travel overseas, but it is only since about 150 years, and especially in the second half of the 20th century, that technical advances and the extreme increase in global marine trade led to the exponential increase of marine species introductions (Carlton & Geller 1993, Bax et al. 2003).

Of the hundreds of species that get introduced to habitats out of their native range, only a small fraction actually establishes permanently in their new environment, and an even smaller fraction reaches high population densities and/or successfully disperses over wider ranges with adverse impacts on the recipient system – consequently termed 'invasive species' (Sakai et al. 2001, Colautti & MacIsaac 2004). The increasing field of invasion biology uses various approaches, e.g. ecology, physiology, evolution and genetics, to investigate mechanisms and consequences of the establishment of NIS. Finding answers to the questions what makes certain species successful invaders and how invasion processes actually happen, is a main focus of invasion biology. These often include aspects that also allow to predict impacts of invasive species on the invaded communities and may disclose starting points for possible management strategies (e.g. Bremner 2008, Williams & Grosholz 2008). Furthermore, the study of biological invasions offers model systems to better understand general biological processes such as species interactions, physiological and ecological adaptations, and evolutionary processes (Ruiz et al. 2000, Stachowicz et al. 2002, Facon et al. 2006). While marine systems globally belong to the most heavily invaded ones, they have long been underrepresented in invasion biology studies compared to terrestrial and limnic systems, but this discrepancy is reduced by a quickly growing body of literature in recent years (Grosholz & Ruiz 1996, Ruiz et al. 2000, Chan & Briski 2017).

Even though it is difficult to identify universal factors and traits that lead to high invasion success due to their apparent dependency on the individual conditions of each invasion event (Sakai et al. 2001), some general patterns regularly occur in this context. These include for example common invasion pathways and vectors (Katsanevakis et al. 2013), anthropogenic alterations or perturbations of recipient habitats (Bax et al. 2000, Briggs 2012, Mineur et al. 2012), high ecological and physiological plasticity of successful invaders (Hänfling et al. 2011, Parker et al. 2013, Tepolt & Somero 2014), and the general nature of interactions between native and non-native as well as among non-native species (Snyder & Evans 2006, Johnson et al. 2009, Briggs 2010). This review aims to give an introductory overview of important aspects of successful marine invasions, including human impacts, species' traits and interactions, and invasion genetics. The second part of this review copes with ecological and socio-economic consequences of marine invasions and their implications for policy and management and closes with an outlook on future developments of the phenomenon under the perspectives of ongoing global (esp. climate) change.

1.2. Promoters of Successful Spread and Establishment

1.2.1. Vectors, Pathways and Altered Habitats – Human Impacts

Anthropogenic impacts are by definition major prerequisites for the occurrence of marine NIS, as only they allow species to reach regions beyond their natural range and dispersal limits. Besides obvious examples of direct species transportation, either intentional or unintentional, human impacts on marine habitats can also indirectly act as strong promoters of the spread and establishment of marine NIS.

Ship traffic is the most important vector of species' introductions. Ships act as vectors in two ways. First, their hulls provide a habitat for fouling communities of sessile species, which are transported between ports and may eventually get removed or detached, or release offspring into a new environment (Ruiz et al. 1997, Gollasch 2002). If the fouling layer is thick enough, also mobile species can survive transoceanic transport in sheltered cavities, as for example the Asian crab *Hemigrapsus takanoi*, which was first recorded in Europe in 1994 on a ship's hull (Gollasch 1999, then identified as *H. penicillatus*). Second, and even more important, the exchange of huge amounts of ballast water holds the potential for all species with (at least temporal) planktonic or swimming lifestyle to be taken up in one port and be released in another. Since the 1880s, when seawater started to replace solid ballast, the number of marine NIS and the frequency of introductions are constantly increasing (Carlton & Geller 1993, Ruiz et al. 1997, Ruiz & Smith 2005, Wolff 2005). The ongoing trend to ever more and bigger – increasing ballast water volume and thus the number of transported organisms – and faster – increasing the survival probability of transported organisms – vessels, further fuels this trend. The importance of international ship traffic for the dispersal of marine NIS is also underlined by the fact that especially international ports and their surroundings have often turned into hot-spots for exotic species, and that the dispersal routes of many species follow the main transoceanic shipping routes (Briggs 2012, Seebens et al. 2013). While the big container vessels and other large trading ships account to a large extent for primary species introductions across continents, regional traffic of smaller ships is an important vector for the secondary spread of marine NIS. Recent studies showed that especially recreational boating is an important driver of regional dispersal of non-native species (e.g. Clarke Murray et al. 2011, Hänfling et al. 2011).

Aquaculture is another important vector for marine NIS, which as well accounts for a rising number of introductions parallel to the global growth of this economy during the last decades (Naylor et al. 2001). Organisms with a planktonic larval stage are especially prone to ‘spill over’ from their culture areas into the surrounding habitats. This introduction pathway led for example to the invasion of the Pacific oyster *Magallana gigas* (formerly *Crassostrea gigas*) along the southeastern coast of the European North Sea. A series of warm summers following the introduction of *M. gigas* (which was initially believed to not be able to reproduce in the cold climate of the North Sea) promoted their dispersal, highlighting how a combination of human actions, environmental changes and species’ traits can lead to a successful invasion (Diederich et al. 2005, Smaal et al. 2009). Aquaculture is not only a vector for the cultured target species, but often also unintentionally introduces organisms which are associated with them if they are not vigorously cleaned before transportation. This is particularly true for invasive ecosystem-engineers like reef-building mollusks, which, once established, provide favourable conditions for the species they brought along, eventually resulting in profound shifts towards NIS-dominated communities (e.g. Ruiz et al. 2000).

Floating (plastic) litter is a vector recently gaining attention. While the marine litter problem is mostly discussed under the aspect of pollution and the hazardous effects of microplastic accumulation, larger pieces of litter are also a possible habitat for fouling organisms, which might then be transported over large distances by oceanic currents. Recent studies found a variety of species from different taxonomic groups (including bryozoans, barnacles and mollusks) settling on macroplastic, with a considerable proportion of marine NIS among them (Barnes & Milner 2005, Gregory 2009, Gil & Pfaller 2016). While driftwood and other debris may already historically have played a role in the cosmopolitan distribution of species like *Teredo navalis* (Bivalvia, Myoida) or *Lepas anatifera* (Crustacea, Pedunculata), the recent extreme increase in amounts of marine litter may to lead to a future increase in numbers on marine NIS dispersed by this vector (Gregory 2009).

Even though being no ‘classical’ vector, canals are a major introduction pathway for marine NIS. The best known example is the Suez Canal, connecting the Mediterranean Sea to the Red Sea and Indian Ocean, which accounts for the vast majority of species invasions to the Mediterranean by migration through the canal (Lessepsian migration) (Galil 2009). The Baltic Sea, as another example, was invaded by numerous ponto-caspian species since it is connected to the Black Sea by a system of canals and rivers (Leppäkoski et al. 2002, Katsanevakis et al. 2013). Additionally, the Kiel Canal provides a shortcut route between the southwestern Baltic and the southeastern North Sea. It likely served as invasion pathway for numerous species native or invasive to the Atlantic, like the crabs *Rhithropanopeus harrisii* and *H. takanoi* (Fowler et al. 2013, Geburzi et al. 2015). An interesting case in this context is the shrimp *Palaemon elegans*, of which an Atlantic type invaded the Baltic Sea from the west, and a Mediterranean/Black Sea-type invaded from the southeast (Reuschel et al. 2010). Besides opening routes for the active migration or natural (e.g. larval) dispersal processes, canals also increase the probability for successful ship-mediated introductions, as they shorten transportation times, thus increasing survival probabilities e.g. for organisms in ballast water tanks.

Besides human-mediated transportation or migration, anthropogenic habitat changes are another type of human environmental impacts which can facilitate the establishment of marine NIS. The construction of harbors and coastal defense structures such as groines or seawalls on sedimentary coasts or in estuaries for example adds artificial rocky habitats to naturally soft-bottomed environments. Such new habitats are often rapidly colonized by non-native species as native species are less adapted to their conditions (Mineur et al.

2012). They may also serve as ‘stepping stones’ for the dispersal of rocky-shore species (Landschoff et al. 2013), a function which is also currently investigated with regard to the increasing number of offshore wind farms (Petersen & Malm 2006). Water pollution and eutrophication are discussed as additional anthropogenic impacts which lead to disturbances of marine ecosystems and communities, making them more receptive to invasions (Reise et al. 2006, Briggs 2007).

1.2.2. Life History, Ecology and Physiology – Species’ Traits

Of the high numbers of transported and introduced species, only a small proportion successfully establishes and becomes invasive. Several ecological and life-history traits regularly occur in marine invasive species from different taxa and can therefore be associated with their success. Overall, the following traits and examples show that plasticity, for example in life-history strategies, behavior and physiology, is a key feature of successful invaders.

Many of these traits are associated with reproduction, as in the end reproductive success is the one factor determining whether a species successfully establishes and spreads. Invaders are often characterized by an *r*-selected breeding strategy (early maturity, short generation time, high fecundity, rapid growth rates) or the ability to switch between *r*- and *K*-selected strategies (reviewed in Sakai et al. 2001), enabling them to develop a high propagule pressure even from small founder populations. The ability of females to produce several broods per season when environmental conditions allow for it has as well been often observed (Hines 1986, Anderson & Epifanio 2010, van den Brink et al. 2013). For crabs in particular, Zeng et al. (2014) also discuss the ability to switch between two strategies of resource allocation – capital and income breeding – as a potential promoter of invasiveness. In some cases, also seasonality seems to have facilitated the establishment of NIS. Temporally shifted breeding periods are for example believed to reduce competition between early juveniles of native crabs and the invasive *Carcinus maenas* in Australia (Garside et al. 2015).

Having a planktonic larval stage is a common feature of many marine taxa, but it provides particular benefits for establishing NIS by increasing their dispersal abilities. This trait mostly affects the secondary regional spread after the initial introduction of a species. Some marine NIS possess a significantly longer duration of larval development compared to native species of the same taxonomic group, which is regarded as a mean of further enhancing the dispersal potential (Roman & Palumbi 2004, Viard et al. 2006, Delaney et al. 2012, Katsanevakis et al. 2013). The choice of recruitment sites by the last larval stages is often positively influenced by chemical signals released by conspecifics. For some successful invaders, however, cues from suitable habitat act as strong as recruitment enhancers as conspecific cues, a mechanism that enhances a species' ability to colonize new habitats (O'Connor 2007, Anderson & Epifanio 2009).

Resource utilization and food preference is another set of traits where successful invaders often show high levels of plasticity. Omnivory (in animals) and the ability to quickly adapt to a changed food supply is often observed (e.g. Blasi & O'Connor 2016) and allows NIS to avoid food competition with native species of the same guild. On the other hand, newly arrived species can also cause dietary shifts in native species, being both beneficial for themselves, but detrimental for the native competitor (Griffen et al. 2011).

Species physiology is an important component in determining its ability to take hold in a new habitat. Temperature and salinity are two factors highly regarded in limiting an organism's ability to expand its range as a NIS, as is the case in *Mytilus* studies (Pickens 1965, Helm & Trueman 1967, Coleman & Trueman 1971, Stickle & Sabourin 1979, Nicholson 2002, Braby & Somero 2006b). *Mytilus trossulus* is native to the North Pacific, however, it has been replaced along the California coastline from the Mexican border to Monterey Bay after the introduction of *Mytilus galloprovincialis*, a Mediterranean native, to Southern California via shipping in the 1900s (McDonald & Koehn 1988, Geller 1999). A habitat mosaic exists in San Francisco and Monterey Bay, which are both characterised by varying abiotic environmental conditions (Braby & Somero 2006a). While the invasive mussel (*M. galloprovincialis*) is genetically inclined to high temperature thermal tolerance, *M. trossulus* is well adapted to areas achieving a critical salinity level, making the matrix of habitats in Monterey and San Francisco Bay a mixed mosaic, where otherwise *M. galloprovincialis* had displaced it along the southern coast given its thermal tolerance acclimation advantage (Braby & Somero 2006b).

1.2.3. Competition, Facilitation and Parasitism – Species' Interactions

Wherever NIS are introduced, they develop interactions with both native and other non-native species in their new habitat. At the same time, important interactions with species in their native range may fall away. Either way, shifts in the interaction regimes of NIS during the invasion process are probably among the most important factors determining the long-term potential for a successful establishment after initial introduction.

Many invasive species are known as strong competitors, having negative effects on native species occupying the same niche, which in turn facilitates their own or their offspring's establishment. Common mechanisms are superiority in the competition for food and shelter, or for optimal settlement space in the case of sessile animals and plants, respectively (Ruiz et al. 1999, Jensen et al. 2002, Levin et al. 2002, van den Brink et al. 2012, Katsanevakis et al. 2013). Several studies also reported direct predation pressure by invaders on native species within the same guild (Ruiz et al. 1999, and references therein). Some authors (Briggs 2010) relate the strong competitiveness of many NIS to a regularly observed biogeographical pattern of marine invasions: They often originate from regions with high biodiversity and are therefore well adapted to strong competition. This makes them superior over native species of their recipient regions which are often characterized by lower biodiversity. This pattern is also addressed as 'enemy-release-hypothesis' (Bax et al. 2001, Brockerhoff & McLay 2011), and assumed to significantly contribute to the observation that successful invaders often 'perform better' (grow bigger, reproduce more) in their invaded compared to their native ranges (Parker et al. 2013).

On the other hand, also positive interactions may enhance invasion success of marine NIS. They have been described to occur among species invading the same region, where the establishment of a first species (often an ecosystem-engineer, see above) facilitates subsequent invasions of further species (Fridley et al. 2007, Altieri & Irving 2017). The initial invader might either provide directly beneficial effects for subsequent invaders (e.g. habitat or food) or exert detrimental effects for native competitors of subsequent invaders (e.g. predation, pathogens, structural habitat changes). Such cascading effects have led to the assumption that increasingly invaded systems become more susceptible to further introductions, cumulating in ‘invasional meltdown’ scenarios (Simberloff & Von Holle 1999, Grosholz 2005). Empirical evidence for ‘invasional meltdown’ is however scarce (Simberloff 2006, Briggs 2012). At the same time, an increasing number of studies report both negative interactions between NIS (Lohrer & Whitlatch 2002, Griffen et al. 2008, Griffen 2016) and positive effects of NIS on native species (Rodriguez 2006, and references therein). In summary, these studies underline the complexity of species interactions in the context of NIS establishment, making predictions on general interaction patterns and long-term invasion success extremely difficult.

Parasitism is another type of species’ interactions with the potential to strongly affect invasion success. Just like being released from enemies, a release from parasites often occurs during the translocation process of many species, resulting in a much lower parasite load of introduced compared to native populations (Snyder & Evans 2006, McDermott 2011, Fowler et al. 2013). Direct positive effects of reduced parasite load include for example increased survival and fecundity (especially when released from sterilising parasites). Even more important are the indirect effects by the reduced need to invest in parasite defense, allowing organisms to reallocate those resources to traits like growth or reproduction (Goedknegt et al. 2016). Reduced investment in parasite defense however results in higher susceptibility to parasite infections, which may in turn negatively impact establishment success (Keogh et al. 2016). Introduced non-native parasites on the other hand can reach extreme invasion success when they are able to infect native species which are closely related to their original host, but have only weak defensive traits due to the lack of coevolution (examples in Ruiz et al. 1999, Feis et al. 2016). This could theoretically even promote the invasion success of the original host, which may gain competitive advantages over its native relative by being better adapted to infections.

1.2.4. Selection, Multiple Introductions and Hybridisation – Invasion Genetics

Species introductions have the potential to trigger rapid evolutionary changes and adaptation processes acting on the genetic level. Invasion genetics therefore play an important role in determining long-term success of species introductions and their evolutionary consequences for the respective species (Holland 2000, Geller et al. 2010).

Usually, introduction and colonisation processes of species into new habitats are associated with a considerable reduction of genetic diversity by strong genetic drift or bottleneck effects. One would therefore expect to regularly observe negative effects of genetic depletion in newly establishing populations, especially a reduced ability to adapt to changing environmental conditions. This seems, however, often not to be the case (examples in Tsutsui et al. 2000, Hänfling 2007). Possible reasons are for example co-segregation of fixed loci or changes in frequencies of rare (recessive) alleles caused by the reduction of population size, leading to an actual increase in additive genetic diversity (Hänfling 2007, Facon et al. 2008, and references therein). If, by chance events, advantageous genotypes develop under these conditions, they can rapidly become fixed in a small founder population due to the strong selective forces. Multiple introductions of the same species can further mitigate possibly negative effects of small founder populations. They will often be not recognised as long as no genetic studies are performed (‘cryptic invasions’), but are likely to occur in many introduced species. If repeated introductions originate from different source populations, this leads to an admixture of genotypes, holding the potential to strongly increase the adaptive abilities of the species by novel combination of alleles (Hänfling 2007, Herborg et al. 2007, Chan & Briski 2017). This is believed to considerably contribute to the invasiveness of global invaders like the European shore crab *Carcinus maenas* (Geller et al. 1997, Roman 2006).

Hybridisation between native species and NIS regularly occurs in animals with external fertilisation like mollusks and fish, and especially in plants. From the invader’s perspective, it increases the chances to successfully establish despite small founder population sizes either by introgression of native alleles which enhance adaptive evolution, or by the development of new hybrid lineages combining beneficial traits from both parental lineages (Sakai et al. 2001, Hänfling 2007). The latter can occasionally lead to hybrid superiority and eventually result in the displacement of native species by newly evolved hybrids. This has been for example observed for cordgrass, *Spartina sp.*, in Great Britain and North America, where hybrids between native and invasive species disperse more successfully than their parent species (Huxel 1999, Williams & Grosholz 2008, and references therein).

1.3. Why Does It Matter?

1.3.1. Ecological Impacts

The evidence is overwhelming that NIS invasions are a significant stressor to marine communities and has been observed in invasions by plants, fish, crabs, snails, clams, mussels, bryozoans and nudibranchs (Ruiz et al. 1999). Invasive species are the second greatest cause of extinction in world fish (Clavero & García-Berthou 2005). Furthermore, anthropogenic derived disturbances and the introduction of new species are skewing food webs towards a loss of higher trophic groups and a gain in lower order consumers, the effects of which are not yet fully understood (Byrnes et al. 2007). Species most responsible for dramatic ecosystem alterations are NIS that act as ecosystem engineers.

An ecosystem engineer is an organism that alters the availability of resources to other species. Jones et al. (1994) described ecosystem engineers as falling into two categories, autogenic and allogenic. Autogenic engineers change the environment through their own physical structure. Coral for example, provides a habitat for many reef dwelling species. Allogenic engineers alter the environment by transforming living or non-living materials between physical states, as is the case for sea urchins that alter the environment by eating kelp, which would otherwise be providing a habitat for organisms as autogenic engineers (Jones et al. 1994). Broadly speaking, NIS as ecosystem engineers can provide both positive and negative impacts on their environments.

Pacific oysters (*Magallana gigas*) have been introduced globally for aquaculture purposes and have in some cases established wild oyster beds among its introduced ranges (Lejart & Hily 2011). The impact of Pacific oysters has varied from displacement of *Sabellaria* reefs, a species of conservation importance, to increases in sessile invertebrate diversity via secondary settlement on oyster shells (Olyarnik et al. 2009, Herbert et al. 2016).

Apart from the introduction of ecosystem engineers themselves, the introduction of pathogens can indirectly cause a significant alteration to the physical environment by infecting ecosystem engineers. The introduced protistan pathogens *Haplosporidium nelsoni* and *Perkinsus marinus* were partly responsible for the decline of the Virginia oyster (*Crassostrea virginica*) (Crooks 2002), historically an important ecosystem engineer in the Chesapeake Bay. The Chesapeake Bay has seen a decrease in over 90 % of its oyster population in the last century and the pathogen introduction has been recorded as a dominant factor of mortality. Additionally, results of the pathogen introduction have limited the physical structure of oysters as a habitat and as a filter feeder, thus altering the benthic and planktonic food webs (Ruiz et al. 1999).

1.3.2. The Economy and Human Health

Social and economic impacts are linked to invasive species altering fisheries, aquaculture, tourism and marine infrastructure activities. Human health is also impacted when the consequence of these alterations results in lost revenue and potentially, a direct decrease in human health (Bax et al. 2001).

The economy drives the exchange of goods across the globe via shipping routes and trade and with it come new NIS. In some instances, NIS wreak economic havoc at home by altering ecosystems and reducing exportable fish and shellfish markets through competition and disease. Few studies focus on the economic impacts of aquatic species alone and even fewer separate out marine from freshwater species impacts. Marine invasions pose an additional challenge because of the widely dispersing planktonic larvae of many marine species.

The European green crab *Carcinus maenas*, ranked in the IUCN list of the world's worst invasive alien species (Lowe et al. 2004) has had quite an economic impact on the North Atlantic since emerging from its native European range over 200 years ago (Carlton & Cohen 2003). The estimated annual losses to shellfisheries on the East Coast of the United States due to predation alone range from US$ 14.7 to US$ 18.7 million a year and up to US$ 805.9 million during the period from 1975 to 2005 (Abt Associates Inc. 2008). In addition to loss of profit from shellfish sales, green crabs are also responsible for the loss of eelgrass in restoration projects through bioturbation activities such as foraging and burrowing (Davis et al. 1998). The associated costs from these activities range from US$ 60'150 to US$ 77'433 as an estimate for the year 2006 (Abt Associates Inc. 2008). Apart from the costs associated with direct shellfish predation and eelgrass restoration projects there are also projected costs for handling further losses from the NIS. Expenditures for a proposed monitoring and control program to the US Environmental Protection Agency would cost US$ 285'000 per year (Abt Associates Inc. 2008). The European green crab is just one NIS, in one country and calculations are based on only the known impacts. In any given 24 hour period 10'000 different species are being transported across bio-geographic zones (Carlton 1999) and as demonstrated by the green crab, the economic cost of a single NIS can be quite significant, highlighting the need for effective control and management implementation.

Keller et al. (2011) highlights the possibility that there may be economic benefits in some cases. The release of the Red king crab *Paralithodes camtschaticus* into the Barents Sea and subsequent expansion into the Norwegian coast provided an income of over 9 million EUR for fisherman (Galil 2009). While the Red king crab represents one lone example of economic benefit, it is heavily outweighed by the estimated annual 2.2 billion EUR per year that aquatic NIS cost Europe (Kettunen et al. 2008). While known impacts of NIS can be calculated to the dollar, other factors may not have a monetary label, especially those concerning human health. Lafferty & Kuris (1996) describe the risk that the Chinese mitten crab *Eriocheir sinensis* in California presents as a second intermediate host for the Oriental lung fluke (*Paragonimus westermani*) which can cause paralysis in humans. If the mitten crab becomes widely abundant, it may serve as a suitable host for the native North American lung flukes and increase the potential for infection in humans (Lafferty & Kuris 1996). Another example of NIS posing a risk to human health include the establishment of the Zebra mussel (*Dreissena polymorpha*) and the Quagga mussel (*Dreissna bugensis*) in Lake Erie. The non-natives provide a link between contaminants bound to sediment in the water column up trophic levels to top predators including smallmouth bass *Micropterus dolomieui* and humans (Hogan et al. 2007).

1.3.3. Management and Policy

As Thresher & Kuris (2004) summarise, there are management efforts in outbreaks across the globe. From the marine alga *Caulerpa taxifolia* in California, the Mediterranean Sea and Australia (Meinesz et al. 2001, Cheshire et al. 2002, Williams & Grosholz 2002), to the Asian whelk *Rapana venosa* in the Chesapeake Bay (Mann & Harding 2000), to the Asian mussel *Perna viridis* in Cairns, Australia (Thresher & Kuris 2004), marine invasions are truly a global challenge. In meeting this challenge, the study highlights four key differences between management in marine and terrestrial invasion approaches. The first is that the ocean is perceived as an open system and that due to global patterns of circulation, pelagic larvae and large-scale migrations, local eradication efforts are futile. The open nature of the ocean establishes a defeatist attitude among public managers. Another challenge to marine invasions is who should bear the burden of cost. The benefits of management actions can be widespread and therefore which parties should be involved in paying for them can become convoluted. Furthermore, the public perceives the ocean and open coastline to be pristine, allowing invasions to go largely unnoticed by the public. Finally, scientific literature on the biology of most marine taxa is limited, making decisions and predicting outcomes of management practices difficult to predict (Thresher & Kuris 2004). Despite these additional challenges in managing marine invasions, there have been approaches developed which have produced mixed results.

Lovell et al. (2006) highlight some of the policies developed to limit the spread of NIS. Two main approaches to international policy have been to focus on shipping vectors as a means of distribution and by limiting the amount of imports via quarantine bans or tariffs (Lovell et al. 2006). Despite regulations, trade among the North Atlantic Free Trade Area (NAFTA) countries spreads invasive species that were introduced as a result of trade with countries outside of NAFTA (Perrault et al. 2003). It is in this manner that NIS can be introduced and widely distributed among trade partners who themselves adhere to stringent treaty regulations.

Secord (2003) highlights the options that a mere handful of marine focused studies of bioinvasions have recommended. Recommendations are centred around prevention and control. The least disturbing to the environment is the prevention of invasions in the first place. This may be manifested through public education and outreach programs, ballast water exchange and treatment options, and regulation in the aquaculture industry (Secord 2003). Second, is the eradication of small invasive populations while they are still susceptible to localised chemical or mechanical control methods. This may take form through shading algal species, chlorine treatment of marinas, or the physical smashing of individual snail hosts of an invasive parasite (Bax 1999, Culver & Kuris 2000, Dalton 2000). Invasions can further be augmented through biocontrol using native species. The advantage of this method is that it introduces no further NIS however, the implications for affected community dynamics may be put at risk. Finally, the most risky method to local ecosystem dynamics is through the introduction of other NIS to eat, parasitise, infect or compete with the invader (Secord 2003).

Returning to the example of *C. maenas*, one considered bioinvasion control for this species was the introduction of the castrating barnacle *Sacculina carcini*. After infiltrating the crab body, the parasite castrates both male and females, effectively prohibiting its victims from reproducing (Lafferty & Kuris 1996). The danger in this form of bioinvasion control is how it would impact native as well as commercially valuable crabs or shrimp (Secord 2003).

1.3.4. Future Implications in light of Climate Change

Future studies should consider marine invasions in light of changing climate conditions. As sea level rises, how will it influence the expansion of NIS ranges? If bodies of water are connected by larger channels and increased water flow, how will this impact the spread of future invasions? Will prone to acclimatizing NIS thrive when new areas of land sink beneath the rising oceans?

Educating the public on marine NIS is an important step towards keeping invasions in check. The ocean is large and peering out at it from on land, it can seem undisturbed and peaceful. Beneath the surface, however, NIS are thriving under rocks and floating through the water column as microscopic plankton. Using molecular genetic techniques, we can first study the past and use it to understand how established NIS came to be. Through public education and supporting research, we can monitor ongoing invasions and implement management actions. Finally, by carefully predicting climatic conditions of the future, we can hypothesize about the course of future marine invasions and begin to plan future management campaigns in light of global climate change.

Climate change is expected to impact aquatic invasions by warming water temperatures, altering water flow patterns and increasing storm events (Poff et al. 2002). Aquatic systems that are naturally saline will likely increase in salinity, though whether this will necessarily allow marine species to invade inland waters is still largely unknown (Rahel & Olden 2008). Climate change will also result in physiological changes, which will become apparent at the population level and as seen by shifts in abundance, timing of annually recurring events and distribution and dispersion of organisms (Doney et al. 2012). As Occhipinti-Ambrogi (2007) highlights, the range expansions of populations of NIS has already been observed to be coupled with increasing water temperatures. The Mediterranean Sea has witnessed the establishment of alien microalgae species, an increase that is largely attributed to increased water temperatures (Gómez & Claustre 2003). Other microalgae whose spread is also thought to be linked to increasing water temperatures has impacted human health. The NIS *Ostreopsis armata*, which bloomed in the Ligurian sea, caused respiratory illness in tourists exposed to it (Occhipinti-Ambrogi 2007).

One of the most comprehensive models for predicting the fate of marine invasions found that overall, there would be a high species turnover rate attributed to invasions and extinctions by the mid 21st century. In considering the distributional ranges of 1’066 marine fish and invertebrates for 2050 in a bioclimate envelope model, Cheung et al. (2009) found that patterns of species invasion as well as turnover (accounting for invading and locally extinct species) were predicted in high latitude regions of the Arctic and Southern Ocean and that combined with global extinctions, invasions and extinctions will amount to a turnover of 60 % of the present biodiversity. The potential disruptions in ecosystem services in the future based on this model remain yet to be known. With a growing world population and in light of a changing global climate, studies are needed to better understand how marine invasions will further impact our environment and economy, and how managers can better prepare for future invasions.

The studied species

Hemigrapsus takanoi Asakura & Watanabe, 2005 and *Hemigrapsus sanguineus* (De Haan, 1835) are classified within the familiy Varunidae (H. Milne Edwards, 1853), superfamily Grapsoidea of the true crabs (Brachyura) (Ng et al. 2008). The genus *Hemigrapsus* (Dana, 1851) currently comprises 13 living species (Davie & Türkay 2017), all of which are native to coastal regions around the Pacific Ocean. To date, *H. takanoi* and *H. sanguineus* are the only representatives of this genus that have been reported as non-native species. In the following, I will briefly describe the two species' main characteristics and give an overview of their invasion history.

Hemigrapsus takanoi

Hemigrapsus takanoi, the 'Brush-clawed shore crab' is a small to medium-sized crab, males growing to maximally 30 mm carapace width (CW) and about 20 mm on average, females growing to maximally 20 mm CW and about 15 mm on average (Noël et al. 1997, Gothland et al. 2014, own observations). It has a rectangular carapace shape with three anterolateral teeth on either side, a character which it shares with the other *Hemigrapsus* species. By this, they can easily be distinguished from all coastal crab species in its northern European range (Fig. I.1).

The carapace coloration of *H. takanoi* is usually brown, sometimes greenish or greyish or even almost black, often with two white dorsal spots of varying size and shape. The infraorbital ridge, running along the front below the eyes is interrupted twice on its distal end, which is the most reliable character to discriminate *H. takanoi* from *H. sanguineus* in both sexes and all ages (compare Breton et al. 2002). A further distinct character in male crabs are the prominent patches of soft setae in their chelar joints (Fig. I.2 A and B).

Hemigrapsus takanoi was only in 2005 separated from *H. penicillatus* (De Haan, 1835) and described as own species, based on molecular evidence

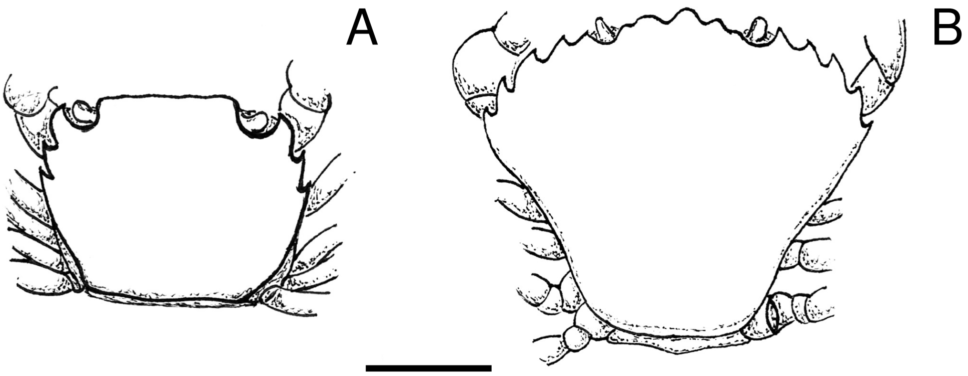


Fig. I.1. Comparison of carapace shapes of *Hemigrapsus spp.* (A), and the most common European coastal crab, the shore crab *C. maenas* (B); scale bar 10 mm.

(Asakura & Watanabe 2005). Though initially doubted (Sakai 2007), further molecular studies confirmed the separation (Mingkid et al. 2006, Asakura et al. 2008, Yamasaki et al. 2011, Markert et al. 2014) and the status of *H. takanoi* as separate species is now widely accepted. However, morphological characteristics proposed to distinguish the two sibling species (including relative size of the chelar setal patch in males, pigmentation patterns, and gonopod morphology) show some overlap or are missing in some individuals, and are thus not fully reliable (Mingkid et al. 2006, Markert et al. 2014, Makino et al. 2015). All European samples of *H.penicillatus*/*H. takanoi* that have been included in molecular investigations have been identified as *H. takanoi*, which is thus assumed to be the only of these two species invasive in Europe (Asakura & Watanabe 2005, Markert et al. 2014, Makino et al. 2017).

Due to its only recent description and its great morphological similarity with *H. penicillatus*, distribution data of *H. takanoi* in its native range are still incomplete. Data on its ecology in the native range are as well scarce, but may be assumed to be similar to *H. penicillatus*. Its currently reported distribution includes the Pacific coast of southern Russia, the Korean Peninsula, northern China and the whole Japanese archipelago, as well as Taiwan (Asakura & Watanabe 2005, Lee et al. 2013, Marin 2013, Makino et al. 2017) (Fig. I.3 A). *Hemigrapsus takanoi* inhabits the mid to lower intertidal zone and shallow subtidal areas and shows high tolerance towards brackish conditions, occurring in bays and estuaries at salinities as low as 7 PSU (Mingkid et al. 2006). Compared to *H. sanguineus*, it prefers more sheltered areas with less wave exposure and muddier sediment (Mingkid et al. 2006, Dauvin et al. 2009, own observations).

Aspects of the reproductive biology of *H. takanoi* have been studied by van den Brink et al. (2013) in the invaded Dutch delta (Netherlands), who reported a reproductive period of about five months (April–September), and the ability of females to produce consecutive broods (up to four) after a single copulation. These data are consistent with data on *H. penicillatus* in Japan (Pillay & Ono 1978, Fukui 1988).

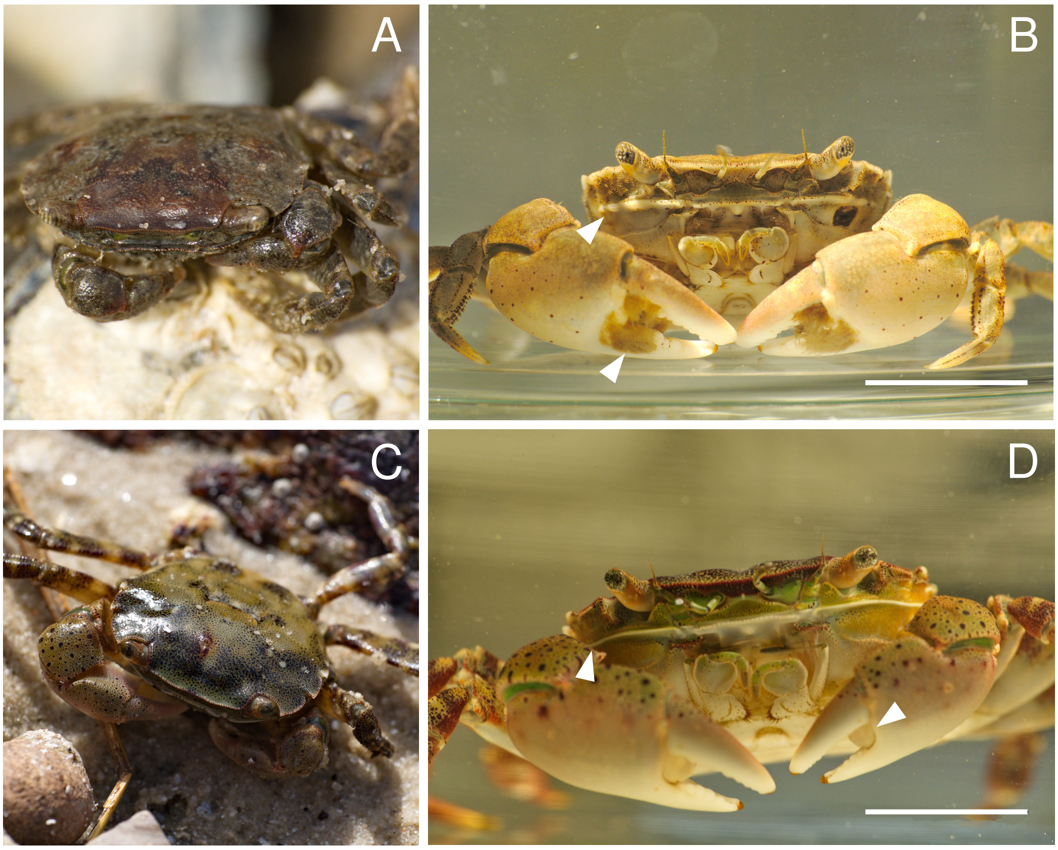


Fig. I.2. Discrimination between *Hemigrapsus spp.*: *H. takanoi*, habitus with typical coloration (A), and male, frontal view, arrows indicating the distally interrupted infraorbital ridge and the chelar setal patch (B). *H. sanguineus*, habitus with typical coloration (C), and male, frontal view, arrows indicating the uninterrupted infraorbital ridge and the chelar vesicle (D). Scale bars 10 mm.

Hemigrapsus sanguineus

Hemigrapsus sanguineus, the 'Asian shore crab' growths slightly larger than *H. takanoi*, the males reaching up to > 40 mm CW and about 25 mm on average, the females reaching up to 30 mm CW and about 20 mm on average (Epifanio 2013, own observations). Carapace shape and the presence of three anterolateral carapace teeth are as in *H. takanoi* (Fig. I.1 B). The coloration of *H. sanguineus* is typically dark greenish-brown or dark purple-brown, the walking legs showing an alternating pattern of dark and brighter bands. Brighter, reddish-brown and very dark colour morphs can also be found. The infraorbital ridge in *H. sanguineus* is distally uninterrupted and narrows out into a thin line, thus being clearly distinct from *H. takanoi*. In contrast to *H. takanoi* males, male *H. sanguineus* exhibit a fleshy, membranous vesicle in their chelar joints (Fig. I.2 C and D, compare Breton et al. 2002).

The native range of *H. sanguineus* encompasses the Pacific coasts of southern Russia, the Korean peninsula, Japan, Taiwan and the Chinese mainland coast as far south as Hong Kong (Epifanio 2013) (Fig. I.3 A). Thus, it largely occurs sympatrically with *H. takanoi*, a fact that most likely promoted their joint invasion to Europe. Like its congener, it occurs from the mid inter- to the shallow subtidal, but does prefer rockier areas with higher wave exposure and coarser sediment structure (Lohrer et al. 2000, Dauvin et al. 2009).

For *H. sanguineus* data from both, native and invaded ranges report reproduction periods between three and eight months, depending on average seasonal water temperatures (Pillay & Ono 1978, Fukui 1988, McDermott 1998b). Females of *H. sanguineus* are as well capable of consecutive breeding and may produce up to four consecutive broods per season, each one with up to 40'000 eggs (Fukui 1988, Anderson & Epifanio 2010, Epifanio 2013), which illustrates the high reproductive potential of this species.

Invasion history of *Hemigrapsus spp.*

The first record of a *Hemigrapsus spp.* as non-native species was the discovery of a single *H. sanguineus* at the mouth of the Delaware River, New Jersey, on the Atlantic coast of the United States in 1988, followed by the discovery of a breeding population at the same site in 1990 (McDermott 1991). During the following years, *H. sanguineus* rapidly extended its invaded range north-and southwards, in 1993 already occurring from Virginia to Massachusetts (Epifanio 2013). Its current range encompasses over 1’300 km of coastline from North Carolina to the Gulf of Maine (McDermott 1998a, Epifanio 2013, Lord & Williams 2016) (Fig. I.3 B). A recent study by Blakeslee et al. (2017) identified the New York/Long Island region as a possible ‘hot spot’ of multiple introductions of *H. sanguineus* to the northwestern Atlantic.

In Europe, *H. takanoi* was the first of the two species to be reported. In 1993, Gollasch (1999) found several individuals in the hull fouling of a ship at Bremerhaven (Germany), and one year later, Noël et al. (1997) detected a breeding population of *H. takanoi* close to La Rochelle on the French Atlantic coast, which was suspected to originate from the above-mentioned vessel (Gollasch 1999). By then, the crabs were identified as *H. penicillatus*, but later reassigned to the newly described *H. takanoi* (see above). Within only two years, *H. takanoi* occurred along the whole Bay of Biscay coast, from southern Brittany to northern Spain (Noël et al. 1997). In 1999, *H. takanoi* and *H. sanguineus* were simultaneously found at the port of Le Havre in northern France, several sites along the French coast of the English channel, and in the Dutch delta system (Breton et al. 2002, Wolff 2005, Kerckhof et al. 2007, Dauvin et al. 2009). Between 2004 and 2006, both species reached the western Wadden Sea (northern Netherlands) and continued their simultaneous northeasterly spread into the North Sea, reaching the central Wadden Sea (Lower Saxony, Germany) in 2007 (Obert et al. 2007), and the northern Wadden Sea (Schleswig-Holstein, Germany) and the island of Helgoland in the German Bight in 2008 (Landschoff et al. 2013, Jungblut et al. 2017).

After subsequent further spread following initial discoveries *H. takanoi* currently almost continuously occurs from Northern Spain to southern Denmark, along more than 2’500 km of coastline, and *H. sanguineus* from the Cotentin peninsula in France to southern Denmark, along more than 1’500 km of coastline. Further single records of *H. sanguineus* exist from the Mediterranean (Schubart 2003) and Black Seas (Micu et al. 2010). More recently, both species have also been recorded at several sites in Great Britain (Seeley et al. 2015, Wood et al. 2015, Ashelby et al. 2017), and there is anecdotal evidence for its occurrence on the west coast of Sweden (M. Berggren, Sven Lovén Centre for Marine Science, Kristineberg; cited in Jungblut et al. 2017) (Fig. I.3 C). The new records of *H. takanoi* from the Baltic Sea included in this thesis, can be seen as the ‘next step’ in the species’ invasion history.

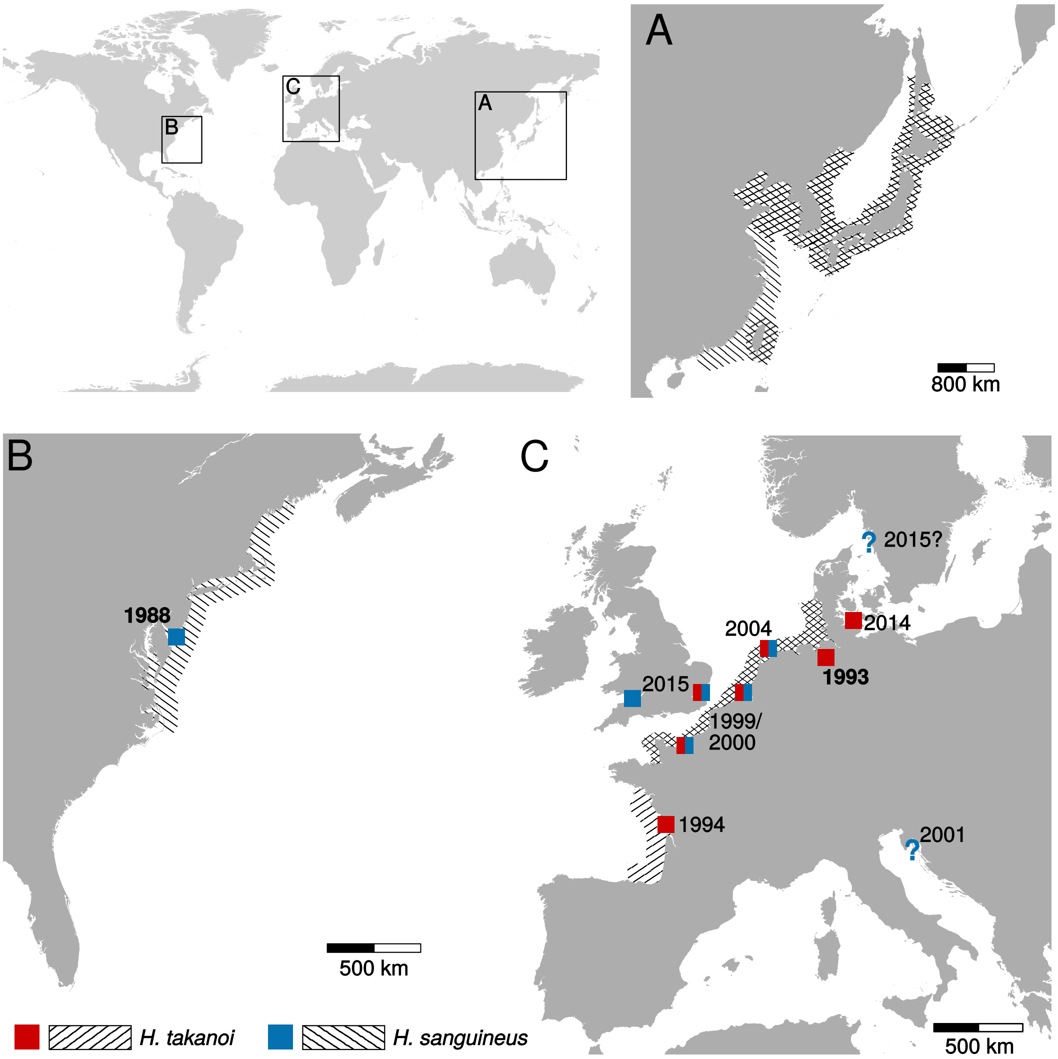


Fig. I.3. Global distribution of *H. takanoi* and *H. sanguineus*: Native Asian range (A), invaded North American (B) and European (C) ranges. Squares indicate site and year of important new records, question marks indicate unsure establishment status. For references, see text.

The study area

Geographically, this work focuses on the establishment and spread of *Hemigrapsus spp.* in German coastal waters. These are to the west the Wadden Sea, as part of the southeastern North Sea, and to the east the southwestern Baltic Sea. Despite their different and unique hydrographic and ecological characteristics, both ecosystems show parallels in terms of natural history and being geologically very young systems, a fact with direct implications for their receptivity for species' invasions. Their main characteristics will be shortly described in the following.

The Wadden Sea

The Wadden Sea, located in the southeastern North Sea along the coasts of the Netherlands, Germany and Denmark, is the largest uninterrupted intertidal ecosystem in the world. Stretching out between the mainland coast and a chain of islands and sand banks over a length of *c.* 500 km, it comprises about 4'700 km^2 of intertidal sand and mud flats (Reise et al. 2010). Due to its unique character, large parts of the Wadden Sea are protected as national parks and have further been declared a UNESCO world natural heritage in 2008. Acknowledging this uniqueness, the three states bordering the Wadden Sea commonly develop and follow concepts for the protection, management and sustainable use of this ecosystem (CWSS 2010).

Geologically, the Wadden Sea is a young ecosystem, which only formed about 8'000 years ago, after the last glaciation period. During this time, its vast tidal flats developed as a consequence of a balance between sea level rise and sedimentation processes (Reise et al. 2010). As a consequence of its young age and high dynamics, as well as anthropogenic influences and frequent perturbations throughout its history, the species communities of the Wadden Sea have been subjected to constant changes, with extinctions, immigrations and introductions being common phenomena (Reise et al. 1999, Lotze et al. 2005).

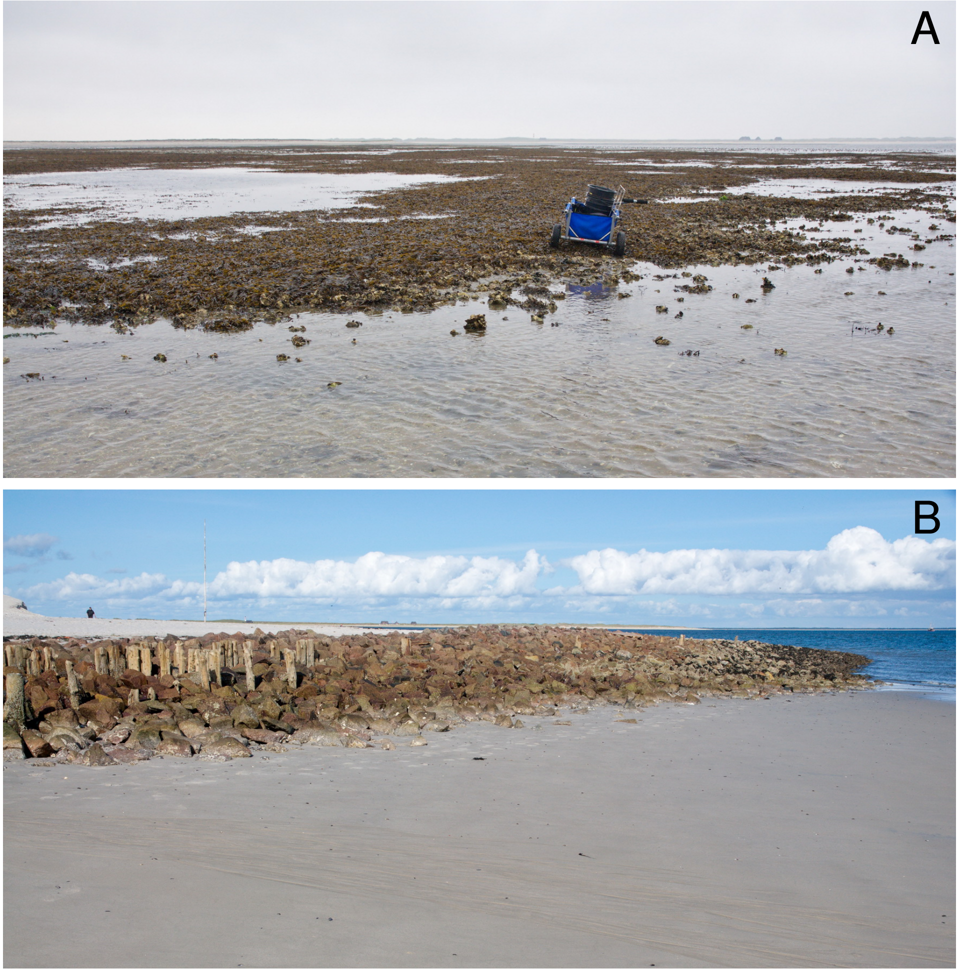


Fig. I.4. Typical habitats of *Hemigrapsus spp.* in the Wadden Sea (observed at the island of Sylt): Intertidal oyster-mussel-reef on a tidal flat, dominated by *H. takanoi* (A), and boulder revetment on an exposed shore, dominated by *H. sanguineus* (B).

Following the global trend, numerous non-native and invasive species have established in and spread over the Wadden Sea during the 20th century. Buschbaum et al. (2012) recorded 66 nonnative macrobenthic species throughout the Wadden Sea, and Gittenberger et al. (2010) reported 64 non-native plant and animal species in the comparatively small Dutch part of the Wadden Sea alone. Several factors are attributed to the high prevalence of species' invasions to the Wadden Sea: First, its location adjacent to one of the most heavily shipped sea areas worldwide, in the vicinity of major European ports, which guarantees a continuous supply of new species (Reise et al. 1999, Gollasch 2006). Second, its high productivity in combination with a relatively species-poor community, the latter being attributed to the young age of the Wadden Sea and anthropogenic species depletions and extinctions. In combination, these two factors are assumed to facilitate the establishment of additional species and the ecosystem to sustain them (Reise et al. 1999, Lotze et al. 2005, Buschbaum et al. 2012). Third, the effect of direct and indirect anthropogenic habitat changes. These are large-scale introductions of artificial hard-bottom substrate in the form of harbour facilities and coastal defence structures, replacing significant parts of the natural sedimentary habitats (Lotze et al. 2005, Reise 2005); and the introduction and spread of the Pacific oyster *Magallana gigas* Salvi & Mariottini, 2016 (formerly *Crassostrea gigas* [Thunberg, 1793]) which, as an ecosystem engineer, accounted for large-scale structural transformations of intertidal mussel beds, the only natural hard-bottom habitats in the Wadden Sea (Markert et al. 2010, Reise et al. 2017). Such habitat changes are believed to have paved the way for the introduction and establishment of high numbers of hard-bottom species in the naturally soft-sediment dominated Wadden Sea, *Hemigrapsus spp.* being a prominent example (Buschbaum et al. 2012, Landschoff et al. 2013).

Consequently, *H. takanoi* and *H. sanguineus* are observed in intertidal and shallow subtidal (rarely) hard-bottom habitats throughout the Wadden Sea. They occur sympatrically in principle, but at a given site, one species is usually observed to be dominant according to hydrographic conditions as described above (Landschoff et al. 2013, own observations) (Fig. I.4).

The Baltic Sea

Similar to the Wadden Sea, also the Baltic Sea is a geologically young system, which began to develop at the end of the last glaciation, about 12’000 years ago. Since then, it underwent several major transformations between limnic, brackish and marine conditions. The current coastlines developed only about 7’000 years ago, when the connection to the Atlantic Ocean/North Sea via the Kattegat and Belt Sea opened, and the current brackish conditions developed as late as about 3’000–4’000 years ago (Rheinheimer & Nehring 1995, Bonsdorff 2006). Covering approx. 412’000 km^2, it is today one of the largest brackish water bodies in the world (Jazdzewski & Grabowski 2011).

The ecological conditions of the Baltic Sea are dominated by two strong gradients, temperature and salinity, that occur from the southwest to the northeast, which to large parts determine species' distributions in the Baltic Sea. The climate of the southwestern Baltic Sea is temperate and under oceanic influence, while it becomes colder to the eastern and northern parts, which are under continental and (sub)polar influence. Even more important for aquatic organisms is the salinity gradient, reaching from almost fully marine conditions (*c.* 25 PSU) in the transition zone to the North Sea (Kattegat), over 9–7 PSU in the central Baltic Sea to almost limnic conditions (3–1 PSU) in its northern- and easternmost parts (Jazdzewski & Grabowski 2011). In the southwestern Baltic Sea, the area under investigation in this thesis, the salinity ranges between about 7 and 20 PSU, also showing some seasonal variation (Jazdzewski & Grabowski 2011, Lennartz et al. 2014).

Even more than in the Wadden Sea, the species communities of the Baltic Sea are shaped by a history of species' immigrations and introductions as a consequence of the biological transformations and – in more recent times – human impacts (Leppäkoski et al. 2002, Bonsdorff 2006). The evolutionary very recent stabilisation of the current ecological conditions and its characteristic of being a largely enclosed body of water have left the Baltic Sea relatively species-poor with many free functional and ecological niches being open for species' invasions (Leppäkoski et al. 2002, Bonsdorff 2006). Concerning human impacts, artificial waterways are of particular importance for invasions to the Baltic Sea. To the west, the Kiel Canal, connecting the Kiel Fjord/southwestern Baltic Sea with the Elbe estuary/southeastern North Sea, significantly shortens migration and transport routes from an highly invaded area (see above) to the Baltic Sea. To the east, a system of canals and rivers in eastern and central Europe opens several pathways for the immigration and introduction of Ponto-Caspian species (from the Black and Azov Sea region) to the southern and eastern Baltic Sea (Leppäkoski et al. 2002, Jazdzewski & Grabowski 2011, Katsanevakis et al. 2013). These species are often well pre-adapted to the conditions in the Baltic Sea, as their native ranges are as well characterised by brackish conditions with varying salinities. Consequently, the bigger part of the approximately 100 non-native species occurring in the Baltic Sea is of Ponto-Caspian origin (Leppäkoski et al. 2002, Gollasch 2006).

With about 20 species, crustaceans represent about one fifth of the Baltic Seas' total count of non-native species, amphipods, shrimp (Palaemonidae) and crabs being the most numerous subgroups. Many of them are assumed to impact food chains or having habitat-modifying abilities (e.g. Olenin &

Fig. I.5. Marinas are typical habitats of *H. takanoi* in the soutwestern Baltic Sea. The crabs inhabit shallow near-shore areas with rocks and mussel clumps, as well as fouling communities on harbour facilities. (Strande harbour, Kiel Fjord; picture taken by N. Weiler.)

Leppäkoski 1999, Ojaveer et al. 2007, Jazdzewski & Grabowski 2011, Fowler et al. 2013, Forsström et al. 2015). In the southwestern Baltic Sea, two introduced crab species have been reported so far, the North American mud crab *Rhithropanopeus harrisii* (Gould, 1841) and the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853. *Hemigrapsus takanoi* is now reported as the third brachyuran invader to this region (see chapter 2). As the Baltic Sea is a non-tidal sea, *H. takanoi* here occurs in shallow near-shore waters. Up until now, it has been exclusively found in harbours/marinas or their vicinity (Fig. I.5).

Aims of the thesis

The overarching research question of this thesis can be put very simply:

"How do they do it?"

Specifically, with this work, I investigate underlying factors and mechanisms of the successful establishment and spread of the coastal crabs *Hemigrapsus takanoi* and *H. sanguineus* in German coastal waters. Being one of the most recent biological invasions of brachyuran species, their case offers a unique opportunity to study an early-phase invasion process in this important group of invasive species. While the introduction and subsequent spread of *H. takanoi* and *H. sanguineus* along European coasts is well-documented, comparatively few European studies have been investigating aspects of their biology with regard to their high invasion success.

Therefore, I used a combination of ecological and genetic approaches to jointly analyse reproductive traits, early life history and the genetic population structure and diversity of *Hemigrapsus spp.*, which are presumed crucial factors during initial establishment processes. The research was conducted along the following questions:

- Is *H. takanoi* able to establish a reproducing population and to further spread into the Baltic Sea?

  Following the first records of *H. takanoi* in the Baltic Sea (part II, chapter 2), a plankton sampling study in Kiel Fjord aimed to morphologically describe the larvae of *H. takanoi* and to reconstruct its larval cycle in order to confirm or reject the species' ability to reproduce under the conditions of the Baltic Sea. The results of this study are presented in part II, chapter 3.

  To detect a possible spread of *H. takanoi* further into the Baltic Sea, selected sites along the southwestern Baltic Sea coast were monitored for the occurrence of the crab. The results are included in part III, chapter 5.

- Do temporal reproductive patterns and interactions during early life-history contribute to the invasion success of *Hemigrapsus spp.*?

  In the Wadden Sea, *Hemigrapsus spp.* rapidly established and now maintains very dense populations, despite the presence of the native *C. maenas*, itself being an infamous global invader. A 2-year sampling series was conducted in the northern Wadden Sea, investigating the timing of juvenile recruitment. Additional field and laboratory experiments investigated interactions between native and invasive crabs during their early life-history. These investigations aimed to clarify the hypothesised existence of mechanisms reducing competition between native and invasive crabs, thus facilitating the invaders' establishment. The results of these studies are presented in part III, chapter 4.

- Is there genetic evidence for population structuring of *H. takanoi*, and what can be inferred from it on invasion history in Europe and the recent invasion of the Baltic Sea?

  With these last two questions, the scope of research is broadened from the individual to the population level, and set from establishment processes to invasion dynamics and history. A genetic study, using microsatellites on *H. takanoi* samples from across Europe, aimed to reconstruct possible invasion events and pathways, with a special focus on the origin of the Baltic Sea populations. This study is presented in part III, chapter 5.

Answering these questions allows for assessing possible impacts and the potential of further spread of *Hemigrapsus spp.*, and furthermore increases our general understanding of brachyuran biological invasions.

References

Abt Associates Inc. (2008): *Ecological and Economic Impacts and Invasion Management Strategies for the European Green Crab.* Tech. report, Cambridge, MA, 51 pp.

Altieri, A.H. & Irving, A.D. (2017): Species coexistence and the superior ability of an invasive species to exploit a facilitation cascade habitat. *PeerJ* 5, e2848, doi: 10.7717/peerj.2848.

Anderson, J.A. & Epifanio, C.E. (2009): Induction of metamorphosis in the Asian shore crab *Hemigrapsus sanguineus*: Characterization of the cue associated with biofilm from adult habitat. *Journal of Experimental Marine Biology and Ecology* 382 (1), 34–39, doi: 10.1016/j.jembe.2009.10.006.

Anderson, J.A. & Epifanio, C.E. (2010): Mating and sperm storage of the Asian shore crab *Hemigrapsus sanguineus*. *Journal of Shellfish Research* 29 (2), 497–501, doi: 10.2983/035.029.0228.

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Asakura, A., Yamasaki, I., Mingkid, W.M. & Watanabe, S. (2008): Revalidation of *Hemigrapsus takanoi* Asakura & Watanabe, 2005: A rebuttal to "Sakai (2007): Comments on an invalid nominal species, *Hemigrapsus takanoi* Asakura & Watanabe, 2005, a synonym of *Hemigrapsus penicillatus* (De Haan, 1835)". *Crustaceana* 81 (10), 1263–1273, doi: 10.1163/156854008X374603.

Ashelby, C.W., Sewell, J., Rostron, J., Shrubsole, R., Child, T. & Clark, P.F. (2017): Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90 (6), 695–708, doi: 10.1163/15685403-00003692.

Barnes, D.K.A. & Milner, P. (2005): Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. *Marine Biology* 146 (4), 815–825, doi: 10.1007/s00227-004-1474-8.

Bax, N.J. (1999): Eradicating a dreissenid from Australia. *Dreissena!* 10 (3), 1–5.

Bax, N.J., Carlton, J.T., Mathews-Amos, A., Haedrich, R.L., Howarth, F.G., Purcell, J.E., Rieser, A. & Gray, A. (2001): The Control of Biological Invasions in the World's Oceans. *Conservation Biology* 15 (5), 1234–1246, doi: 10.1046/j.1523-1739.2001.99487.x.

Bax, N.J., Hayes, K., Marshall, A., Parry, D. & Thresher, R.E. (2000): Man-made marinas as sheltered islands for alien marine organisms: Establishment and eradication of an alien invasive marine species. In: Veitch, C.R. & Clout, M.N. (eds.) *Turning the tide: the eradication of invasive species.* IUCN SSC Invasive Specialist Group, Gland, Cambridge, pp. 26–39, doi: 10.1366/0003702991946064.

Bax, N.J., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. (2003): Marine invasive alien species: a threat to global biodiversity. *Marine Policy* 27 (4), 313–323.

Blakeslee, A.M.H., Kamakura, Y., Onufrey, J., Makino, W., Urabe, J., Park, S., Keogh, C.L., Whitman Miller, A., Minton, M.S., Carlton, J.T. & Miura, O. (2017): Reconstructing the Invasion History of the Asian shorecrab, *Hemigrapsus sanguineus* (De Haan 1835) in the Western Atlantic. *Marine Biology* 164, 47, doi: 10.1007/s00227-017-3117-x.

Blasi, J.C. & O'Connor, N.J. (2016): Amphipods as potential prey of the Asian shore crab *Hemigrapsus sanguineus*: Laboratory and field experiments. *Journal of Experimental Marine Biology and Ecology* 474, 18–22, doi: 10.1016/j.jembe.2015.09.011.

Bonsdorff, E. (2006): Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. *Journal of Experimental Marine Biology and Ecology* 330, 383–391, doi: 10.1016/j.jembe.2005.12.041.

Braby, C.E. & Somero, G.N. (2006a): Ecological gradients and relative abundance of native (*Mytilus trossulus*) and invasive (*Mytilus galloprovincialis*) blue mussels in the California hybrid zone. *Marine Biology* 148 (6), 1249–1262, doi: 10.1007/s00227-005-0177-0.

Braby, C.E. & Somero, G.N. (2006b): Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology* 209 (13), 2554–2566, doi: 10.1242/jeb.02259.

Brawley, S.H., Coyer, J.A., Blakeslee, A.M.H., Hoarau, G., Johnson, L.E., Byers, J.E., Stam, W.T. & Olsen, J.L. (2009): Historical invasions of the intertidal zone of Atlantic North America associated with distinctive patterns of trade and emigration. *Proceedings of the National Academy of Sciences* 106 (20), 8239–8244, doi: 10.1073/pnas.0812300106.

Bremner, J. (2008): Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology* 366, 37–47, doi: 10.1016/j.jembe.2008.07.007.

Breton, G., Faasse, M., Noël, P.Y. & Vincent, T. (2002): A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1), 184–189, doi: 10.1651/0278-0372(2002)022[0184:ANACIE]2.0.CO;2.

Briggs, J.C. (2007): Marine biogeography and ecology: invasions and introductions. *Journal of Biogeography* 34, 193–198, doi: 10.1111/j.1365-2699.2006.01632.x.

Briggs, J.C. (2010): Marine biology: the role of accommodation in shaping marine biodiversity. *Marine Biology* 157, 2117–2126, doi: 10.1007/s00227-010-1490-9.

Briggs, J.C. (2012): Marine species invasions in estuaries and harbors. *Marine Ecology Progress Series* 449, 297–302, doi: 10.3354/meps09553.

Brockerhoff, A.M. & McLay, C.L. (2011): Human-Mediated Spread of Alien Crabs. In: Galil, B.S., Clark, P.F. & Carlton, J.T. (eds.) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts.* Invading Nature – Springer Series in Invasion Ecology 6, Springer Netherlands, pp. 27–106, doi: 10.1007/978-94-007-0591-3_2.

Buschbaum, C., Lackschewitz, D. & Reise, K. (2012): Nonnative macrobenthos in the Wadden Sea ecosystem. *Ocean & Coastal Management* 68, 89–101, doi: 10.1016/j.ocecoaman.2011.12.011.

Byrnes, J.E., Reynolds, P.L. & Stachowicz, J.J. (2007): Invasions and extinctions reshape coastal marine food webs. *PloS one* 2 (3), e295, doi: 10.1371/journal.pone.0000295.

Carlton, J.T. (1999): The scale and ecological consequences of biological invasions in the World's oceans. In: Sandlund, O.T., Schei, P.J. & Viken, Å. (eds.) *Invasive species and biodiversity management.* Kluwer Academic Publishers, Dordrecht, pp. 195–212.

Carlton, J.T. & Cohen, A.N. (2003): Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. *Journal of Biogeography* 30 (12), 1809–1820, doi: 10.1111/j.1365-2699.2003.00962.x.

Carlton, J.T. & Geller, J.B. (1993): Ecological roulette: the global transport of nonindigenous marine organisms. *Science* 261, 78–82, doi: 10.1126/science.261.5117.78.

Chan, F.T. & Briski, E. (2017): An overview of recent research in marine biological invasions. *Marine Biology* 164, 121, doi: 10.1007/s00227-017-3155-4.

Cheshire, A., Westphalen, G., Boxall, V., Marsh, R., Gilliland, J., Collings, G., Seddon, S. & Loo, M. (2002): *Caulerpa taxifolia in West Lakes and the Port River: distribution and eradication options.* Tech. report, South Australian Research and Development Institute, Aquatic Sciences & PIRSA Fisheries, Marine Habitat Program, Adelaide.

Cheung, W.W.L., Lam, V.W.Y., Sarmiento, J.L., Kearney, K., Watson, R. & Pauly, D. (2009): Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* 10 (3), 235–251, doi: 10.1111/j.1467-2979.2008.00315.x.

Clarke Murray, C., Pakhomov, E.A. & Therriault, T.W. (2011): Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distributions* 17 (6), 1161–1172, doi: 10.1111/j.1472-4642.2011.00798.x.

Clavero, M. & García-Berthou, E. (2005): Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* 20 (3), 110, doi: 10.1016/j.tree.2005.01.003.

Colautti, R.I. & MacIsaac, H.J. (2004): A neutral terminology to define 'invasive' species. *Diversity and Distributions* 10 (2), 135–141, doi: 10.1111/j.1366-9516.2004.00061.x.

Coleman, N. & Trueman, E.R. (1971): The effect of aerial exposure on the activity of the mussels *Mytilus edulis* L. and *Modiolus modiolus* (L.) *Journal of Experimental Marine Biology and Ecology* 7 (3), 295–304, doi: 10.1016/0022-0981(71)90011-6.

Comittee on Ships' Ballast Operations, Marine Board & Comission on Engineering and Technical Systems, eds. (1996): *Stemming the Tide: Controlling Introductions of Nonindigenous Species by Ships' Ballast Water*. National Academy of Sciences, Washington DC, 160 pp., doi: 10.17226/5294.

Crooks, J.A. (2002): Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos* 97, 153–166.

Culver, C.S. & Kuris, A.M. (2000): The apparent eradication of a locally established introduced marine pest. *Biological Invasions* 2, 245–253, doi: 10.1023/A:1010082407254.

CWSS (2010): *Sylt Declaration. Ministerial Council Declaration of the Eleventh Trilateral Governmental Conference on the Protection of the Wadden Sea*. Common Wadden Sea Secretariat, Wilhelmshaven.

Dalton, R. (2000): Researchers criticize response to killer algae. *Nature* 406, 447–447.

Dauvin, J.-C., Tous Rius, A. & Ruellet, T. (2009): Recent expansion of two invasive crab species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions* 4 (3), 451–465, doi: 10.3391/ai.2009.4.3.3.

Davie, P. & Türkay, M. (2017): *Hemigrapsus* Dana, 1851. Accessed through: World Register of Marine Species, http://www.marinespecies.org/aphia.php?p=taxdetails%7B%5C&%7Did=106964 (visited on 05/01/2018).

Davis, R.C., Short, F.T. & Burdick, D.M. (1998): Quantifying the Effects of Green Crab Damage to Eelgrass Transplants. *Restoration Ecology* 6 (3), 297–302, doi: 10.1046/j.1526-100X.1998.00634.x.

De Castro, M.C.T., Fileman, T.W. & Hall-Spencer, J.M. (2017): Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review. *Marine Pollution Bulletin* 116, 41–47, doi: 10.1016/j.marpolbul.2016.12.048.

Delaney, D.G., Edwards, P.K. & Leung, B. (2012): Predicting regional spread of non-native species using oceanographic models: validation and identification of gaps. *Marine Biology* 159, 269–282, doi: 10.1007/s00227-011-1805-5.

Diederich, S., Nehls, G., van Beusekom, J.E. & Reise, K. (2005): Introduced Pacific oysters (*Crassostrea gigas*) in the northern Wadden Sea: Invasion accelerated by warm summers? *Helgoland Marine Research* 59 (2), 97–106, doi: 10.1007/s10152-004-0195-1.

Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J. & Talley, L.D. (2012): Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science* 4, 11–37, doi: 10.1146/annurev-marine-041911-111611.

Epifanio, C.E. (2013): Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: A review. *Journal of Experimental Marine Biology and Ecology* 441, 33–49, doi: 10.1016/j.jembe.2013.01.010.

Facon, B., Genton, B.J., Shykoff, J., Jarne, P., Estoup, A. & David, P. (2006): A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology and Evolution* 21 (3), 130–135, doi: 10.1016/j.tree.2005.10.012.

Facon, B., Pointier, J.P., Jarne, P., Sarda, V. & David, P. (2008): High Genetic Variance in Life-History Strategies within Invasive Populations by Way of Multiple Introductions. *Current Biology* 18 (5), 363–367, doi: 10.1016/j.cub.2008.01.063.

Feis, M.E., Goedknegt, M.A., Thieltges, D.W., Buschbaum, C. & Wegner, K.M. (2016): Biological invasions and host-parasite coevolution: different coevolutionary trajectories along separate parasite invasion fronts. *Zoology* 119 (4), 366–374, doi: 10.1016/j.zool.2016.05.012.

Forsström, T., Fowler, A.E., Manninen, I. & Vesakoski, O. (2015): An introduced species meets the local fauna: predatory behavior of the crab *Rhithropanopeus harrisii* in the Northern Baltic Sea. *Biological Invasions* 17 (9), 2729–2741, doi: 10.1007/s10530-015-0909-0.

Fowler, A.E., Forsström, T., von Numers, M. & Vesakoski, O. (2013): The North American mud crab *Rhithropanopeus harrisii* (Gould, 1841) in newly colonized Northern Baltic Sea: distribution and ecology. *Aquatic Invasions* 8 (1), 89–96, doi: 10.3391/ai.2013.8.1.10.

Fridley, J.D., Stachowicz, J.J., Naeem, S., Sax, D.F., Seabloom, E.W., Smith, M.D., Stohlgren, T.J., Tilman, D. & Von Holle, B. (2007): The Invasion Paradox: Reconciling Pattern and Process in Species Invasions. *Ecology* 88 (1), 3–17.

Fukui, Y. (1988): Comparative Studies on the Life History of the Grapsid Crabs (Crustacea, Brachyura) Inhabiting Intertidal Cobble and Boulder Shores. *Publications of the Seto Marine Biological Laboratory* 33 (4/6), 120–162.

Galil, B.S. (2009): Taking stock: inventory of alien species in the Mediterranean sea. *Biological Invasions* 11 (2), 359–372, doi: 10.1007/s10530-008-9253-y.

Garside, C.J., Glasby, T.M., Stone, L.J. & Bishop, M.J. (2015): The timing of *Carcinus maenas* recruitment to a south-east Australian estuary differs to that of native crabs. *Hydrobiologia* 762, 41–53, doi: 10.1007/s10750-015-2332-z.

Geburzi, J.C., Graumann, G., Köhnk, S. & Brandis, D. (2015): First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Records* 4 (2), 103–107, doi: 10.3391/bir.2015.4.2.06.

Geller, J.B. (1999): Decline of a Native Mussel Masked by Sibling Species Invasion. *Conservation Biology* 13 (3), 661–664, doi: 10.1046/j.1523-1739.1999.97470.x.

Geller, J.B., Darling, J.A. & Carlton, J.T. (2010): Genetic perspectives on marine biological invasions. *Annual Review of Marine Science* 2, 367–93, doi: 10.1146/annurev.marine.010908.163745.

Geller, J.B., Walton, E.D., Grosholz, E.D. & Ruiz, G.M. (1997): Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* 6, 901–906, doi: 10.1046/j.1365-294X.1997.00256.x.

Gil, M.A. & Pfaller, J.B. (2016): Oceanic barnacles act as foundation species on plastic debris: implications for marine dispersal. *Scientific Reports* 6, 19987, doi: 10.1038/srep19987.

Gittenberger, A., Rensing, M., Stegenga, H. & Hoeksema, B. (2010): Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33, 21–76.

Goedknegt, M.A., Feis, M.E., Wegner, K.M., Luttikhuizen, P.C., Buschbaum, C., Camphuysen, K.C.J., van der Meer, J. & Thieltges, D.W. (2016): Parasites and marine invasions: Ecological and evolutionary perspectives. *Journal of Sea Research* 113, 11–27, doi: 10.1016/j.seares.2015.12.003.

Gollasch, S. (1999): The Asian decapod *Hemigrapsus penicillatus* (De Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgoländer Meeresuntersuchungen* 52, 359–366, doi: 10.1007/BF02908909.

Gollasch, S. (2002): The importance of Ship Hull Fouling as a Vector of Species Introductions into the North Sea. *Biofouling* 18 (2), 105–121.

Gollasch, S. (2006): Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60 (2), 84–89, doi: 10.1007/s10152-006-0022-y.

Gómez, F. & Claustre, H. (2003): The genus *Asterodinium* (Dinophyceae) as a possible biological indicator of warming in the western Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom* 83 (1), 173–174, doi: 10.1017/S0025315403006945h.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

Gregory, M.R. (2009): Environmental implications of plastic debris in marine settings - entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philosophical transactions of the Royal Society B* 364 (1526), 2013–2025, doi: 10.1098/rstb.2008.0265.

Griffen, B.D. (2016): Scaling the consequences of interactions between invaders from the individual to the population level. *Ecology and Evolution* 6 (6), 1769–1777, doi: 10.1002/ece3.2008.

Griffen, B.D., Altman, I., Hurley, J. & Mosblack, H. (2011): Reduced fecundity by one invader in the presence of another: A potential mechanism leading to species replacement. *Journal of Experimental Marine Biology and Ecology* 406 (1-2), 6–13, doi: 10.1016/j.jembe.2011.06.005.

Griffen, B.D. & Byers, J.E. (2009): Community impacts of two invasive crabs: The interactive roles of density, prey recruitment, and indirect effects. *Biological Invasions* 11 (4), 927–940, doi: 10.1007/s10530-008-9305-3.

Griffen, B.D., Guy, T. & Buck, J.C. (2008): Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology* 77, 32–40, doi: 10.1111/j.1365-2656.2007.01304.x.

Grosholz, E.D. (2005): Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences* 102 (4), 1088–1091, doi: 10.1073/pnas.0308547102.

Grosholz, E.D. & Ruiz, G.M. (1996): Predicting the impact of introduced marine species: lessons from the multiple invasions of the european green crab *Carcinus maenas*. *Biological Conservation* 78, 59–66.

Grosholz, E.D., Ruiz, G.M., Dean, C.A., Shirley, K.A., Maron, J.L. & Connors, P.G. (2000): The Impacts of a Nonindigenous Marine Predator in a California Bay. *Ecology* 81 (5), 1206–1224.

Hänfling, B. (2007): Understanding the establishment success of non-indigenous fishes: Lessons from population genetics. *Journal of Fish Biology* 71, 115–135, doi: 10.1111/j.1095-8649.2007.01474.x.

Hänfling, B., Edwards, F. & Gherardi, F. (2011): Invasive alien Crustacea: dispersal, establishment, impact and control. *BioControl* 56, 573–595, doi: 10.1007/s10526-011-9380-8.

Helm, M.M. & Trueman, E.R. (1967): The effect of exposure on the heart rate of the mussel, *Mytilus edulis* L. *Comparative Biochemistry and Physiology* 21 (1), 171–177, doi: 10.1016/0010-406X(67)90126-0.

Herbert, R.J.H., Humphreys, J., Davies, C.J., Roberts, C., Fletcher, S. & Crowe, T.P. (2016): Ecological impacts of non-native Pacific oysters (*Crassostrea gigas*) and management measures for protected areas in Europe. *Biodiversity and Conservation* 25, 2835–2865, doi: 10.1007/s10531-016-1209-4.

Herborg, L.-M., Weetman, D., van Oosterhout, C. & Hänfling, B. (2007): Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology* 16 (2), 231–242, doi: 10.1111/j.1365-294X.2006.03133.x.

Hines, A.H. (1986): Larval patterns in the life histories of Brachyuran crabs (Crustacea, Decapoda, Brachyura). *Bulletin of Marine Science* 39 (2), 444–466.

Hogan, L.S., Marschall, E., Folt, C. & Stein, R.A. (2007): How Non-native Species in Lake Erie Influence Trophic Transfer of Mercury and Lead to Top Predators. *Journal of Great Lakes Research* 33 (1), 46–61, doi: 10.3394/0380-1330(2007)33[46:HNSILE]2.0.CO;2.

Holland, B.S. (2000): Genetics of marine bioinvasions. *Hydrobiologia* 420, 63–71, doi: 10.1023/A:1003929519809.

Hollebone, A.L. & Hay, M.E. (2007): Propagule pressure of an invasive crab overwhelms native biotic resistance. *Marine Ecology Progress Series* 342, 191–196, doi: 10.3354/meps342191.

Huxel, G.R. (1999): Rapid displacement of native species by invasive species: Effects of hybridization. *Biological Conservation* 89, 143–152, doi: 10.1016/S0006-3207(98)00153-0.

Jazdzewski, K. & Grabowski, M. (2011): Alien Crustaceans Along the Southern and Western Baltic Sea. In: Galil, B.S., Clark, P.F. & Carlton, J.T. (eds.) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts.* Springer Netherlands, Dordrecht, pp. 323–344, doi: 10.1007/978-94-007-0591-3.

Jensen, G.C., McDonald, P.S. & Armstrong, D.A. (2002): East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp. Marine Ecology Progress Series* 225, 251–262, doi: 10.3354/meps225251.

Johnson, P.T.J., Olden, J.D., Solomon, C.T. & Vander Zanden, M.J. (2009): Interactions among invaders: Community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia* 159 (1), 161–170, doi: 10.1007/s00442-008-1176-x.

Jones, C.G., Lawton, J.H. & Shachak, M. (1994): Organisms as ecosystem engineers. *Oikos* 69, 373–386.

Jungblut, S., Beermann, J., Boos, K., Saborowski, R. & Hagen, W. (2017): Population development of the invasive crab *Hemigrapsus sanguineus* (De Haan, 1853) and its potential native competitor *Carcinus maenas* (Linnaeus, 1758) at Helgoland (North Sea) between 2009 and 2014. *Aquatic Invasions* 12 (1), 85–96, doi: 10.3391/ai.2017.12.1.0.

Katsanevakis, S., Zenetos, A., Belchior, C. & Cardoso, A.C. (2013): Invading European Seas: Assessing pathways of introduction of marine aliens. *Ocean & Coastal Management* 76, 64–74, doi: 10.1016/j.ocecoaman.2013.02.024.

Keller, R.P., Geist, J., Jeschke, J.M. & Kühn, I. (2011): Invasive species in Europe: Ecology, status, and policy. *Environmental Sciences Europe* 23, 23, doi: 10.1186/2190-4715-23-23.

Keogh, C.L., Miura, O., Nishimura, T. & Byers, J.E. (2016): The double edge to parasite escape: invasive host is less infected but more infectable. *Ecology* 38 (1), 42–49, doi: 10.1002/ecy.1953.

Kerckhof, F., Haelters, J. & Gollasch, S. (2007): Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions* 2 (3), 243–257.

Kettunen, M., Genovesi, P., Gollasch, S., Pagad, S., Starfinger, U., ten Brink, P. & Shine, C. (2008): *Technical support to EU strategy on invasive species (IAS) - Assessment of the impacts of IAS in Europe and the EU.* Final report for the European Commission, Institute for European Environmental Policy (IEEP), Brussels, 44 pp. + annexes.

Lafferty, K.D. & Kuris, A.M. (1996): Biological Control of Marine Pests. *Ecology* 77 (7), 1989–2000.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Lee, S., Lee, S.-K., Rho, H.S. & Kim, W. (2013): New Report of the Varunid Crabs, *Hemigrapsus takanoi* and *Sestrostoma toriumii* (Crustacea: Decapoda: Varunidae) from Korea. *Animal Systematics, Evolution and Diversity* 29 (2), 152–159, doi: 10.5635/ASED.2013.29.2.152.

Lejart, M. & Hily, C. (2011): Differential response of benthic macrofauna to the formation of novel oyster reefs (*Crassostrea gigas*, Thunberg) on soft and rocky substrate in the intertidal of the Bay of Brest, France. *Journal of Sea Research* 65 (1), 84–93.

Lennartz, S.T., Lehmann, A., Herrford, J., Malien, F., Hansen, H.-P., Biester, H. & Bange, H.W. (2014): Long-term trends at the Boknis Eck time series station (Baltic Sea), 1957–2013: does climate change counteract the decline in eutrophication? *Biogeosciences* 11 (22), 6323–6339, doi: 10.5194/bg-11-6323-2014.

Leppäkoski, E., Gollasch, S., Gruszka, P., Ojaveer, H., Olenin, S. & Panov, V.E. (2002): The Baltic – a sea of invaders. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1175–1188, doi: 10.1139/f02-089.

Levin, P.S., Coyer, J.A., Petrik, R. & Good, T.G. (2002): Community-Wide Effects of Nonindigenous Species on Temperate Rocky Reefs. *Ecology* 83 (11), 3182–3193.

Lockwood, J.L., Cassey, P. & Blackburn, T.M. (2009): The more you introduce the more you get: The role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions* 15 (5), 904–910, doi: 10.1111/j.1472-4642.2009.00594.x.

Lohrer, A.M., Fukui, Y., Wada, K. & Whitlatch, R.B. (2000): Structural complexity and vertical zonation of intertidal crabs, with focus on habitat requirements of the invasive Asian shore crab, *Hemigrapsus sanguineus* (de Haan). *Journal of Experimental Marine Biology and Ecology* 244 (2), 203–217.

Lohrer, A.M. & Whitlatch, R.B. (2002): Interactions Among Aliens: Apparent Replacement of One Exotic Species By Another. *Ecology* 83 (3), 719–732.

Lord, J.P. & Williams, L.M. (2016): Increase in density of genetically diverse invasive Asian shore crab (*Hemigrapsus sanguineus*) populations in the Gulf of Maine. *Biological Invasions* 19 (4), 1153–1168, doi: 10.1007/s10530-016-1304-1.

Lotze, H.K., Reise, K., Worm, B., van Beusekom, J.E., Busch, M., Ehlers, A., Heinrich, D., Hoffmann, R.C., Holm, P., Jensen, C., Knottnerus, O.S., Langhanki, N., Prummel, W., Vollmer, M. & Wolff, W.J. (2005): Human transformations of the Wadden Sea ecosystem through time: A synthesis. *Helgoland Marine Research* 59 (1), 84–95, doi: 10.1007/s10152-004-0209-z.

Lovell, S.J., Stone, S.F. & Fernandez, L. (2006): The Economic Impacts of Aquatic Invasive Species: A Review of the Literature. *Agricultural and Resource Economics Review* 35 (1), 195–208, doi: 10.1017/S1068280500010157.

Lowe, S., Browne, M., Boudjelas, S. & De Poorter, M. (2004): *100 of the World's Worst Invasive Species: A selection from the Global Invasive Species Database*. The Invasive Species Specialist Group (ISSG) of the World Conservation Union (IUCN), Auckland, 12 pp.

Makino, W., Kan, K., Sato, M., Mukai, Y., Kaiser, F., Katsube, T., Suzuki, T. & Urabe, J. (2015): Usefulness of the size of dark spots on the body surface as a diagnostic character distinguishing two morphologically similar *Hemigrapsus* species (Decapoda: Brachyura: Varunidae). *Plankton & Benthos Research* 10 (1), 45–54.

Makino, W., Miura, O., Kaiser, F., Geffray, M., Katsube, T. & Urabe, J. (2017): Evidence of multiple introductions and genetic admixture of the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae) along the Northern European coast. *Biological Invasions* 20 (4), 825–842, doi: 10.1007/s10530-017-1604-0.

Mann, R. & Harding, J.M. (2000): Invasion of the North American Atlantic coast by a large predatory Asian mollusc. *Biological Invasions* 2, 7–22.

Marin, I.N. (2013): New data on the distribution of hairy-clawed shore crabs of the genus *Hemigrapsus* (Decapoda: Varunidae) along the Russian mainland coast of the Sea of Japan. *Russian Journal of Marine Biology* 39 (4), 301–305, doi: 10.1134/S106307401304007X.

Markert, A., Raupach, M.J., Segelken-Voigt, A. & Wehrmann, A. (2014): Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatus* (De Haan, 1835) versus *Hemigrapsus takanoi* Asakura & Watanabe 2005. *Organisms Diversity & Evolution*, doi: 10.1007/s13127-014-0176-4.

Markert, A., Wehrmann, A. & Kröncke, I. (2010): Recently established *Crassostrea*-reefs versus native *Mytilus*-beds: Differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight). *Biological Invasions* 12, 15–32, doi: 10.1007/s10530-009-9425-4.

McDermott, J.J. (1991): A Breeding Population of the Western Pacific Crab *Hemigrapsus sanguineus* (Crustacea: Decapoda: Grapsidae) Established on the Atlantic Coast of North America. *Biological Bulletin* 181, 195–198.

McDermott, J.J. (1998a): The western Pacific brachyuran (*Hemigrapsus sanguineus*: Grapsidae), in its new habitat along the Atlantic coast of the United States: geographic distribution and ecology. *ICES Journal of Marine Science* 55, 289–298.

McDermott, J.J. (1998b): The western Pacific Brachyuran *Hemigrapsus sanguineus* (Grapsidae) in its new habitat along the Atlantic coast of the United States: reproduction. *Journal of Crustacean Biology* 18 (2), 308–316.

McDermott, J.J. (2011): Parasites of shore crabs in the genus *Hemigrapsus* (Decapoda: Brachyura: Varunidae) and their status in crabs geographically displaced: a review. *Journal of Natural History* 45 (39-40), 2419–2441, doi: 10.1080/00222933.2011.596636.

McDonald, J.H. & Koehn, R.K. (1988): The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. *Marine Biology* 99 (1), 111–118, doi: 10.1007/BF00644984.

Meinesz, A., Belsher, T., Thibaut, T., Antolic, B., Mustapha, K.B., Cinelli, F., Cottalorda, J.-M., Djellouli, A., Abed, A.E., Orestano, C., Grau, A.M., Ivesa, L., Jaklin, A., Langar, H., Massuti-Pascual, E., Peirano, A., Tunesi, L., Vaugelas, J.D., Zavodnik, N. & Zuljevic, A. (2001): The introduced green alga *Caulerpa taxifolia* continues to spread in the Mediterranean. *Biological Invasions* 3, 201–210.

Micu, D., Niţă, V. & Todorova, V. (2010): First record of the Japanese shore crab *Hemigrapsus sanguineus* (de Haan, 1835) (Brachyura: Grapsoidea: Varunidae) from the Black Sea. *Aquatic Invasions* 5 (2), doi: 10.3391/ai.2010.5.2.

Mineur, F., Cook, E.J., Minchin, D., Bohn, K., Macleod, A. & Maggs, C.A. (2012): Changing Coasts: Marine aliens and artificial structures. *Oceanography and Marine Biology: An Annual Review* 50, 189–234.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Miron, G., Audet, D., Landry, T. & Moriyasu, M. (2005): Predation Potential of the Invasive Green Crab (*Carcinus maenas*) and Other Common Predators on Commercial Bivalve Species Found on Prince Edward Island. *Journal of Shellfish Research* 24 (2), 579–586, doi: 10.2983/0730-8000(2005)24[579:PPOTIG]2.0.CO;2.

Naylor, R.L., Williams, S.L. & Strong, D.R. (2001): Aquaculture – A Gateway for Exotic Species. *Science* 294 (5547), 1655–1656.

Ng, P.K.L., Davie, P.J.F. & Guinot, D. (2008): Systema Brachyurorum: Part I . an Annotated Checklist of Extant Brachyuran Crabs of the World. *The Raffles Bulletin of Zoology* 17, 1–286.

Nicholson, S. (2002): Ecophysiological aspects of cardiac activity in the subtropical mussel *Perna viridis* (L.) (Bivalvia: Mytilidae). *Journal of Experimental Marine Biology and Ecology* 267 (2), 207–222, doi: 10.1016/S0022-0981(01)00362-8.

Noël, P.Y., Tardy, E. & D'Udekem d'Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Obert, B., Herly, M. & Grotjahn, M. (2007): First records of two crabs from the North West Pacific *Hemigrapsus sanguineus* and *H. takanoi* at the coast of Lower Saxony, Germany. *Wadden Sea Newsletter* 1, 21–22.

Occhipinti-Ambrogi, A. (2007): Global change and marine communities: Alien species and climate change. *Marine Pollution Bulletin* 55, 342–352, doi: 10.1016/j.marpolbul.2006.11.014.

O'Connor, N.J. (2007): Stimulation of molting in megalopae of the Asian shore crab *Hemigrapsus sanguineus*: physical and chemical cues. *Marine Ecology Progress Series* 352, 1–8, doi: 10.3354/meps07315.

Ojaveer, H., Gollasch, S., Jaanus, A., Kotta, J., Laine, A.O., Minde, A., Normant, M. & Panov, V.E. (2007): Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea - a supply-side invader? *Biological Invasions* 9, 409–418, doi: 10.1007/s10530-006-9047-z.

Olenin, S. & Leppäkoski, E. (1999): Non-native animals in the Baltic Sea: alteration of benthic habitats in coastal inlets and lagoons. *Hydrobiologia* 393, 233–243, doi: 10.1023/A:1003511003766.

Olyarnik, S.V., Bracken, M.E.S., Byrnes, J.E., Hughes, A.R., Hultgren, K.M. & Stachowicz, J.J. (2009): Ecological Factors Affecting Community Invasibility. In: Rilov, G. & Crooks, J.A. (eds.) *Biological Invasions in Marine Ecosystems.* Ecological Studies 204, Springer-Verlag, Berlin, Heidelberg, pp. 215–238, doi: 10.1007/978-3-540-79236-9_12.

Panning, A. (1938): The Chinese mitten crab. In: *Annual Report* Smithsonian Institution, Washington DC, pp. 361–375.

Papacostas, K.J., Rielly-Carroll, E.W., Georgian, S.E., Long, D.J., Princiotta, S.D., Quattrini, A.M., Reuter, K.E. & Freestone, A.L. (2017): Biological mechanisms of marine invasions. *Marine Ecology Progress Series* 565, 251–268, doi: 10.3354/meps12001.

Parker, J.D., Torchin, M.E., Hufbauer, R.A., Lemoine, N.P., Alba, C., Blumenthal, D.M., Bossdorf, O., Byers, J.E., Dunn, A.M., Heckman, R.W., Hejda, M., Jarošík, V., Kanarek, A.R., Martin, L.B., Perkins, S.E., Pyšek, P., Schierenbeck, K., Schlöder, C., van Klinken, R., Vaughn, K.J., Williams, W. & Wolfe, L.M. (2013): Do invasive species perform better in their new ranges? *Ecology* 94 (5), 985–994.

Perrault, A., Bennett, M., Burgiel, S., Delach, A. & Muffett, C. (2003): Invasive Species, Agriculture and Trade: Case Studies from the NAFTA Context. In: *Second North American Symposium on Assessing the Environmental Effects of Trade.* North American Commission for Environmental Cooperation, Mexico City, Mexico, 55pp.

Petersen, J.K. & Malm, T. (2006): Offshore Windmill Farms: Threats to or Possibilities for the Marine Environment. *Royal Swedish Academy of Sciences* 35 (2), 75–80, doi: 10.1579/0044-7447(2006)35.

Pickens, P.E. (1965): Heart Rate of Mussels as a Function of Latitude, Intertidal Height, and Acclimation Temperature. *Physiological Zoology* 38 (4), 390–405, doi: 10.1086/physzool.38.4.30152416.

Pillay, K.K. & Ono, Y. (1978): The breeding cycles of two species of grapsid crabs (Crustacea: Decapoda) from the North Coast of Kyushu, Japan. *Marine Biology* 45, 237–248.

Poff, N.L., Brinson, M.M. & Day, J.W. (2002): *Aquatic ecosystems & Global climate change: Potential Impacts on Inland Freshwater and Coastal Wetland Ecosystems in the United States*. Tech. report, Pew Center on Global Climate Change, 45 pp., doi: 10.1039/b211160h.

Rahel, F.J. & Olden, J.D. (2008): Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* 22 (3), 521–533, doi: 10.1111/j.1523-1739.2008.00950.x.

Reise, K. (2005): Coast of change: Habitat loss and transformations in the Wadden Sea. *Helgoland Marine Research* 59 (1), 9–21, doi: 10.1007/s10152-004-0202-6.

Reise, K., Baptist, M., Burbridge, P., Dankers, N., Fischer, L., Flemming, B., Oost, A.P. & Smit, C. (2010): The Wadden Sea – A Universally Outstanding Tidal Wetland. *Wadden Sea Ecosystem* 29, 7–24.

Reise, K., Buschbaum, C., Büttger, H. & Wegner, K.M. (2017): Invading oysters and native mussels: From hostile takeover to compatible bedfellows. *Ecosphere* 8 (9), e01949, doi: 10.1002/ecs2.1949.

Reise, K., Gollasch, S. & Wolff, W.J. (1999): Introduced marine species of the North Sea coasts. *Helgoländer Meeresuntersuchungen* 52 (1993), 219–234.

Reise, K., Olenin, S. & Thieltges, D.W. (2006): Are aliens threatening European aquatic coastal ecosystems? *Helgoland Marine Research* 60, 77–83, doi: 10.1007/s10152-006-0024-9.

Reuschel, S., Cuesta, J.A. & Schubart, C.D. (2010): Marine biogeographic boundaries and human introduction along the European coast revealed by phylogeography of the prawn Palaemon elegans. *Molecular Phylogenetics and Evolution* 55 (3), 765–775, doi: 10.1016/j.ympev.2010.03.021.

Rheinheimer, G. & Nehring, D., eds. (1995): *Meereskunde der Ostsee*. 2nd ed. Springer, Berlin, Heidelberg, 334 pp.

Rodriguez, L.F. (2006): Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions* 8 (4), 927–939, doi: 10.1007/s10530-005-5103-3.

Roman, J. (2006): Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society B* 273, 2453–2459, doi: 10.1098/rspb.2006.3597.

Roman, J. & Palumbi, S.R. (2004): A global invader at home: Population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology* 13 (10), 2891–2898, doi: 10.1111/j.1365-294X.2004.02255.x.

Rudnick, D., Veldhuizen, T., Tullis, R., Culver, C.S., Hieb, K. & Tsukimura, B. (2005): A life history model for the San Francisco Estuary population of the Chinese mitten crab, *Eriocheir sinensis* (Decapoda: Grapsoidea). *Biological Invasions* 7 (2), 333–350, doi: 10.1007/s10530-004-2286-y.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D. & Hines, A.H. (1997): Global Invasions of Marine and Estuarine Habitats by Non-Indigenous Species: Mechanisms, Extent, and Consequences. *American Zoologist* 37, 621–632, doi: 10.1093/icb/37.6.621.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J. & Hines, A.H. (2000): Invasion of Coastal Marine Communities in North America: Apparent Patterns, Processes, and Biases. *Annual Review of Ecology and Systematics* 31, 481–531, doi: 10.2307/annurev.ecolsys.37.091305.30000016.

Ruiz, G.M., Fofonoff, P.W., Hines, A.H. & Grosholz, E.D. (1999): Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. *Limnology and Oceanography* 44 (3, part 2), 950–972, doi: 10.4319/lo.1999.44.3_part_2.0950.

Ruiz, G.M. & Smith, G. (2005): *Biological Study of Container Vessels at the Port of Oakland*. Tech. report, Oakland, OR, 155 pp.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. (2001): The Population Biology of Invasive Species. *Annual Review of Ecology and Systematics* 32, 305–332, doi: 10.1146/annurev.ecolsys.32.081501.114037.

Sakai, K. (2007): Comments on an invalid nominal species, *Hemigrapsus takanoi* Asakura & Watanabe, 2005, a synonym of *Hemigrapsus penicillatus* (de Haan, 1835) (Decapoda, Brachyura, Grapsidae). *Crustaceana* 80 (4), 503–508.

Schubart, C.D. (2003): The East Asian shore crab *Hemigrapsus sanguineus* (Brachyura: Varunidae) in the Mediterranean Sea: an independent human-mediated introduction. *Scientia Marina* 67 (2), 195–200.

Secord, D. (2003): Biological control of marine invasive species: cautionary tales and land-based lessons. *Biological Invasions* 5 (1-2), 117–131, doi: 10.1023/A:1024054909052.

Seebens, H., Gastner, M.T. & Blasius, B. (2013): The risk of marine bioinvasion caused by global shipping. *Ecology Letters* 16 (6), 782–790, doi: 10.1111/ele.12111.

Seeley, B., Sewell, J. & Clark, P.F. (2015): First GB records of the invasive Asian shore crab, Hemigrapsus sanguineus from Glamorgan, Wales and Kent, England. *Marine Biodiversity Records* 8, e102, doi: 10.1017/S1755267215000809.

Simberloff, D. (2006): Invasional meltdown 6 years later: Important phenomenon, unfortunate metaphor, or both? *Ecology Letters* 9 (8), 912–919, doi: 10.1111/j.1461-0248.2006.00939.x.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B.S., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. & Vilà, M. (2013): Impacts of biological invasions: What's what and the way forward. *Trends in Ecology and Evolution* 28 (1), 58–66, doi: 10.1016/j.tree.2012.07.013.

Simberloff, D. & Von Holle, B. (1999): Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1, 21–32.

Smaal, A.C., Kater, B.J. & Wijsman, J. (2009): Introduction, establishment and expansion of the Pacific oyster *Crassostrea gigas* in the Oosterschelde (SW Netherlands). *Helgoland Marine Research* 63 (1), 75–83, doi: 10.1007/s10152-008-0138-3.

Snyder, W.E. & Evans, E.W. (2006): Ecological Effects of Invasive Arthropod Generalist Predators. *Annual Review of Ecology, Evolution, and Systematics* 37 (2006), 95–122, doi: 10.2307/annurev.ecolsys.37.091305.30000006.

Stachowicz, J.J., Fried, H., Osman, R.W. & Whitlatch, R.B. (2002): Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. *Ecology* 83 (9), 2575–2590, doi: 10.2307/3071816.

Stickle, W.B. & Sabourin, T.D. (1979): Effects of salinity on the respiration and heart rate of the common mussel, *Mytilus edulis* L., and the black chiton, *Katherina tunicata* (Wood). *Journal of Experimental Marine Biology and Ecology* 41 (3), 257–268, doi: 10.1016/0022-0981(79)90135-7.

Tepolt, C.K. & Somero, G.N. (2014): Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*. *The Journal of Experimental Biology* 217 (7), 1129–1138, doi: 10.1242/jeb.093849.

Thresher, R.E. & Kuris, A.M. (2004): Options for managing invasive marine species. *Biological Invasions* 6 (3), 295–300, doi: 10.1023/B:BINV.0000034598.28718.2e.

Tsutsui, N.D., Suarez, A.V., Holway, D.A. & Case, T.J. (2000): Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences* 97 (11), 5948–5953, doi: 10.1073/pnas.100110397.

Van den Brink, A.M., Godschalk, M., Smaal, A.C., Lindeboom, H. & McLay, C.L. (2013): Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of the Marine Biological Association of the United Kingdom* 93 (1), 189–196, doi: 10.1017/S0025315412000446.

Van den Brink, A.M., Wijnhoven, S. & McLay, C.L. (2012): Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73, 126–136, doi: 10.1016/j.seares.2012.07.006.

Viard, F., Ellien, C. & Dupont, L. (2006): Dispersal ability and invasion success of *Crepidula fornicata* in a single gulf: Insights from genetic markers and larval-dispersal model. *Helgoland Marine Research* 60 (2), 144–152, doi: 10.1007/s10152-006-0033-8.

Vitousek, P.M., D’Antonio, C.M., Loope, L.L. & Westbrooks, R. (1996): Biological Invasions as Global Environmental Change. *American Naturalist* 84 (5), 468–478.

Walton, W.C., MacKinnon, C., Rodriguez, L.F., Proctor, C. & Ruiz, G.M. (2002): Effect of an invasive crab upon a marine fishery: green crab *Carcinus maenas*, predation upon a venerid clam, *Katelysia scalarina*, in Tasmania (Australia). *Journal of Experimental Marine Biology and Ecology* 272 (2), 171–189, doi: 10.1016/S0022-0981(02)00127-2.

Williams, S.L. & Grosholz, E.D. (2002): Preliminary reports from the *Caulerpa taxifolia* invasion in southern California. *Marine Ecology Progress Series* 233, 307–310, doi: 10.3354/meps233307.

Williams, S.L. & Grosholz, E.D. (2008): The invasive species challenge in estuarine and coastal environments: Marrying management and science. *Estuaries and Coasts* 31 (1), 3–20, doi: 10.1007/s12237-007-9031-6.

Wolff, W.J. (2005): Non-indigenous marine and estuarine species in The Netherlands. *Zoologische Mededelingen Leiden* 79 (1), 1–116.

Wood, C.A., Bishop, J.D.D., Davies, C.J., Delduca, E.L., Hatton, J.C., Herbert, R.J.H. & Clark, P.F. (2015): *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4 (2), 109–113, doi: 10.3391/bir.2015.4.2.07.

Yamasaki, I., Doi, W., Mingkid, W.M., Yokota, M., Strüssmann, C.A. & Watanabe, S. (2011): Molecular-Based Method To Distinguish the Sibling Species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of Crustacean Biology* 31 (4), 577–581, doi: 10.1651/10-3366.1.

Zeng, Y., McLay, C.L. & Yeo, D.C.J. (2014): Capital or income breeding crabs: who are the better invaders? *Crustaceana* 87 (14), 1648–1656, doi: 10.1163/15685403-00003385.

Zenni, R.D. & Nuñez, M.A. (2013): The elephant in the room: The role of failed invasions in understanding invasion biology. *Oikos* 122, 801–815, doi: 10.1111/j.1600-0706.2012.00254.x.

Part II

An invasion process in real-time: *Hemigrapsus takanoi* in the Baltic Sea

2. First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea

Jonas C. Geburzi, Garrit Graumann, Stefanie Köhnk and Dirk Brandis

*Zoologisches Museum der Christian-Albrechts-Universität zu Kiel,
Hegewischstr. 3, 24105 Kiel, Germany*

Published in:
BioinvasionsRecords (2015) Volume 4, Issue 2: 103-107
doi: 10.3391/bir.2015.4.2.06

Abstract

Twenty years after its initial introduction to Europe, the Asian brush clawed crab *Hemigrapsus takanoi* is now a successful invader of the European Atlantic and North Sea coasts from northern Spain to southern Denmark. In July 2014, this species was recorded in the Baltic Sea for the first time, as eight individuals were found in the inner Kiel Fjord, Schleswig-Holstein, Germany. To date, a total number of 19 individuals have been collected. Findings of an ovigerous female and of juvenile crabs among the collected individuals indicate that *H. takanoi* most likely already reproduces successfully in the Baltic Sea.

Key words: Asian brush-clawed crab, Brachyura, Baltic Sea, invasive, non-indigenous

2.1. Introduction

Since the mid-1990s, the Asian brush-clawed crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005, native to the Western Pacific, has been invading the intertidal zone of the European Atlantic and North Sea coasts. Initially identified as *H. penicillatus* (DeHaan, 1835) (Gollasch 1999, Noël et al. 1997), the invasive populations in Europe were later reassigned to a newly-described sibling species of *H. penicillatus*, *H. takanoi* (Asakura & Watanabe 2005). Recent morphological and genetic studies confirmed the identification of *H. takanoi* as the invasive brush-clawed crab in Europe (Asakura et al. 2008, Markert et al. 2014, Yamasaki et al. 2011).

The first record of *H. takanoi* in Europe was based on several individuals found on a ship's hull at Bremerhaven harbour (Germany) in 1993 (Gollasch 1999). A reproducing population of *H. takanoi* was found close to La Rochelle (France) in 1994, from where it spread rapidly along the French and Spanish Bay of Biscay coast (Noël et al. 1997), expanding as far north as the Morbihan Gulf in southern Brittany (Noël & Gruet 2008). In 1999, the species was found at Le Havre harbour (France) and in the Oosterschelde (Netherlands), together with another invasive Asian crab, *Hemigrapsus sanguineus* (DeHaan, 1835) (Breton et al. 2002, Gittenberger et al. 2010). Both species then almost simultaneously expanded their range along the French coast of the English Channel and northeastwards into the North Sea. *Hemigrapsus takanoi*, in particular, spread along the Bay of Seine coast to the eastern Cotentin peninsula and further north towards the Belgian border, while only single individuals were reported from the western Cotentin coast (Dauvin & Delhay 2010, Dauvin et al. 2009, Gothland et al. 2014). It reached Belgian waters in 2003 (Kerckhof et al. 2007), the Dutch Wadden Sea between 2004 and 2006 (Gittenberger et al. 2010), the German Wadden Sea in 2007 (Obert et al. 2007), and finally the islands of Rømø and Fanø (Denmark) in the northernmost part of the Wadden Sea in 2011/12 (K. Reise, Alfred-Wegener-Insititute List/Sylt, pers. comm. 2014). Thus, the current distribution of *H. takanoi* in Europe includes the Atlantic coast from northern Spain to southern Brittany, the continental English Channel and southern North Sea coast from the Cotentin peninsula to the Dutch Delta, and the whole Wadden Sea.

The Baltic is, as a geologically young sea that underwent several major hydrological conversions, strongly influenced by the immigration and establishment of species from adjacent water bodies (Leppäkoski et al. 2002). Human-mediated introductions account for about 100 non-indigenous species that have invaded the Baltic Sea since the 19th century (Leppäkoski et al. 2002). Among these are two non-indigenous brachyuran crabs that developed invasive populations in the Baltic Sea over several decades: The North American mud crab *Rhithropanopeus harrisii* (Gould, 1841), initially found in the Kiel Canal in 1936 (Schubert 1936), and the Vistula Lagoon in 1948 (Jazdzewski & Grabowski 2011); and the Chinese mitten crab *Eriocheir sinensis* (H. Milne Edwards, 1853), found for the first time in the Baltic Sea in 1926 (Jazdzewski & Grabowski 2011). In this study, we report the first occurrence of *H. takanoi* as a third invasive brachyuran crab in the Baltic Sea.

2.2. Methods

The first crab specimens were collected 8 July 2014 while sampling seafloor and fouling communities on the quay walls and footbridge poles of a marina in the innermost part of Kiel Fjord, Schleswig-Holstein, Germany (54°19.669 N, 10°08.847 E; Figure 1) with a scraping scoop (mesh size 0.5 mm). Water depth at this site was about 2 m, and the seafloor consisted of fine sediment with scattered small boulders and mussel clumps. The water temperature in the inner Kiel Fjord ranges between 2.8 °C in February and 19.5 °C in July (1.5 m water depth, monthly average 2014; data provided by GEOMAR Helmholtz-Centre for Ocean Research Kiel, Research Division 1, Marine Meteorology 2015). The salinity in this part of the Baltic Sea (measured at Boknis Eck time series station) ranges between 13.5 in May and 19.2 in January and November (1 m water depth, 10-year average 2004 – 2014; Lennartz et al. 2014).

Additional sampling at the same site, as well as rapid assessment surveys around the Kiel Canal sluices and at Strande and Laboe at the entrance of Kiel Fjord, were performed in the following weeks (Figure 2.1; Appendix 2.1). These surveys were conducted by one or two collectors, sampling each site for 40 minutes (one collector) or 20 minutes (two collectors), respectively. Samples were taken from the seafloor, boulder revetments, and harbour buildings in near-shore shallow water up to 2.5 m depth, again using scraping scoops of 0.5 mm mesh size.

The collected crabs were identified, sex determined, and their carapace width (CW) was measured to the nearest 0.1 mm. For species identification, the

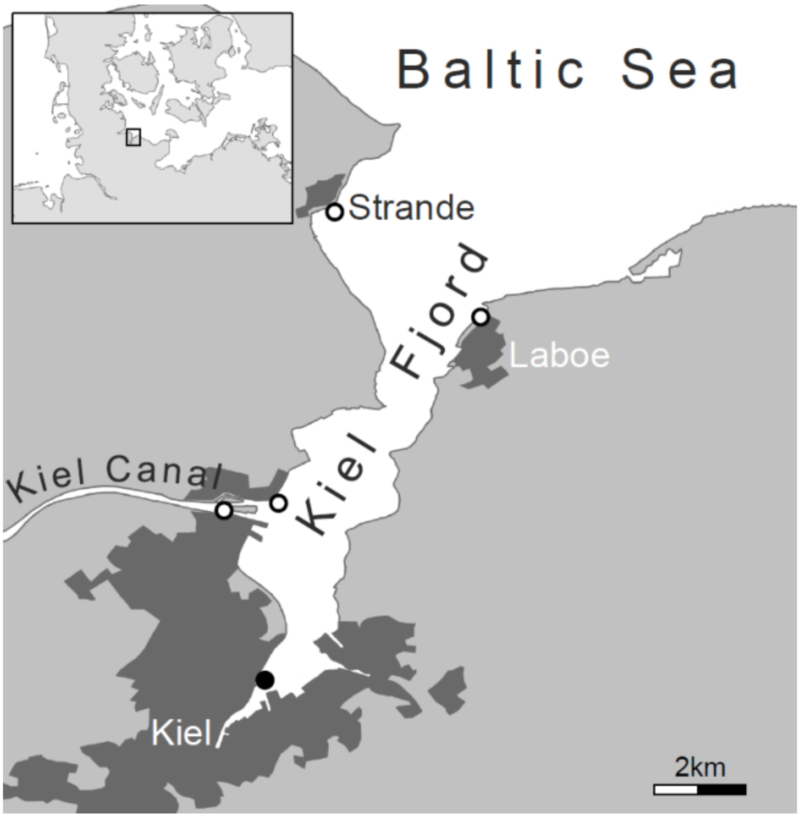


Fig. 2.1. Kiel Fjord sampling sites in summer 2014. Solid circle: H. *takanoi* present; open circles: *H. takanoi* not present. Small map: situation of Kiel Fjord in the southwestern Baltic Sea.

characters given in Breton et al. (2002) and Asakura & Watanabe (2005) were used, especially the presence of three lateral spines on the dorsal carapace (Figure 2A), of an interrupted infraorbital ridge (both sexes; Figure 2B), and of a chelar setal patch (only in males; Figure 2C). All individuals were fixed in 12 % formalin solution and stored in 75 % ethanol at the Zoological Museum Kiel (catalogue numbers ZMK 3500 to ZMK 3503).

2.3. Results and Discussion

During the summer 2014, the first records of *H. takanoi* in the Baltic Sea were made at a marina in the inner Kiel Fjord, 20 years after the species' initial invasion to Europe. Two individuals, an adult male (11.2 mm CW; Figure 2.2 C) and an ovigerous (egg-carrying) female (10.2 mm CW; Figure 2.2 D) were captured

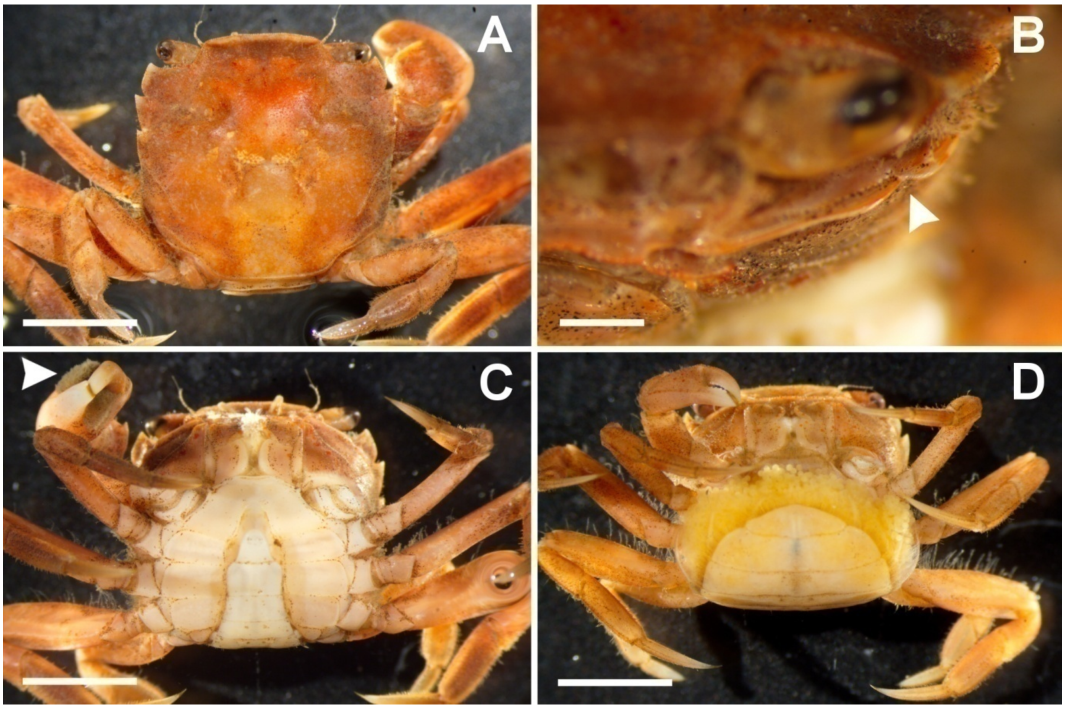


Fig. 2.2. *Hemigrapsus takanoi* collected in Kiel Fjord 8 July 2014: A) male, dorsal view, scale bar 5 mm; B) male, detail of the cephalothorax, frontal view with interrupted suborbital ridge (arrow), scale bar 1 mm; C) male, ventral view with chelar setal patch visible (arrow), scale bar 5 mm; D) ovigerous female, ventral view, scale bar 5 mm. Photographs by J. Geburzi.

on 8 July 2014. Seventeen other individuals were captured from the same spot during subsequent samplings from July to early November. Among them were several juvenile crabs measuring less than 5 mm CW (see Appendix 2.1 for details). No *H. takanoi* were found during the snapshot-survey around the Kiel Canal sluices and also none at Strande and Laboe at the entrance of Kiel Fjord, even though these places offer suitable habitat with extensive boulder revetments and other hard structures in the littoral and shallow sublittoral zone.

In an earlier study, Noël et al. (1997) concluded that expansion of the distribution of *H. takanoi* into the Baltic Sea was likely due to the species' tolerance of low salinity. The *H. takanoi* population in Tokyo Bay lives in waters with salinity values as low as 7 (Mingkid et al. 2006), which is considerably lower than the salinity of 16 recorded in the inner Kiel Fjord in July 2014 (monthly average, data provided by GEOMAR Helmholtz-Centre for Ocean Research Kiel, Research Division 3, Marine Ecology 2014). Thus, it can be assumed that

H. takanoi will be able to establish a reproducing population in the western and southern Baltic Sea. The collection of an ovigerous female and of several juveniles (< 10 mm CW), the smallest measuring only 4.1 mm CW, supports this assumption. However, the further expansion of *H. takanoi* into the Baltic Sea might be restricted by the salinity gradient occurring from the south-western towards the northeastern part (Leppäkoski et al. 2002).

The Kiel Canal between Brunsbüttel on the Elbe estuary and Kiel, connecting the North and Baltic Seas, formed most likely the invasion pathway of *H. takanoi*. With more than 30’000 ships using the canal annually (WSD Nord 2012), it is one of the most frequented artificial waterways worldwide and is assumed to be a major invasion pathway into the Baltic Sea (Leppäkoski et al. 2002). There are three possible ways how *H. takanoi* could have crossed the canal: First, crabs could have spread naturally along the canal from west to east. This appears to be unlikely because we would have expected to find *H. takanoi* around the sluices rather than in the innermost part of Kiel Fjord. Likewise, Landschoff et al. (2013) found no *H. takanoi* at Brunsbüttel, on the western entry to the canal. Second, crab larvae could have been transported in ballast water of ships coming in from one of the North Sea or European Atlantic ports. In such a case, the initial introduction would have happened in 2013 or earlier taking into account the findings of several adult and apparently reproducing crabs. Third, adult crabs could have been transported as hull fouling of a ship, probably a recreational boat or a cruise ship. We assume this vector to be the most likely due to the fact that the only spot where *H. takanoi* has been found to date is a marina in close proximity to cruise ship terminals. Recreational ship traffic plays an important role in the short- and medium-distance secondary spread of invasive crustaceans (Hänfling et al. 2011). During the summer months, the Kiel Canal is used extensively by recreational boats travelling between the Wadden and Baltic Seas. The fact that *H. takanoi* was not reported from Kiel Fjord before summer 2014 suggests a recent introduction, while at the same time the collection of different size juvenile crabs indicates successful reproduction of *H. takanoi* for at least one season. These observations point towards an extremely rapid establishment of a reproducing population that was facilitated by the introduction of adult individuals.

The first occurrence of *H. takanoi* in Kiel Fjord offers an opportunity to study mechanisms and impacts of a crustacean invasion into a new ecosystem from its very beginning. A careful study of the population development and expansion of *H. takanoi* is suggested, including monitoring of suitable habitats, especially harbours, in the southwestern Baltic Sea during the next few years. Of particular interest are possible interactions of *H. takanoi* with the native European shore crab *Carcinus maenas*, as well as with the two other brachyuran invaders, *Eriocheir sinensis* and *Rhithropanopeus harrisii*, as studies in the Netherlands (van den Brink et al. 2012) and France (Gothland et al. 2014) have already shown the strong competitive abilities of *H. takanoi* might have negative impacts on other crab species.

Acknowledgements

The authors thank R. Kreschel, S. Hayer and M. Wirth for their collecting efforts, F. Nevoigt and M. Paulsen for providing temperature and salinity data for Kiel Fjord and K. Reise for information on the most recent observations of *Hemigrapsus spp.* in the northern Wadden Sea. We also thank two anonymous reviewers for their very helpful remarks and suggestions on the manuscript. J. Geburzi gratefully thanks the German Environmental Foundation (DBU) for financial support.

References

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Asakura, A., Yamasaki, I., Mingkid, W.M. & Watanabe, S. (2008): Revalidation of *Hemigrapsus takanoi* Asakura & Watanabe, 2005: A rebuttal to "Sakai (2007): Comments on an invalid nominal species, *Hemigrapsus takanoi* Asakura & Watanabe, 2005, a synonym of *Hemigrapsus penicillatus* (De Haan, 1835)". *Crustaceana* 81 (10), 1263–1273, doi: 10.1163/156854008X374603.

Breton, G., Faasse, M., Noël, P.Y. & Vincent, T. (2002): A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1), 184–189, doi: 10.1651/0278-0372(2002)022[0184:ANACIE]2.0.CO;2.

Dauvin, J.-C. & Delhay, J.-B. (2010): First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern Cotentin, Normandy, western English Channel. *Marine Biodiversity Records* 3, 1–3, doi: 10.1017/S1755267210000928.

Dauvin, J.-C., Tous Rius, A. & Ruellet, T. (2009): Recent expansion of two invasive crab species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions* 4 (3), 451–465, doi: 10.3391/ai.2009.4.3.3.

Gittenberger, A., Rensing, M., Stegenga, H. & Hoeksema, B. (2010): Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33, 21–76.

Gollasch, S. (1999): The Asian decapod *Hemigrapsus penicillatus* (De Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgoländer Meeresuntersuchungen* 52, 359–366, doi: 10.1007/BF02908909.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

Hänfling, B., Edwards, F. & Gherardi, F. (2011): Invasive alien Crustacea: dispersal, establishment, impact and control. *BioControl* 56, 573–595, doi: 10.1007/s10526-011-9380-8.

Jazdzewski, K. & Grabowski, M. (2011): Alien Crustaceans Along the Southern and Western Baltic Sea. In: Galil, B.S., Clark, P.F. & Carlton, J.T. (eds.) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts.* Springer Netherlands, Dordrecht, pp. 323–344, doi: 10.1007/978-94-007-0591-3.

Kerckhof, F., Haelters, J. & Gollasch, S. (2007): Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions* 2 (3), 243–257.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Lennartz, S.T., Lehmann, A., Herrford, J., Malien, F., Hansen, H.-P., Biester, H. & Bange, H.W. (2014): Long-term trends at the Boknis Eck time series station (Baltic Sea), 1957–2013: does climate change counteract the decline in eutrophication? *Biogeosciences* 11 (22), 6323–6339, doi: 10.5194/bg-11-6323-2014.

Leppäkoski, E., Gollasch, S., Gruszka, P., Ojaveer, H., Olenin, S. & Panov, V.E. (2002): The Baltic – a sea of invaders. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1175–1188, doi: 10.1139/f02-089.

Markert, A., Raupach, M.J., Segelken-Voigt, A. & Wehrmann, A. (2014): Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatus* (De Haan, 1835) versus *Hemigrapsus takanoi* Asakura & Watanabe 2005. *Organisms Diversity & Evolution*, doi: 10.1007/s13127-014-0176-4.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Noël, P.Y. & Gruet, Y. (2008): Progression du crabe introduit *Hemigrapsus takanoi* Asukura & Watanabe 2005 (Crustacé, Décapode) vers le nord du Golfe de Gascogne.

Bulletin de la Société des Sciences Naturelles de l'Ouest de la France, Nouvelle Série 30, 141–148.

Noël, P.Y., Tardy, E. & D'Udekem d'Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Obert, B., Herly, M. & Grotjahn, M. (2007): First records of two crabs from the North West Pacific *Hemigrapsus sanguineus* and *H. takanoi* at the coast of Lower Saxony, Germany. *Wadden Sea Newsletter* 1, 21–22.

Schubert, K. (1936): *Pilumnopeus tridentatus* Maitland, eine neue Rundkrabbe in Deutschland. *Zoologischer Anzeiger* 11/12, 320–323.

Van den Brink, A.M., Wijnhoven, S. & McLay, C.L. (2012): Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73, 126–136, doi: 10.1016/j.seares.2012.07.006.

WSD Nord (2012): *Jahresbericht 2012*. Tech. report, Wasser- und Schifffahrtsdirektion Nord, Kiel, 114 pp.

Yamasaki, I., Doi, W., Mingkid, W.M., Yokota, M., Strüssmann, C.A. & Watanabe, S. (2011): Molecular-Based Method To Distinguish the Sibling Species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of Crustacean Biology* 31 (4), 577–581, doi: 10.1651/10-3366.1.

Appendix

Tab. 2.1. Records of *Hemigrapsus takanoi* in Kiel Fjord in summer and fall 2014, for locations see map (Figure 2.1); N: number of individuals, m: male, f: female, ov: ovigerous, j: juvenile, ZMK No.: catalogue number Zoological Museum Kiel.

Location	Sampling site coordinates		Date	N	CW [mm]	ZMK No.
	Latitude, N	Longitude, E				
inner Kiel Fjord	54°19.669’	10°08.847’	08 Jul 2014	1 m, 1 f (ov)	10.2–11.2	ZMK 3550
inner Kiel Fjord	54°19.669’	10°08.847’	17 Jul 2014	3 m, 2 f	11.1–15.1	ZMK 3501
Laboe, beach	54°24.638’	10°13.554’	19 Jul 2014	–		
Strande, harbour	54°26.093’	10°10.230’	19Jul 2014	–		
inner Kiel Fjord	54°19.669’	10°08.847’	22 Jul 2014	1 m	12.4	ZMK 3502
Kiel Fjord, Holtenau	54°22.139’	10°09.259’	13 Aug 2014	–		
Kiel Canal, Holtenau	54°22.175’	10°08.211’	13 Aug 2014	–		
inner Kiel Fjord	54°19.671’	10°08.844’	26 Sep 2014	1 j	4.1	ZMK 3503
inner Kiel Fjord	54°19.671’	10°08.844’	01 Nov 2014	4 m, 2 f, 4 j	4.6–10.4	ZMK 3504

3. Full larval cycle and small-scale migration patterns of *Hemigrapsus takanoi* larvae in the recently invaded southwestern Baltic Sea

Jonas C. Geburzi[a, b, c], Nena Weiler[a], Günther B. Hartl[b] and Dirk Brandis[a]

[a]Kiel University, Zoological Museum, Hegewischstr. 3, 24105 Kiel, Germany
[b]Kiel University, Zoological Institute, Population Genetics, Am Botanischen Garten 1-9, 24118 Kiel, Germany
[c]Alfred-Wegener-Institute, Helmholtz-Centre for Polar and Marine Research, Wadden Sea Station, Hafenstr. 43, 25992 List/Sylt, Germany

Submitted to:
Aquatic Invasions

Abstract

Constantly high numbers of marine species are transferred to regions way beyond their natural distribution limits by human activities. Whether such introduced species successfully establish and eventually become invasive, crucially depends on their ability to reproduce in their new habitats. In the Baltic Sea, the crab *Hemigrapsus takanoi* is the most recently introduced decapod crustacean species. We here present evidence that *H. takanoi* is able to reproduce in this brackish ecosystem by reconstructing its full larval cycle from extensive plankton samplings in Kiel Fjord, southwestern Baltic Sea. The larval cycle of *H. takanoi* consists of five zoea and one megalopa stage. We provide morphological descriptions of all larval stages, and species-specific characters to distinguish *H. takanoi* larvae from the larvae of other crabs occurring in this

area. A reproductive period of 5 months is derived from the sampling data. Our results further indicate that zoea I stages of *H. takanoi* perform migrations from near-shore hatching sites to more open waters with higher salinity to undergo larval development. We discuss these findings and their implications for possible underlying rapid adaptations of *H. takanoi* larvae to the specific environmental conditions in Kiel Fjord. Three years after the first record, *H. takanoi* can now be considered as fully established in the southwestern Baltic Sea, its case offering an opportunity to study adaptations and impacts of a non-native species from the earliest introduction stages on.

3.1. Introduction

The Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) is the most recent non-native decapod species reported from the Baltic Sea. This species, which was only described in 2005 as a sibling species of *H. penicillatus* (De Haan, 1835) (Asakura & Watanabe 2005), is native to the coasts of far east Russia, the Korean peninsula and Japan (Asakura & Watanabe 2005, Yamasaki et al. 2011, Lee et al. 2013, Marin 2013). In Europe, it has been initially reported from La Rochelle (France) and Bremerhaven (Germany) in the mid-1990s, first identified as *H. penicillatus* (Noël et al. 1997, Gollasch 1999), but later reassigned to *H. takanoi*, (Asakura & Watanabe 2005, Markert et al. 2014), and has since then spread along the Bay of Biscay, the French and Belgian coast of the English Channel, the Dutch Delta, and the whole Wadden Sea from the Netherlands to northern Germany and southern Denmark (Nijland & Beekman 2000, Kerckhof et al. 2007, Noël & Gruet 2008, Dauvin & Delhay 2010, Gittenberger et al. 2010, Landschoff et al. 2013, Gothland et al. 2014). More recently, *H. takanoi* has also been reported from several sites in Great Britain (Wood et al. 2015, Ashelby et al. 2017).

The first record of the species in the Baltic Sea was made in 2014 in Kiel Fjord, Schleswig-Holstein, Germany, including an ovigerous female and several juvenile crabs (Geburzi et al. 2015). *Hemigrapsus takanoi* is thus the fifth crab species occurring in this part of the Baltic Sea, besides the European shore crab *Carcinus maenas* (Linnaeus, 1758), the sandy swimming crab *Liocarcinus depurator* (Linnaeus, 1758), the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853 (another member of the family Varunidae) and the mud crab *Rhithropanopeus harrisii* (Gould, 1841), the latter two being as well non-native species (HELCOM 2012).

Even though the early findings of ovigerous females and juvenile crabs suggested that *H. takanoi* is able to successfully reproduce in the southwestern Baltic Sea, the detection of larvae in the plankton would ultimately confirm this crucial step for a successful establishment as invasive species. As larvae of coastal crabs, particularly the zoeal stages, often have narrower ranges of tolerance regarding temperature and salinity compared to adults, their requirements may also constrain establishment and dispersal of non-native crabs in newly invaded habitats (Anger 2006, and references therein). For several coastal and brackish water crabs, it has been shown that their zoea migrate with the currents to more saline waters (usually further off-shore) to undergo development and return to the adult habitats before metamorphosis (e.g. Anger 1991, Anger et al. 1994, Park et al. 2005, Otto & Brandis 2011).

The larval development of *H. takanoi* has not yet been studied in detail, certainly also due to their recent description as separate species. Morphological descriptions exist for larvae of the sibling species *H. penicillatus* and other *Hemigrapsus spp.* (Hwang & Kim 1995, Kornienko et al. 2008), and it seems legible to assume them to be similar. However, to investigate larval dynamics of *H. takanoi* and their potential role in the further dispersal of this species in the Baltic Sea, species-specific morphological characteristics are needed for all larval stages of *H. takanoi*, to be able to identify them in plankton samples.

In this study, we present evidence that *H. takanoi* undergoes full larval development in the southwestern Baltic Sea, along with morphological descriptions of all six larval stages, the first descriptions of this species' larvae. Furthermore, we present first data on the phenology and small-scale migration patterns of *H. takanoi* larvae in Kiel Fjord. Our results indicate that *H. takanoi* larvae perform vertical and horizontal migrations in adaptation to the specific local hydrographic conditions in order to optimise conditions for their development. The study thus provides first insights into a rapid adaptation process of a newly established species, which might be of high importance for its overall establishment success.

3.2. Methods

3.2.1. Sampling

Sampling for this study was conducted in Kiel Fjord, Schleswig-Holstein, Germany (N 54.36°, E 10.17°) between August 2016 and February 2017. Near-shore samples were taken from the inner part of the fjord (site 'NS', Fig. 3.1 C) with a plankton net (mesh size 55 µm) in the evening hours between 9 and 10 p.m. two to three times a week in August and September 2016, and once a month from October 2016 to February 2017. At each date, one surface sample (0–0.2 m depth, 3 min net tow) and one bottom sample (2–3 m depth, 5 min net tow) were taken. Off-shore surface samples (0–0.4 m depth, 5 min net tow) were taken from the centre of the inner Kiel Fjord (site 'OS', Fig. 3.1 C) with the research vessel RB Polarfuchs (GEOMAR, Helmholtz-Centre for Ocean Research

Fig. 3.1. Location (A) and overview (B) of Kiel Fjord, southwestern Baltic Sea. Inner part of Kiel Fjord (C) with near-shore (NS) and off-shore (OS) sampling areas indicated (hatched areas).

Kiel) with a WP2 net (mesh size 100 µm) and a bongo net (mesh size 300 µm and 500 µm) on 8, 15 and 22 August 2016. The preserved plankton samples are deposited at the Zoological Museum Kiel under the collection numbers ZMK [final collection numbers to be assigned]. Additionally, plankton samples of a weekly sampling series, conducted by GEOMAR, from May, June, July and September 2016 were screened for crab larvae. For each sampling, water temperature (°C) and salinity (PSU, Practical Salinity Unit) (GEOMAR) and air temperature (°C) (Deutscher Wetterdienst [German National Meteorological Service], weather station Kiel-Holtenau) were recorded.

To detect possible diurnal patterns in the occurrence and distribution of larvae, three 24-hour samplings (9 and 26 August and 12 September 2016) were performed at the near-shore sampling site, with one surface sample and one bottom sample taken every two hours. The samplings started at midnight in August and at 11 p.m. in September, respectively.

All samples were fixed in 75 % Ethanol immediately after sampling, and screened for crab larvae under a stereo microscope. Samples from the regular near-shore sampling series and the 24-hour samplings were analysed to exact counts of larvae for each stage. The off-shore samples taken with RB Polarfuchs were analysed semi-quantitatively due to the high numbers of larvae present in these samples.

3.2.2. Identification and description of larvae

Identification of *Hemigrapsus takanoi* larvae was based on morphological characteristics, in particular carapace shape and occurrence of spines, shape and setation of antennule and antenna, the abdomen, and shape and setation of the telson (Fig. 3.2). To discriminate *H. takanoi* larvae from the larvae of other crabs (potentially) occurring in Kiel Fjord, we compared these characteristics with descriptions of the larvae of the European shore crab *Carcinus maenas* (Rice & Ingle 1975), the Chinese mitten crab *Eriocheir sinensis* (Kim & Hwang 1995, Montú et al. 1996) and swimming crabs *Liocarcinus spp.* Stimpson, 1871 (Clark 1984), as well as with descriptions of the closely related *H. sanguineus* (De Haan, 1835) and *H. penicillatus* (Hwang & Kim 1995, Kornienko et al. 2008, Lee & Ko 2008).

To confirm the morphological identification as *H. takanoi* larvae, genomic DNA from subsamples of all zoeal stages and the megalopa was isolated using the Invisorb Tissue Mini Kit (Stratec Molecular, Berlin, Germany). The DNA samples were sequenced on a 583 base pair fragment of the COI gene with the primer pair COL6/COH6 (Schubart 2009). Mastermix preparation and PCR settings followed Schubart (2009), and the checked and trimmed sequences were compared with sequences available on GenBank, using the BLASTN algorithm, version 2.7.1+ (Zhang et al. 2000, NCBI 2017).

Examination of larvae, measurements and photographic documentation were performed under a VHX-5000 MultiScan digital microscope (Keyence, Japan). For scanning electron microscope (SEM) analysis, seven zoea of each stage and three megalopa were dehydrated in 100 % undenatured ethanol (45 min), critical point dried under CO_2 and sputtered with gold-palladium, to be examined under a TM3000 Tabletop Microscope (Hitachi, Japan). Image editing was performed with Adobe Photoshop CS2 (Adobe Systems, USA, 2005).

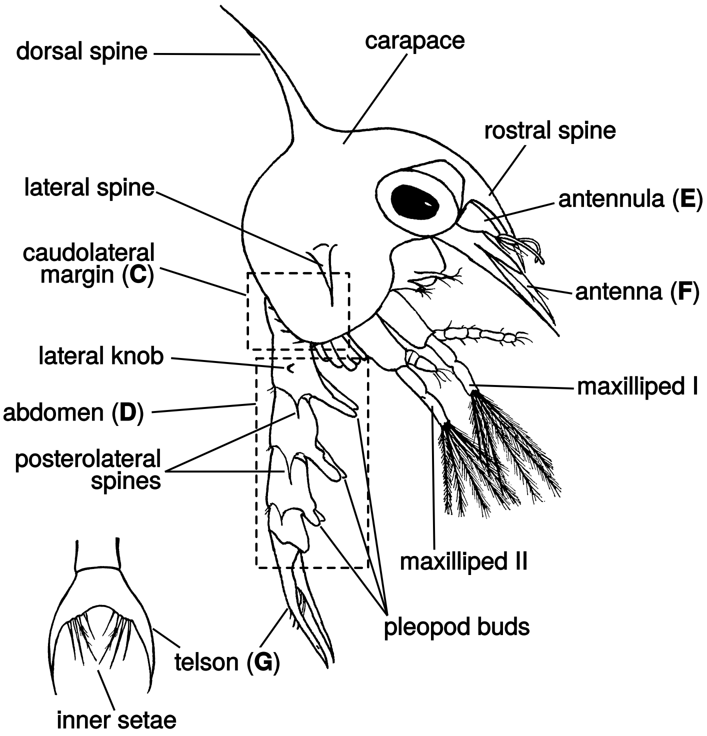


Fig. 3.2. Body parts of brachyuran zoea and main characters used for the identification. Capital letters in brackets refer to sub-figures of Fig. 3.4 to Fig. 3.8 in the results section. Redrawn and modified from Kornienko & Korn (2009).

3.3. Results

3.3.1. Identification and description of *Hemigrapsus takanoi* larval stages

As identified from the plankton samples, larval development of *Hemigrapsus takanoi* includes five zoea stages and a megalopa (Fig. 3.3). Sequencing results of the 583 bp fragment of the COI gene confirmed the morphological identification of larval stages as *H. takanoi* with identity scores of 99 to 100 % to European and Japanese sequences of *H. takanoi* stored on GenBank (Markert et al. 2014, Raupach et al. 2015, NCBI 2017).

The body of zoea is transparent and slightly yellow, with few black chromatophores, which are particularly conspicious at the bases of the spines. The surface of the carapace and spines is smooth. In all zoeal stages, dorsal, rostral and lateral carapace spines are present. The dorsal spine is slightly curved and the rostral spine is longer than antennule and antenna. All zoeal stages show a

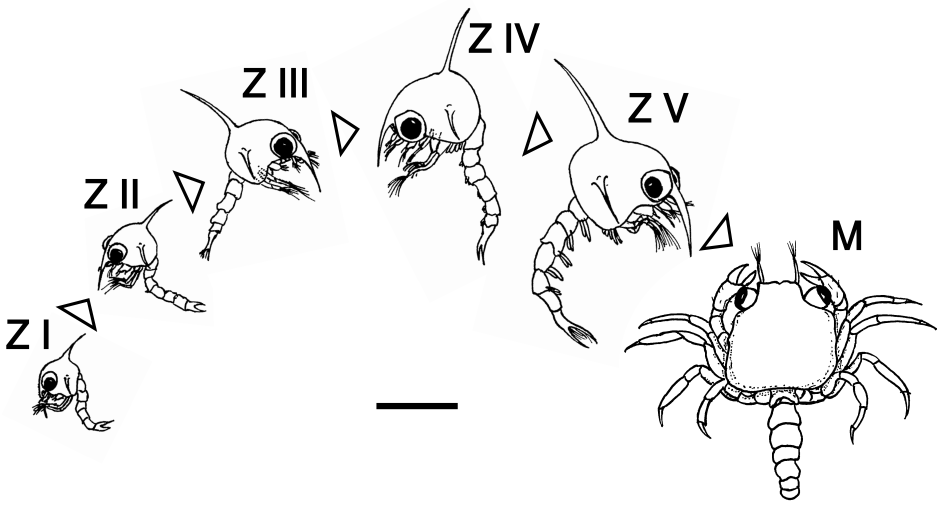


Fig. 3.3. Larval development of *H. takanoi*, consisting of five zoeal stages (Z I to Z V), and a Megalopa stage (M). Redrawn and slightly modified from Anger et al. (1990). Scale bar 1 mm.

pair of lateral knobs pointing frontally on the second abdominal segment, and a pair of much smaller lateral knobs on the third abdominal segment (Fig. 3.4 to Fig. 3.8, D).

In the following, main and diagnostic characteristics for all larval stages of *H. takanoi* are described in detail and are illustrated in Fig. 3.4 to Fig. 3.9. Sub-figures in the illustrations of the zoeal stages (Fig. 3.4 to Fig. 3.8) are all arranged in the same order, also see Fig. 3.2 for orientation. The following abbreviations are used: TL – total length of larva, measured from the base of the rostral spine to the tip of the telson; DS – length of dorsal spine; RS – length of rostral spine; CL – carapace length (only megalopa); CW – carapace width (only megalopa). All measurements are given as mean values ± SD.

Zoea I (Fig. 3.4)

Size: TL 0.96 ± 0.11 mm (n = 25), DS 0.37 ± 0.14 mm (n = 44), RS 0.27 ± 0.13 mm (n = 28)

Carapace (Fig. 3.4 A, B, C): surface smooth; dorsal, lateral and rostral spines well developed, DS longer than RS; surface of all spines is smooth; posteroventral carapace margin with 4 denticles on either side.

Antennule (Fig. 3.4 E): Uniramous, conical shape, with 3 aesthetascs and 1 seta.

Antenna (Fig. 3.4 F): Biramous, long protopod with a marginal row of denticles, shorter exopod with 1 terminal seta.

Abdomen (Fig. 3.4 D): With 5 pleon segments, segments 2 and 3 with lateral knobs.

Telson (Fig. 3.4 G): With 3 pairs of setae along the inner posterior margin; innermost pair of setae partly plumose; 2 rows of denticles along the inner furcal margin.

Zoea II (Fig. 3.5)

Size: TL 1.44 ± 0.23 mm (n = 18), DS 0.59 ± 0.12 mm (n = 42), RS 0.52 ± 0.15 mm (n = 31)

Carapace (Fig. 3.5 A, B, C): Posteroventral carapace margin with 3 denticles on either side; otherwise unchanged.

Antennule (Fig. 3.5 E): Unchanged, larger than in Z I.

Antenna (Fig. 3.5 F): Protopod unchanged, exopod with 2 additional lateral spines; lager than in Z I.

Abdomen (Fig. 3.5 D): unchanged.

Telson (Fig. 3.5 G): unchanged.

Zoea III (Fig. 3.6)

Size: TL 1.97 ± 0.13 mm (n = 10), DS 0.81 ± 0.11 mm (n = 34), RS 0.73 ± 0.13 mm (n = 35)

Carapace (Fig. 3.6 A, B, C): Posteroventral carapace margin with 3 small knobs on either side; otherwise unchanged.

Antennule (Fig. 3.6 E): With 3–4 aesthetascs and 1 seta.

Antenna (Fig. 3.6 F): Beginning development of endopod, exopod with 4–5 terminal setae of various length.

Abdomen (Fig. 3.6 D): With 6 pleon segments, otherwise unchanged.

Telson (Fig. 3.6 G): With 4 pairs of setae along the inner posterior margin, the innermost pair much shorter that the others; otherwise unchanged.

Zoea IV (Fig. 3.7)

Size: TL 2.72 ± 0.23 mm (n = 16), DS 1.05 ± 0.12 mm (n = 44), RS 0.99 ± 0.14 mm (n = 45)

Carapace (Fig. 3.7 A, B, C): Posteroventral carapace margin smooth; otherwise unchanged.

Antennule (Fig. 3.7 E): With 3–4 aesthetascs and 5–7 setae; beginning development of a basal bulge.

Antenna (Fig. 3.7 F): Endopodal bud clearly developed; otherwise unchanged.

Abdomen (Fig. 3.7 D): Pleopodal buds developing on pleon segments 2 to 5; otherwise unchanged.

Telson (Fig. 3.7 G): With 4 pairs of fully developed setae along the inner posterior margin; otherwise unchanged.

Zoea V (Fig. 3.8)

Size: TL 3.42 ± 0.22 mm (n = 21), DS 1.39 ± 0.11 mm (n = 39), RS 1.15 ± 0.14 mm (n = 42)

Carapace (Fig. 3.8 A, B, C): As in Z IV.

Antennule (Fig. 3.8 E): Basal bulge clearly developed; otherwise unchanged.

Antenna (Fig. 3.8 F): Endopod 2-segmented and longer than exopod, otherwise unchanged.

Abdomen (Fig. 3.8 D): Pleopodal buds longer and biramous; otherwise unchanged.

Telson (Fig. 3.8 G): With 5 pairs of setae along the inner posterior margin, the innermost pair much shorter that the others; otherwise unchanged.

Megalopa (Fig. 3.9)

Size: CL 1.47 ± 0.12 mm (n = 23), CW 1.24 ± 0.12 mm (n = 22)

Carapace (Fig. 3.9 A, B): Shape nearly rectangular; rostrum very short and curved ventrally, with a narrow notch at the front.

Antennule (Fig. 3.9 E): Peduncle 3-segmented, the second segment with 2 lateral spines; endopod 2-segmented with 2 short terminal setae; exopod

3-segmented, the second segment with 10-12 aesthetascs and 1 seta on the opposite side, the third segment with 10-12 aesthetascs and 1 terminal seta.

Antenna (Fig. 9 3.9 F): With 10 segments, setal formula 0, 1, 0, 0, 0, 2, 3, 4, 3.

Pereopods (Fig. 3.9 B, C): Dactyli of pereopods 2, 3 and 4 as walking limbs; dactyli of pereopod 1 as chela; chela with several setae.

Abdomen (Fig. 3.9 D): With 6 pleon segments; segments 2 to 5 with biramous pleopods, each with a patch of long terminal setae.

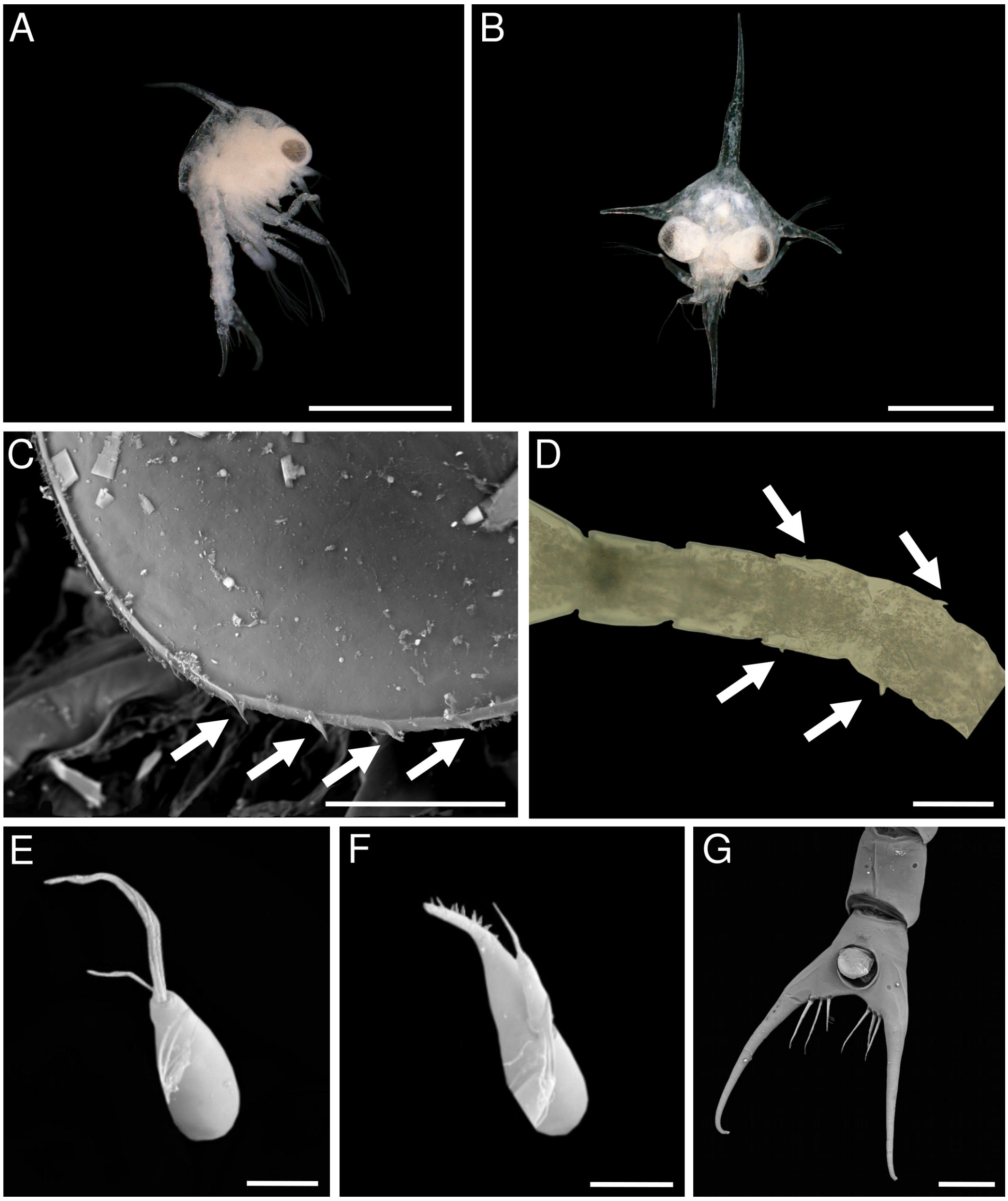


Fig. 3.4. *Hemigrapsus takanoi*, zoea I. A: lateral view; B: frontal view; C: caudolateral carapace margin, lateral view, with 4 denticles (arrwos); D: pleon, dorsal view, with lateral knobs on segments 2 and 3 (arrows); E: antennule; F: antenna; G: telson. Scale bars: A, B 0.5 mm; C, E, F 0.05 mm; D, G 0.1 mm.

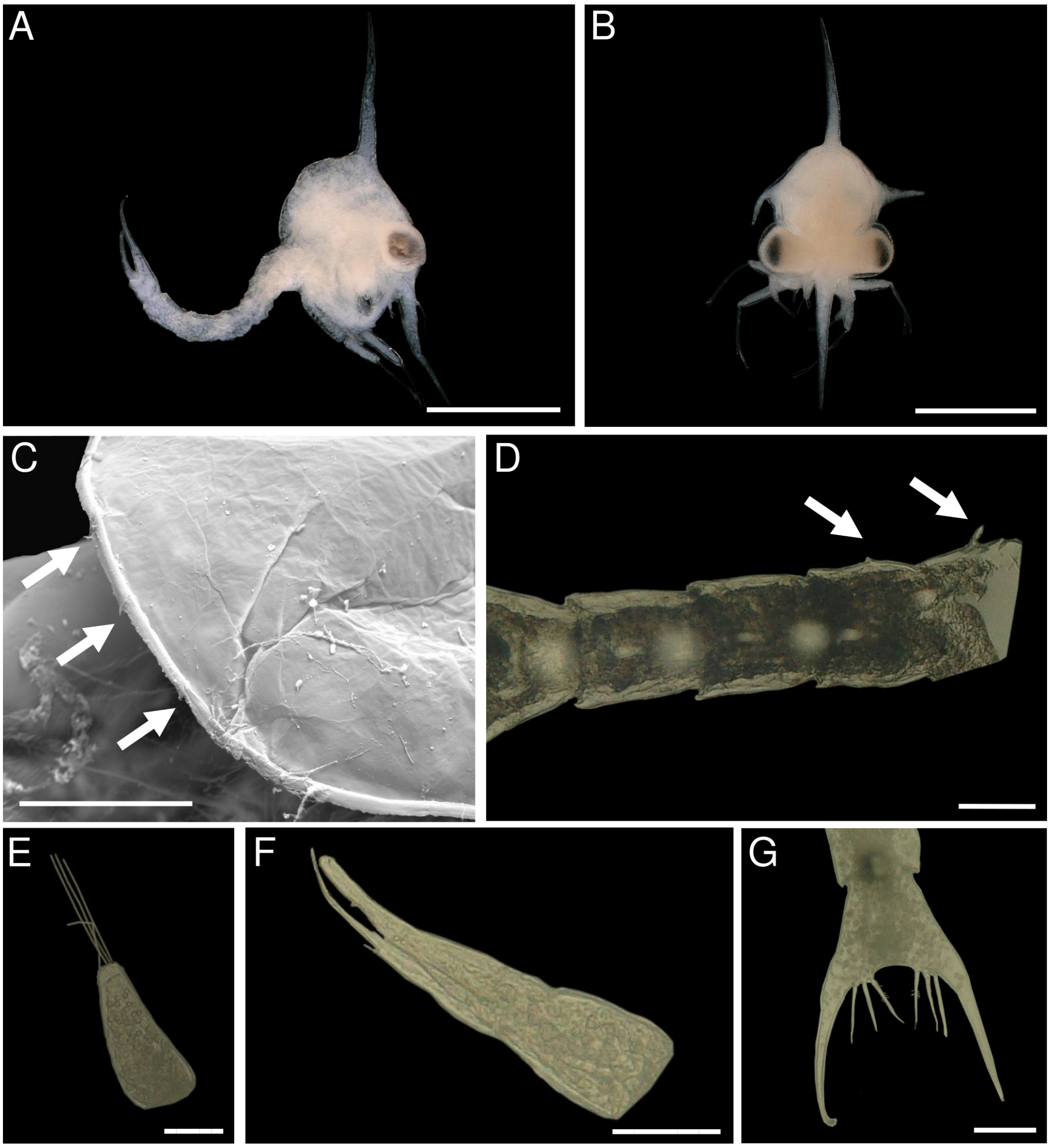


Fig. 3.5. *Hemigrapsus takanoi*, zoea II. A: lateral view; B: frontal view; C: caudolateral carapace margin, lateral view, with 3 denticles (arrwos); D: pleon, dorsal view, with lateral knobs on segments 2 and 3 (arrows); E: antennule; F: antenna; G: telson. Scale bars: A, B 0.5 mm; C, E, F 0.05 mm; D, G 0.1 mm.

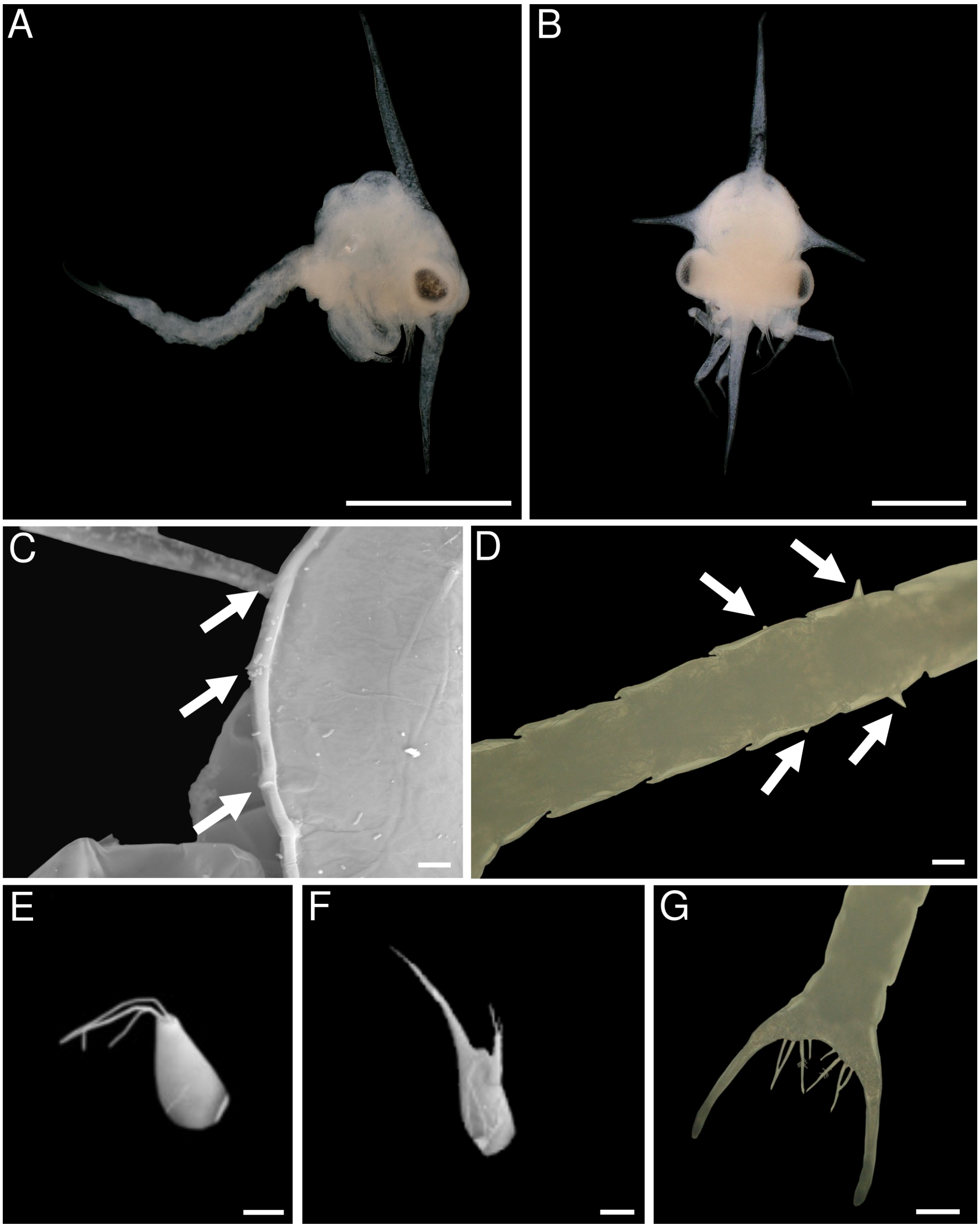


Fig. 3.6. *Hemigrapsus takanoi*, zoea III. A: lateral view; B: frontal view; C: caudolateral carapace margin, lateral view, with 3 knobs (arrwos); D: pleon, dorsal view, with lateral knobs on segments 2 and 3 (arrows); E: antennule; F: antenna; G: telson. Scale bars: A 1 mm; B 0.5 mm; C 0.01 mm; E, F 0.05 mm; D, G 0.1 mm.

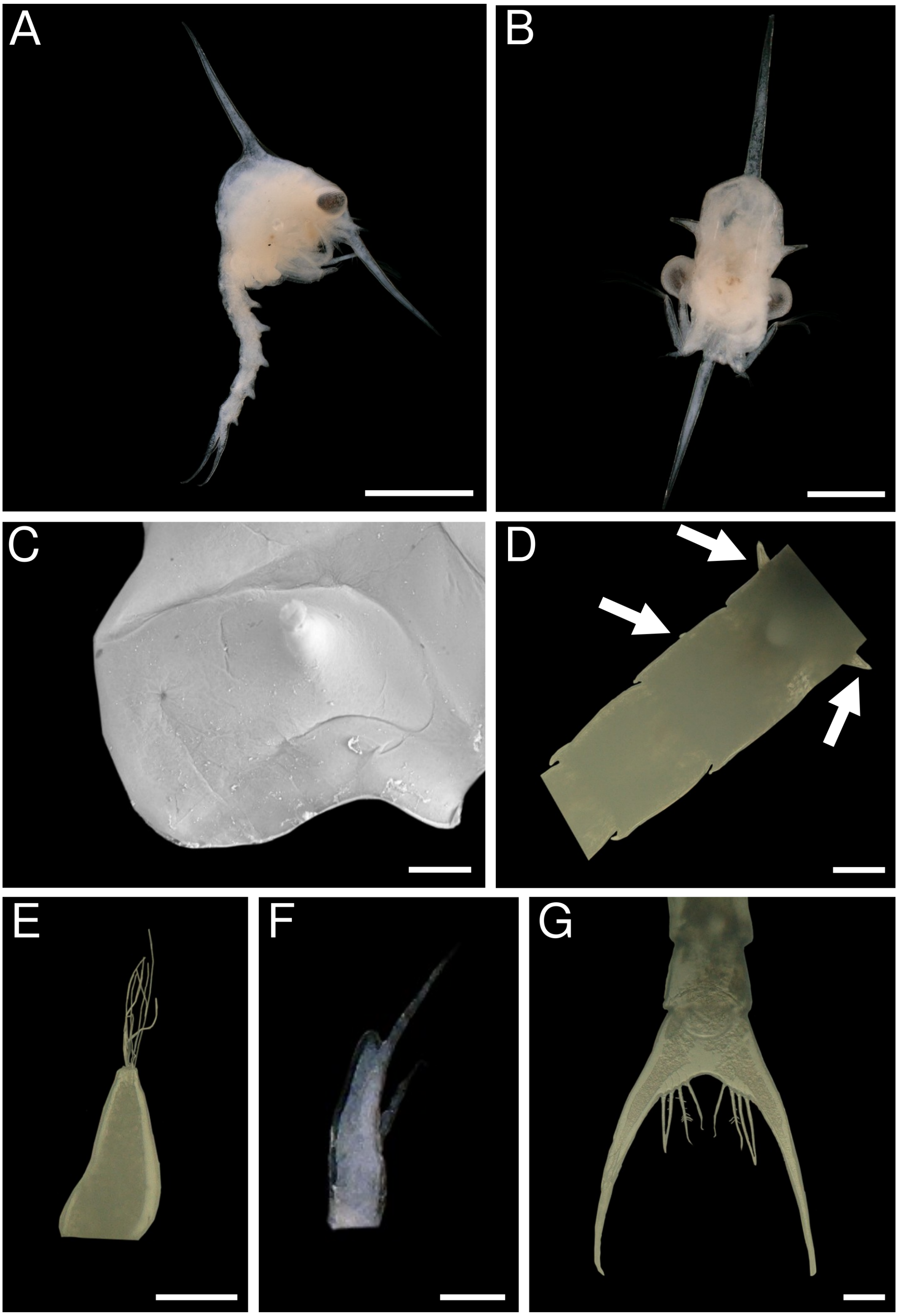


Fig. 3.7. *Hemigrapsus takanoi*, zoea IV. A: lateral view; B: frontal view; C: caudolateral carapace margin, lateral view; D: pleon, dorsal view, with lateral knobs on segments 2 and 3 (arrows); E: antennule; F: antenna; G: telson. Scale bars: A 1 mm; B 0.5 mm; C, D, E, F, G 0.1 mm.

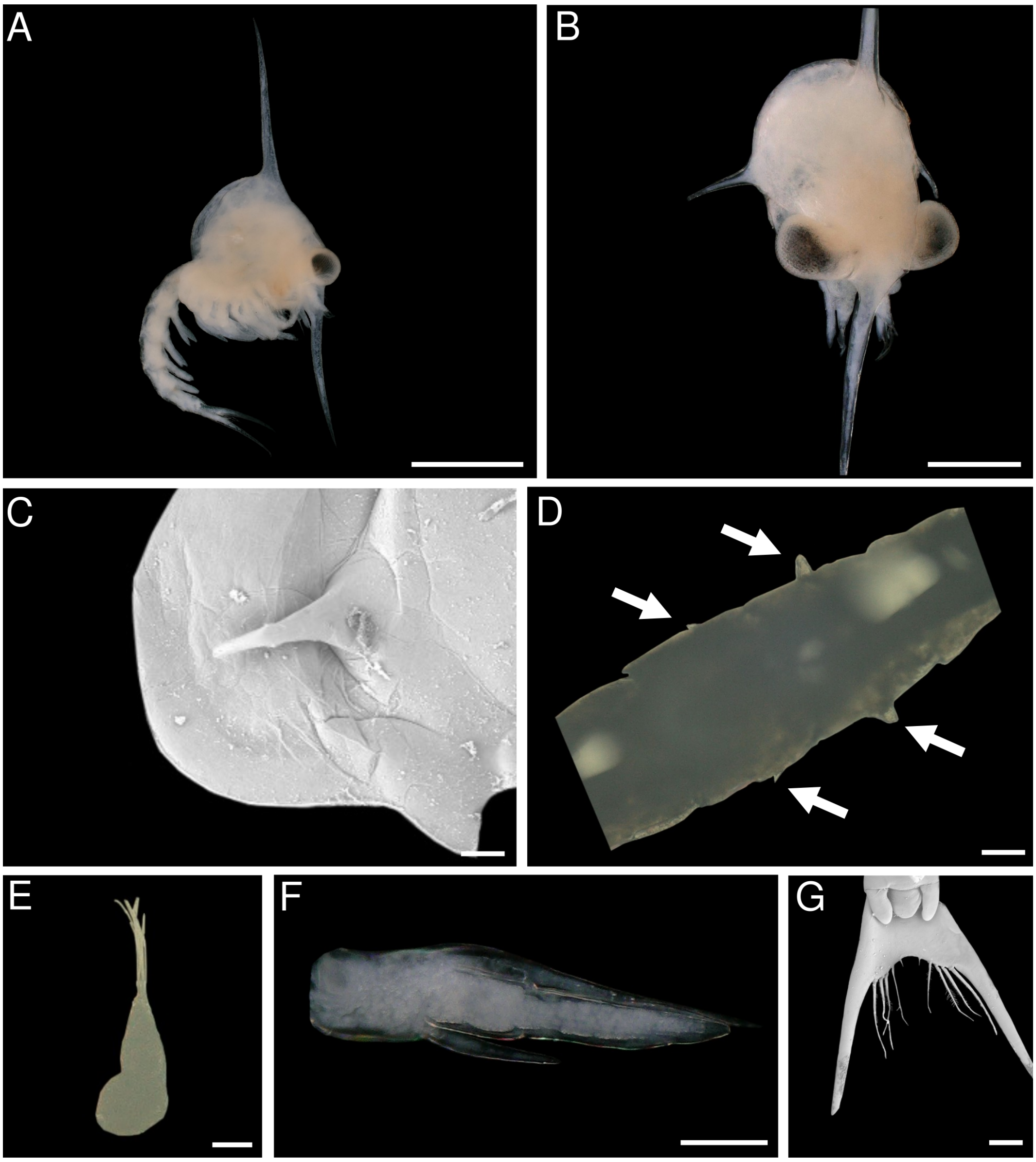


Fig. 3.8. *Hemigrapsus takanoi*, zoea V. A: lateral view; B: frontal view; C: caudolateral carapace margin, lateral view; D: pleon, dorsal view, with lateral knobs on segments 2 and 3 (arrows); E: antennule; F: antenna; G: telson. Scale bars: A 1 mm; B 0.5 mm; C, D, E, F, G 0.1 mm.

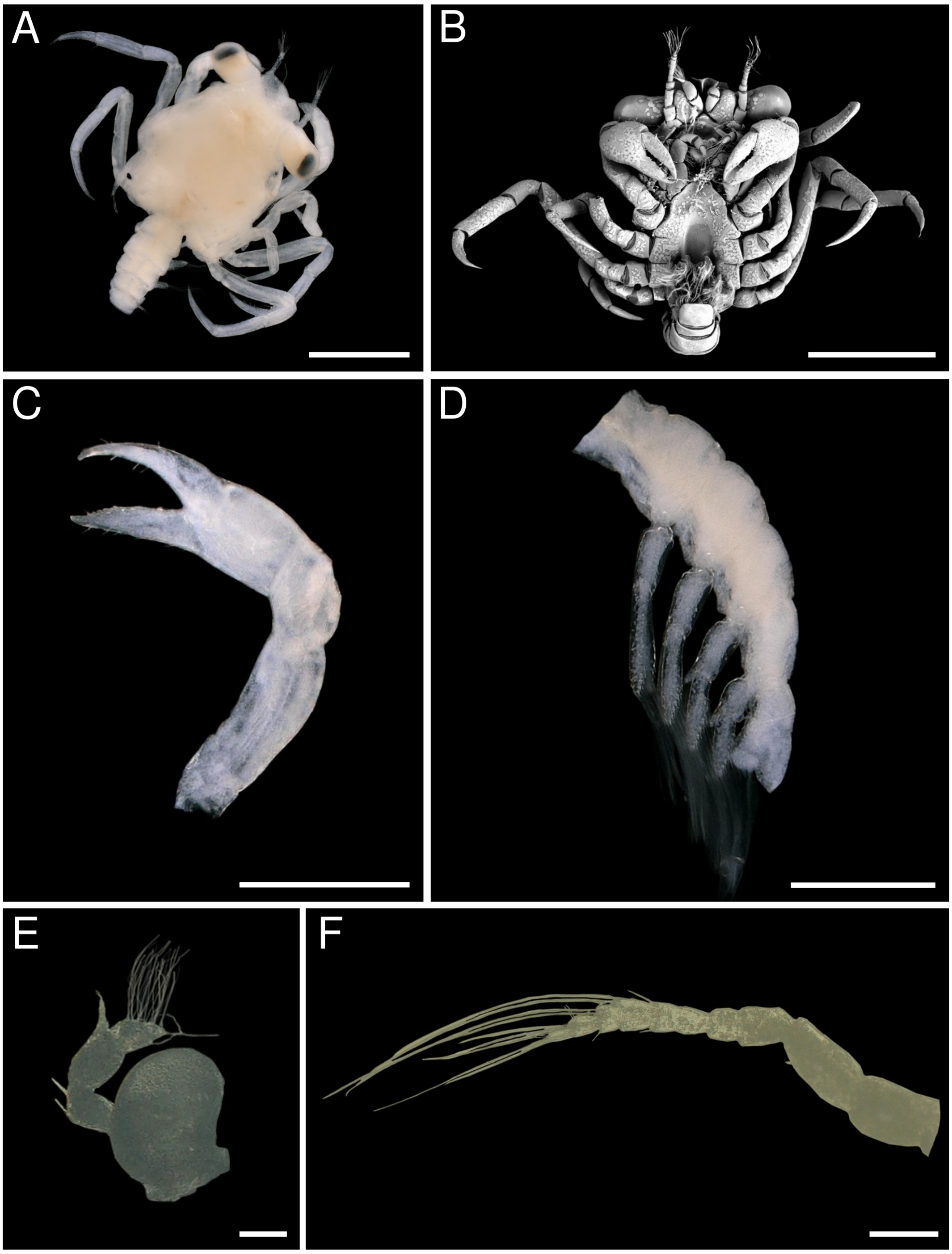


Fig. 3.9. *Hemigrapsus takanoi*, megalopa. A: dorsal view; B: ventral view; C: chela; D: pleon, lateral view; E: antennule; F: antenna. Scale bars: A, B 1 mm; C, D 0.5 mm; E, F 0.1 mm.

3.3.2. Seasonality and migration patterns

The first *H. takanoi* larvae in the near-shore sampling series were collected on 4 August 2016, the last on 30 September 2016, with a clear decrease in numbers already from early September on. From the beginning of October to the end of the monthly sampling series in February 2017, no larvae were collected from the samples. Larvae collected at this site were almost exclusively of Z I stage, with only nine larvae of higher stages being collected throughout the whole sampling series. Distinct peaks in the abundance of Z I larvae were detected in the third week of August and at the beginning of September (Fig. 3.10). A clear diurnal pattern was detected in the three 24-hour samplings at the near-shore site, with much higher abundances during night- than during daytime (Fig. 3.11). In both, the 24-hour samplings and the sampling series, numbers of Z I were marginally higher in the surface compared to the bottom samples, but variation was high. In both sampling series, also few zoea of *C. maenas* were detected.

In the off-shore plankton samples taken with RB *Polarfuchs* in August 2016, high numbers of *H. takanoi* larvae from all stages were detected. Abundances of Z I were estimated between 200 and 300, abundances of all other zoeal stages were estimated between 100 and 200 per sample. Abundance of megalopa was about 30. The additional qualitative screening of samples from the weekly plankton sampling conducted by GEOMAR revealed the first *H. takanoi* zoea to occur by the end of May, the last by the end of September. First megalopa were detected in samples from 25 July, the last in samples from 29 August 2016.

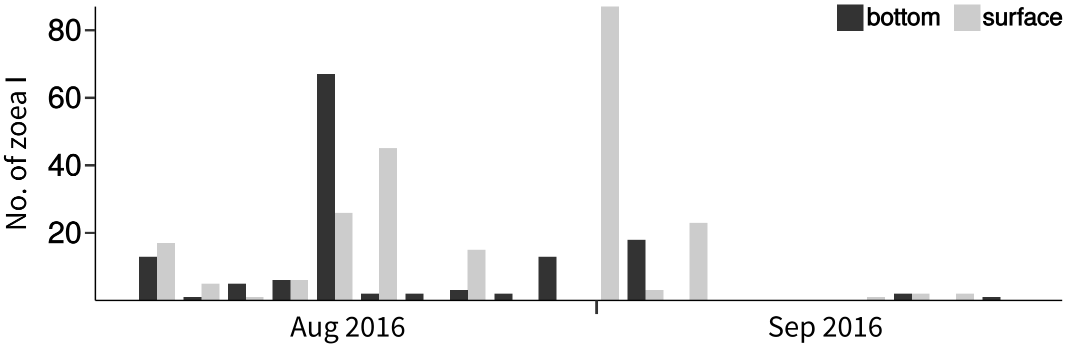


Fig. 3.10. Abundances of zoea I larvae in the near-shore sampling series in August and September 2016 in the surface (light grey) and bottom (dark grey) samples.

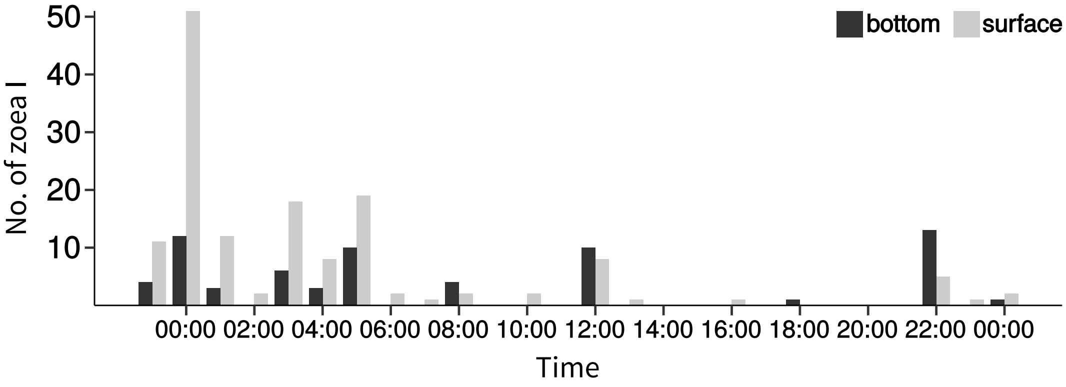


Fig. 3.11. Cumulative result of the three 24-hour sampling series at the near shore sampling site, showing abundances of zoea I larvae in the surface (light grey) and bottom (dark grey) samples during 24 hours.

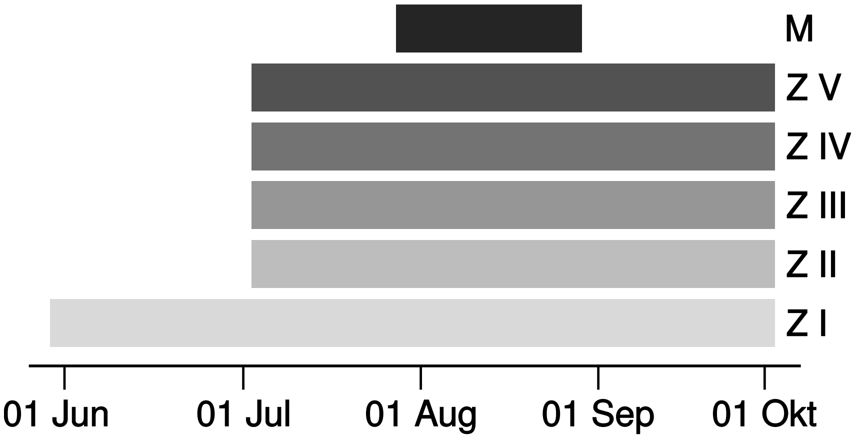


Fig. 3.12. Periods of occurrence of *H. takanoi* larvae, zoea I to megalopa, in the off-shore plankton samples from Kiel Fjord in 2016.

Over the time where *H. takanoi* larvae were detected in the samplings, surface water temperature at the sampling sites was 18°C on average, and always above 15°C. Salinities were about 15 PSU at the near-shore and about 19 PSU at the off-shore site.

3.4. Discussion

3.4.1. Larval morphology

The morphological descriptions given in this study are the first descriptions of the larval stages of *Hemigrapsus takanoi*. The development is as typical for varunid crabs, consisting of five zoea stages and one magelopa. Zoea stages I and II are very difficult to distinguish, as they show some size overlap and the number of denticles on the caudolateral carapace margin (4 in Z I, 3 in Z II) is sometimes difficult to determine due to the small size of the denticles.

Based on larval descriptions in the literature, we were able to identify characteristics to discriminate *H. takanoi* in all larval stages from the other brachyuran larvae (potentially) occurring in Kiel Fjord. Zoea of the native *Carcinus maenas*, which occur at the same time as *H. takanoi*, lack lateral carapace spines, and their megalopa have a distinct, pointy rostrum (compare Rice & Ingle 1975). The zoea of *Eriocheir sinensis* show much stronger setation of the carapace and especially the caudolateral carapace margin, zoea I to II show furthermore rows of denticles on the dorsal and rostral spine (compare Kim & Hwang 1995, Montú et al. 1996). Zoea of *Rhithropanopeus harrisii* are distinct by their very long rostral spine and second antenna (compare Connolly 1925). Furthermore, this species does not occur in Kiel Fjrod directly, but only in the Kiel Canal. The zoea of *Liocarcinus depurator*, which occasionally immigrates into Kiel Fjord from the open Baltic Sea, are similar to *H. takanoi* larvae, but carry distinct patterns of long simple setae on the carapace and dorsal spine (compare Clark 1984).

Hemigrapsus sanguineus has not (yet) been recorded from the Baltic Sea, but occurs sympatrically with *H. takanoi* in the North Sea and English Channel and was therefore also included in the comparison of larval characteristics. Larvae of both *Hemigrapsus spp.* are morphologically very similar, but the following differences could be inferred by comparing our observations of *H. takanoi* to a description of *H. sanguineus* larvae (Kornienko & Korn 2009):

zoea of *H. sanguineus* carry 6–8 denticles on the caudolateral carapace margin, megalopa of *H. sanguineus* differ from *H. takanoi* by the setal formula of the antenna and by having a 4-segmented antennula exopod.

3.4.2. Larval seasonality and migration patterns

The near-shore plankton sampling series conducted in Kiel Fjord between August 2016 and February 2017 indicated a clear seasonality of larval hatching with a peak period of zoea I abundances between late August and early September, and termination of larval hatching by the end of September. Combining the data of the near- and offshore samplings indicates a reproductive period of about 5 months for *H. takanoi* in Kiel Fjord, which is in accordance with literature data from the Netherlands (van den Brink et al. 2013), and the native Japanese range (Fukui 1988, for the closely related *H. penicillatus*). The duration of the reproductive period appears to be largely temperature-dependent, as the occurrence of larvae coincided with water temperatures $\geq$ 15°C, this observation being in accordance with van den Brink et al. (2013). The conspicuous synchronous occurrence of Z II to Z V, which one would usually assume to be temporally shifted from stage to stage might be a sampling artifact due to the very abrupt increase and decline in these stages' abundances. Similarly, the unexpectedly short period of megalopa to be present in the off-shore samples might be an artifact caused by very low densities before and afterwards. However, it could also be an indication of migration of the megalopa (see below). Data of the 24-hour samplings indicate a mainly nocturnal hatching, which has also been observed other species of coastal crabs and is usually related to a reduced predation risk (Anger 2001, Park et al. 2005).

Based on the observational data, we hypothesise the following migration model of *H. takanoi* larvae in Kiel Fjord during the course of larval development (Fig. 3.13): Hatching of Z I occurs nocturnally at near-shore sites inhabited by adult females. Shortly after hatching, the Z I perform a vertical migration to the surface, as indicated by the higher larval densities in the surface- compared to the bottom-samples. Vertical migration is probably linked to better food availability and lower water pressure near the surface (compare Anger 2001). Following this vertical migration, the Z I perform a horizontal migration from the near-shore areas to more open waters in the center of the Fjord, this time following the salinity gradient between the two areas (15 PSU near-shore,

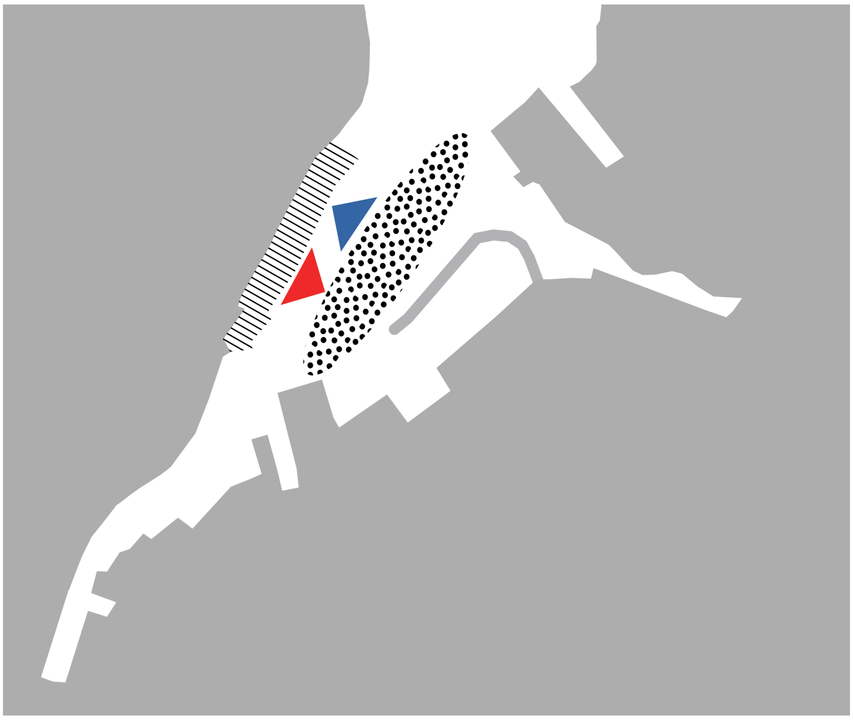


Fig. 3.13. Hypothesised migrations of *H. takanoi* larvae in the inner Kiel Fjord, as derived from plankton samplings. First zoea migrate from low-salinity hatching sites (hashed area) to areas with higher salinity further off-shore (dotted area, red arrow); megalopa migrate in the opposite direction for settlement (blue arrow).

20 PSU off-shore). In the off-shore surface waters, the larvae then undergo the five moults until the megalopa stage, as indicated by the presence of all larval stages in plankton samples from that area. Either the megalopa or the first juvenile crab stage(s) after metamorphosis finally re-migrate to the near-shore habitats. This re-migration however seems to take place on or near the bottom, otherwise, we would have expected to observe more megalopa in the surface near-shore samples. Whether metamorphosis occurs before, during or after re-migration could not be clarified by our data and requires further studies.

Migration patterns similar to the one described here for *H. takanoi* have been described for the larvae of many crabs in coastal and brackish habitats (Anger 1991, Anger et al. 1994, Park et al. 2005). The avoidance of low salinities by zoea has been linked to reduced growth under such conditions as a consequence of higher energy demands for osmoregulation (Anger 2001). Thus, such larval migration behaviour can be assumed to also occur in native populations of *H. takanoi*, as they are reported to inhabit estuaries with salinities as low as

7 PSU (Mingkid et al. 2006). The situation in Kiel Fjord, however, differs in an important point from the situation at other shores. Usually, off- and onshore transport of the larvae are assumed to be passive, driven by respective tides or currents (Anger 1991, Park et al. 2005). In the non-tidal Baltic Sea, and particularly in the enclosed sampling area in the inner Kiel Fjord, directed currents are lacking as a larval transport vector. Thus, the Z I larvae have to rely on other ways of transport to perform their migration. First, they could perform passive, undirected transport driven by variable small-scale wind-driven currents and reach the desired high-salinity zones just by chance. Or, they actively migrate along the salinity gradient. To clarify the mechanism allowing *H. takanoi* larvae to perform their migrations in Kiel Fjord, further investigations are highly encouraged.

Overall, this study confirms the ability of *H. takanoi* to undergo its full reproductive cycle in the southwestern Baltic Sea, thus indicating that this species can be considered as fully established in its new habitat.

Acknowledgements

The authors thank Christian Hesse and the crew of RB *Polarfuchs* (GEOMAR, Helmholtz-Centre for Ocean Research, Kiel) for their great help in the off-shore plankton sampling. Christian Hesse also generously provided access to the GEOMAR plankton sample series. Stefanie Köhnk was of invaluable help for the SEM-analyses, and so was Sarah Hayer for the digital microscopy. We further thank the Institute of Clinical Molecular Biology in Kiel for providing Sanger sequencing as supported in part by the DFG Cluster of Excellence 'Inflammation at Interfaces' and 'Future Ocean', especially S. Greve, S. Arndt and T. Henke for technical support. JCG gratefully thanks the German Environmental Foundation (DBU) for financial support (project no. 20014/335).

References

Anger, K. (1991): Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis*. *Marine Ecology Progress Series* 72, 103–110.

Anger, K. (2001): *The biology of Decapod Crustacean Larvae*. Crustacean Issues 14, A.A. Balkema Publishers, 419 pp., doi: 10.1651/0278-0372(2005)025.

Anger, K. (2006): Contributions of larval biology to crustacean research: a review. *Invertebrate Reproduction and Development* 49 (3), 175–205.

Anger, K., Montú, M., de Bakker, C. & Loureiro Fernandes, L.L. (1990): Larval development of *Uca thayeri* Rathbun, 1900 (Decapoda: Ocypodidae) reared in the laboratory. *Meeresforschung* 32, 276–294.

Anger, K., Spivak, E., Bas, C., Ismael, D. & Luppi, T. (1994): Hatching rhythms and dispersion of decapod crustacean larvae in a brackish coastal lagoon in Argentina. *Helgoländer Meeresuntersuchungen* 48 (4), 445–466, doi: 10.1007/BF02366257.

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Ashelby, C.W., Sewell, J., Rostron, J., Shrubsole, R., Child, T. & Clark, P.F. (2017): Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90 (6), 695–708, doi: 10.1163/15685403-00003692.

Clark, P.F. (1984): A comparative study of zoeal morphology in the genus Liocarcinus (Crustacea: Brachyura: Portunidae). *Zoological Journal of the Linnean Society* 82, 273–290.

Connolly, C.J. (1925): The larval stages and megalops of *Rhithropanopeus harrisi* (Gould). *Contributions to Canadian Biology* 2 (2), 329–334.

Dauvin, J.-C. & Delhay, J.-B. (2010): First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern Cotentin, Normandy, western English Channel. *Marine Biodiversity Records* 3, 1–3, doi: 10.1017/S1755267210000928.

Fukui, Y. (1988): Comparative Studies on the Life History of the Grapsid Crabs (Crustacea, Brachyura) Inhabiting Intertidal Cobble and Boulder Shores. *Publications of the Seto Marine Biological Laboratory* 33 (4/6), 120–162.

Geburzi, J.C., Graumann, G., Köhnk, S. & Brandis, D. (2015): First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Records* 4 (2), 103–107, doi: 10.3391/bir.2015.4.2.06.

Gittenberger, A., Rensing, M., Stegenga, H. & Hoeksema, B. (2010): Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33, 21–76.

Gollasch, S. (1999): The Asian decapod *Hemigrapsus penicillatus* (De Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgoländer Meeresuntersuchungen* 52, 359–366, doi: 10.1007/BF02908909.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

HELCOM (2012): *Checklist of Baltic Sea Macro-species*. Helsinki Commission – Baltic Marine Environment Commission, Helsinki, 203 pp.

Hwang, S.-G. & Kim, C.-H. (1995): Zoeal stages and megalopa of *Hemigrapsus penicillatus* (De Haan, 1835) (Decapoda, Brachyura, Grapsidae) reared in the laboratory. *The Korean Journal of Systematic Zoology* 11 (3), 389–408.

Kerckhof, F., Haelters, J. & Gollasch, S. (2007): Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions* 2 (3), 243–257.

Kim, C.-H. & Hwang, S.-G. (1995): The complete larval development of the Mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853 (Decapoda, Brachyura, Grapsidae) reared in the laboratory and a key to the known zoea of the Varuninae. *Crustaceana* 68 (7), 793–812.

Kornienko, E.S. & Korn, O.M. (2009): Illustrated key for the identification of brachyuran zoeal stages (Crustacea: Decapoda) in the plankton of Peter the Great Bay (Sea of Japan). *Journal of the Marine Biological Association of the United Kingdom* 89 (2), 379–386, doi: 10.1017/S0025315408002762.

Kornienko, E.S., Korn, O.M. & Kashenko, S.D. (2008): Comparative morphology of larvae of coastal crabs (Crustacea: Decapoda: Varunidae). *Russian Journal of Marine Biology* 34 (2), 77–93, doi: 10.1134/S1063074008020016.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Lee, S., Lee, S.-K., Rho, H.S. & Kim, W. (2013): New Report of the Varunid Crabs, *Hemigrapsus takanoi* and *Sestrostoma toriumii* (Crustacea: Decapoda: Varunidae) from Korea. *Animal Systematics, Evolution and Diversity* 29 (2), 152–159, doi: 10.5635/ASED.2013.29.2.152.

Lee, S.H. & Ko, H.S. (2008): First zoeal stages of six species of *Hemigrapsus* (Decapoda: Brachyura: Grapsidae) from the northern Pacific including an identification key. *Journal of Crustacean Biology* 28 (4), 675–685, doi: http://dx.doi.org/10.1651/07-2963.1.

Marin, I.N. (2013): New data on the distribution of hairy-clawed shore crabs of the genus *Hemigrapsus* (Decapoda: Varunidae) along the Russian mainland coast of the Sea of Japan. *Russian Journal of Marine Biology* 39 (4), 301–305, doi: 10.1134/S106307401304007X.

Markert, A., Raupach, M.J., Segelken-Voigt, A. & Wehrmann, A. (2014): Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatus* (De Haan, 1835) versus *Hemigrapsus takanoi* Asakura & Watanabe 2005. *Organisms Diversity & Evolution*, doi: 10.1007/s13127-014-0176-4.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Montú, M., Anger, K. & de Bakker, C. (1996): Larval development of the Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards (Decapoda: Grapsidae) reared in the laboratory. *Helgoländer Meeresuntersuchungen* 50, 223–252.

NCBI (2017): National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/ (visited on 03/12/2017).

Nijland, R. & Beekman, J. (2000): *Hemigrapsus penicillatus* de Haan 1835 waargenomen in Nederland. *Het Zeepaard* 60 (3), 169–171.

Noël, P.Y. & Gruet, Y. (2008): Progression du crabe introduit *Hemigrapsus takanoi* Asukura & Watanabe 2005 (Crustacé, Décapode) vers le nord du Golfe de Gascogne. *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France, Nouvelle Série* 30, 141–148.

Noël, P.Y., Tardy, E. & D'Udekem d'Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Otto, T. & Brandis, D. (2011): First evidence of *Eriocheir sinensis* reproduction from Schleswig-Holstein, Northern Germany, western Baltic Sea. *Aquatic Invasions* 6 (Supplement 1), 65–69, doi: 10.3391/ai.2011.6.S1.015.

Park, S., Epifanio, C.E. & Iglay, R.B. (2005): Patterns of larval release by the Asian shore crab *Hemigrapsus sanguineus* (De Haan): Periodicity at diel and tidal frequencies. *Journal of Shellfish Research* 24 (2), 591–595, doi: http://dx.doi.org/10.2983/0730-8000(2005)24[591:POLRBT]2.0.CO;2.

Raupach, M.J., Barco, A., Steinke, D., Beermann, J., Laakmann, S., Mohrbeck, I., Neumann, H., Kihara, T.C., Pointner, K., Radulovici, A., Segelken-Voigt, A., Wesse, C. & Knebelsberger, T. (2015): The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. *PLoS ONE* 10 (9), 1–23, doi: 10.1371/journal.pone.0139421.

Rice, A.L. & Ingle, R.W. (1975): The larval development of *Carcinus maenas* (L.) and *C. mediterraneus* Czerniavsky (Crustacea, Brachyura, Portunidae) reared in the laboratory. *Bulletin of the British Museum (Natural History)* 28 (3), 101–119.

Schubart, C.D. (2009): Mitochondrial DNA and Decapod Phylogenies: The Importance of Pseudogenes and Primer Optimization. In: Martin, J.W., Crandall, K.A. & Felder, D.L. (eds.) *Decapod Crustacean Phylogenetics.* Crustacean Issues 18, CRC Press, Boca Raton, FL, pp. 47–65, doi: 10.1080/17451001003604796.

Van den Brink, A.M., Godschalk, M., Smaal, A.C., Lindeboom, H. & McLay, C.L. (2013): Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of the Marine Biological Association of the United Kingdom* 93 (1), 189–196, doi: 10.1017/S0025315412000446.

Wood, C.A., Bishop, J.D.D., Davies, C.J., Delduca, E.L., Hatton, J.C., Herbert, R.J.H. & Clark, P.F. (2015): *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4 (2), 109–113, doi: 10.3391/bir.2015.4.2.07.

Yamasaki, I., Doi, W., Mingkid, W.M., Yokota, M., Strüssmann, C.A. & Watanabe, S. (2011): Molecular-Based Method To Distinguish the Sibling Species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of Crustacean Biology* 31 (4), 577–581, doi: 10.1651/10-3366.1.

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000): A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational Biology* 7 (1-2), 203–214, doi: 10.1089/10665270050081478.

Part III

Factors of success: Ecological and genetic aspects of *Hemigrapsus'* establishment

4. Recruitment patterns, low cannibalism and reduced interspecific predation contribute to high invasion success of two Pacific crabs in northwestern Europe

Jonas C. Geburzi[a, b], Dirk Brandis[b] and Christian Buschbaum[a]

[a]*Alfred-Wegener-Institute, Helmholtz-Centre for Polar and Marine Research, Wadden Sea Station, Hafenstr. 43, 25992 List/Sylt, Germany*
[b]*Kiel University, Zoological Institute and Museum, Hegewischstr. 3, 24105 Kiel, Germany*

Published in:
Estuarine, Coastal and Shelf Science (2018) Volume 200: 460-472
doi: 10.1016/j.ecss.2017.11.032

Abstract

Life-history traits and interactions with native species play an important role for the successful establishment of non-native species in new habitats. We investigated the recent successful invasion of the Pacific crabs *Hemigrapsus takanoi* and *H. sanguineus* to the southeastern North Sea coast with respect to their recruitment patterns, as well as interactions of juvenile with sub-adult individuals among the Pacific crabs and with native shore crabs *Carcinus* *maenas*. A field survey of juvenile native and introduced crab abundances (carapace width 1.4–10 mm) was conducted in the northern Wadden Sea, spanning 24 months from 2014 to 2016. The survey revealed different seasonal recruitment patterns of native *C. maenas* and both introduced *Hemigrapsus* species. Native shore crabs showed a single recruitment peak from June to July, while *Hemigrapsus spp.* mainly recruited from August to early September, but recruits occurred in low densities throughout the winter until the end of the following spring season. Field experiments on the effects of larger crabs on the recruitment intensity showed that recruitment of *H. takanoi* was enhanced by the presence of larger congeners, but remained unaffected by larger *C. maenas*. Recruitment of juvenile *C. maenas*, by contrast, was reduced by the presence of larger *Hemigrapsus spp.* Additional laboratory experiments revealed high rates of cannibalism on newly recruited *C. maenas* by subadult conspecifics as well as strong predation by larger *Hemigrapsus spp.* In contrast, newly recruited *Hemigrapsus spp.* had a much lower risk of being preyed on by subadult conspecifics and native shore crabs. Our results suggest that the timing of recruitment in combination with low intraspecific competition and reduced predation pressure by native shore crabs are crucial for the rapid and ongoing establishment of *Hemigrapsus spp.* in the Wadden Sea.

Key words: invasive crustaceans, *Hemigrapsus*, *Carcinus maenas*, recruitment, predation, North Sea, Wadden Sea

4.1. Introduction

Worldwide, marine ecosystems are invaded by a continuously rising number of non-native species, which is in particular true for coastal and estuarine habitats (Ruiz et al. 1997, Gollasch 2006, Williams & Grosholz 2008). The establishment of non-native species can profoundly affect species communities in invaded environments, driven by direct and indirect interactions between native and non-native species (Grosholz et al. 2000, Jensen et al. 2002, Levin et al. 2002, Edelist et al. 2013, Buschbaum et al. 2016, Reise et al. 2017). Life-history traits, especially traits related to reproduction (Lockwood et al. 2005, Bremner 2008), and niche allocation processes (Townsend Peterson 2003, Herborg et al. 2007) are assumed to play an important role for the successful establishment and spread of non-native species. Detailed knowledge of the ecology and life-history of non-native species substantially contributes to the understanding of underlying processes of successful bioinvasions, and can also allow projections on possible effects of newly arrived species on native communities. For example, high fecundity and the duration and timing of reproduction periods have been attributed to invasion success in plants (Rejmanek & Richardson 1996, Gerlach & Rice 2003) as well as freshwater fish (Olden et al. 2006), crayfish (Chucholl 2012) and gammarids (Grabowski et al. 2007, Pöckl 2009).

In European coastal waters, the northwestern Pacific Brush-clawed shore crab *Hemigrapsus takanoi* Asakura and Watanabe 2005 and the Asian shore crab *Hemigrapsus sanguineus* (de Haan 1835), are among the most recent successfully established non-native species. *Hemigrapsus takanoi* was for the first time reported from a ship's hull in Bremerhaven, Germany, in 1993 (Gollasch 1999) and from the Bay of Biscay coast at La Rochelle, France, in 1994 (Noël et al. 1997). Initially identified as *H. penicillatus*, all populations of Brush-clawed shore crabs in Europe have later been assigned to the newly described *H. takanoi* (Asakura & Watanabe 2005, Yamasaki et al. 2011). Within two years, this species had extended its range from northern Spain to southern Brittany (Noël et al. 1997). In 1999, it was reported from Le Havre at the French coast of the English Channel (Breton et al. 2002) and in 2000 from the Dutch Delta (Wolff 2005). In 2006, it was found in the Dutch part of the Wadden Sea (Gittenberger et al. 2010), one year later also on the coast of Lower Saxony, Germany (Obert et al. 2007) and by 2009, it had reached the Sylt-Rømø-Bight between Germany and Denmark in the northern part of the Wadden Sea (Landschoff et al. 2013), thus currently occurring along 2200 km of the European Atlantic and North Sea coastline. Recently, it has also been reported from Great Britain (Wood et al. 2015, Ashelby et al. 2017) and the southwestern Baltic Sea (Geburzi et al. 2015).

Hemigrapsus sanguineus was first recorded at Le Havre and in the Dutch Delta system in 1999 (Breton et al. 2002, Wolff 2005), spreading along the French, Belgian and Dutch coast of the English Channel and the southern North Sea in the following years (Wolff 2005, Kerckhof et al. 2007, Dauvin & Dufossé 2011, Gothland et al. 2013). It reached the Dutch Wadden Sea in 2004 (Gittenberger et al. 2010), German waters in 2006 (Obert et al. 2007) and occurred along the whole German Wadden Sea Coast and on the island of Helgoland in the German Bight by 2009 (Landschoff et al. 2013, Jungblut et al. 2017). In 2012, it was also reported from the Danish Wadden Sea islands Rømø and Fanø (pers. observation) and recently also from Great Britain (Seeley et al. 2015). On the Atlantic coast of North America, *H. sanguineus* was initially reported as an invasive species in 1988 at the mouth of Delaware Bay (McDermott 1998) and is currently distributed in the United States from North Carolina to Maine (Epifanio 2013).

The Wadden Sea as the coastal area of the southeastern North Sea is dominated by unstable sediments, and oyster and mussel reefs are the only naturally occurring extensive hard-bottom substrates. While comparatively species-poor, the proportion of non-native species is high, as reflected by over 60 non-native macrobenthic species alone (Reise et al. 2010, Buschbaum et al. 2012). The native decapod crustacean fauna of the Wadden Sea is dominated by the European shore crab *Carcinus maenas*, which is very abundant in benthic communities both inter- and subtidally, and itself a globally successful invader of coastal ecosystems (Carlton & Cohen 2003). The recent arrival of the two *Hemigrapsus spp.* in this ecosystem allows to simultaneously study interactions between native and invasive and two closely related invasive species, as well as the ongoing establishment process.

Several studies reported detrimental effects of growing *Hemigrapsus* populations on *C. maenas* in North America (Kraemer et al. 2007, O'Connor 2014) and Europe (van den Brink et al. 2012). These can be connected to apparent advantages of *Hemigrapsus spp.* over *C. maenas* in competition for food and shelter (Jensen et al. 2002, Gothland et al. 2014, Hobbs et al. 2017), but also to reduced recruitment success of *C. maenas* as a consequence of predation on early juveniles by *H. sanguineus* (Lohrer & Whitlatch 2002). Early juvenile crabs are an especially sensitive life stage, as they experience high predation pressure (especially from other decapods) and strongly depend on suitable nursery habitats (Moksnes et al. 1998, Lohrer & Whitlatch 2002). They usually occur in high densities but only for a relatively short time, and all processes influencing crab recruitment are assumed to cause effects on the population level (Moksnes 2002).

This study aims to investigate temporal dynamics of the recruitment and early juvenile stages of Asian shore crabs in comparison to European shore crabs, as well as possible competitive interactions affecting early juvenile crabs. The first part of the study comprises regular observations of the abundances of *Hemigrapsus spp.* and *C. maenas* in the intertidal of the northern Wadden Sea between 2014 and 2016, with a special focus on size class distributions and the occurrence of recruitment events. Based on observations of several broods per season regularly occurring in *Hemigrapsus spp.* (Fukui 1988, McDermott 1998, van den Brink et al. 2013) while only one brood per season is common for *C. maenas* in the North Sea (Crothers 1967, Klein Breteler 1976), differences in reproductive cycling of native and non-native species favouring the establishment of *Hemigrapsus spp.* are hypothesised. To the best of our knowledge, the survey data represent the first temporally highly resolved, perennial dataset of *Hemigrapsus spp.* abundances in their invaded European range.

The second part of the study comprises field and laboratory experiments on the influence of subadult and adult crabs on the recruitment success of juveniles, and intra- (e.g. cannibalism) and interspecific predation pressure on newly settled crabs. The latter is a putatively important interaction in crab populations, shaping distribution patterns and abundances of juveniles (Moksnes et al. 1998). We hypothesise differences in the recruitment success of juveniles in response to larger crabs, as well as differential patterns of predation pressure on early juveniles between *C. maenas* and *Hemigrapsus spp.*

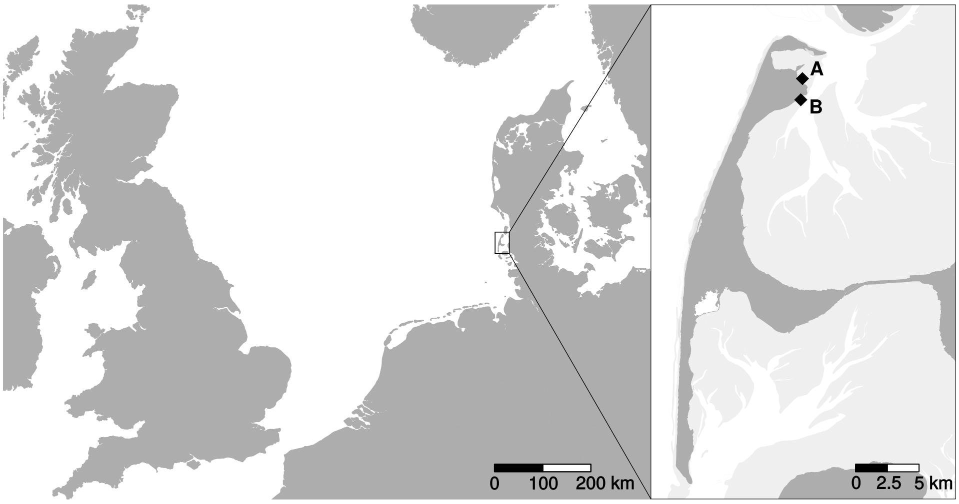


Fig. 4.1. The island of Sylt and its location in the southeastern North Sea. The two sampling sites (A and B) are located at the northern tip of the island. Light grey areas represent tidal flats.

4.2. Materials and Methods

4.2.1. Study area

All field investigations and experiments were carried out in the intertidal zone near the northern part of the island of Sylt in the northern Wadden Sea (Fig. 4.1). This area is dominated by sedimentary soft-bottom habitats with several mixed beds of native blue mussels (*Mytilus edulis*) and introduced Pacific oysters (*Magallana gigas,* Reise et al. 2017), as well as artificial hard structures (boulder groynes), which provide epibenthic hard-bottom habitats in close proximity to each other. It is particularly in these hard bottom habitats where both *Hemigrapsus spp.* occur in the Wadden Sea. On Sylt, the first specimen of *Hemigrapsus spp.* were found in 2008 close to harbours at the southern and northern tips of the island (J. Landschoff, K. Reise pers. comm.). In 2011, *Hemigrapsus spp.* were already found in hard-bottom habitats at 12 sites all along the east coast of Sylt, occurring sympatrically at 5 of these sites and always co-occurring with *C. maenas* (Landschoff et al. 2013).

We selected an intertidal mixed oyster-mussel-bed at the northern tip of the island (N 55.028° E 8.434°; 'site A'), dominated by *H. takanoi*, and boulder groynes in that area (N 55.013° E 8.432°; 'site B'), dominated by *H. sanguineus*, as sampling sites for the survey (Fig. 4.1). At site A, also the field experiment was conducted. Both sites are located between 0.3 and 0.5 m above mean low tide level.

4.2.2. Survey

To gain insight in temporal variations of recruitment and abundances of juvenile crabs, as well as possible differences in habitat preferences between the three crab species in theWadden Sea, a quantitative survey was carried out from December 2014 to December 2016. The survey comprised bi-weekly samplings at the sites A and B. Based on the results of a pilot study, both sites were identified as representative habitats for sampling recruiting and juvenile crabs of all three species (Geburzi 2014).

At each sampling date and site, two samples of 625 cm^2 surface area were taken at low tide by placing a 25 x 25 cm steel frame on the substrate and transferring all substrate and crabs within the frame, including the upper approx. 5 cm of sediment, into plastic buckets. Distance between the two samples was at least 10 m. The samples were transported to the lab and sieved (mesh size 0.5 mm). Remaining crabs in the sieve were identified to species level by using morphological characters. For distinguishing the two *Hemigrapsus* species, we followed the characters given in Breton et al. (2002). The carapace width (CW) of each individual was measured to the nearest 0.01 mm with digital callipers. A stereo microscope was used for species determination and measurements when necessary.

4.2.3. Recruitment experiment

An inclusion-exclusion experiment was conducted in the field during the peak recruitment periods of *C. maenas* and *Hemigrapsus spp.* in summer and autumn 2016 to assess the influence of adult crabs on the recruitment of juveniles. Fully enclosed, cylindrical cages (15 cm diameter, 15 cm height) were constructed of a PVC scaffold and plastic mesh with 3 mm mesh size, which allowed crabs up to 7.5 mm CW to enter the cages (the larger ones by crawling sideways). The following size categories for crabs were defined for this (and the laboratory) experiment: ‘recruits’ ≤ 2 mm CW, ‘juveniles’ 2.1 mm–7.5 mm CW, which together represent ‘0-year crabs’, ‘subadults’ 7.6 mm–9.9 mm CW and ‘adults’ ≥ 10 mm CW.

To provide an attractive substrate for recruiting crabs, the cages were filled with a bottom layer of sand and approximately 4 cm of blue mussels, small oysters and empty shells which were taken from the surrounding area and cleaned thoroughly under running fresh water to remove any crabs. The cages were placed in a flow-through seawater tank for about 36 h to allow the mussels to settle and to produce byssus threads for mutual attachment, which resulted in a natural matrix of mussels, oysters and dead shell material. The experimental setup consisted of five treatments, with six replicates each. Inclusion cages were stocked with (1) nine *C. maenas* (ranging from 14.7 to 25.6 mm CW), (2) nine *H. takanoi* (ranging from 10.1 to 16.4 mm CW) or (3) nine *H. takanoi* (ranging from 10.0 to 16.1 mm CW), respectively. Predator densities in the cages correspond to maximum densities of *Hemigrapsus spp.* in this part of the Wadden Sea, which reach up to 600 crabs m^2 (pers. observation, numbers include all crabs $\geq$ 5 mm CW). Exclusion cages without any crabs served as (4) control treatment to assess the recruitment of 0-year crabs without predation. Open 'cages', consisting of a cage bottom and a 5 cm high mesh ring filled with substrate were used as (5) cage-control treatment to assess natural levels of predation on recruiting crabs at the experimental site.

The closed and open cages were deployed at low tide on the tidal flat along the edge of the oyster-mussel-bed, using iron rods (30 cm length) for fixation. Treatments were arranged in random order and a distance of at least 2 m was kept between the cages to ensure independency of the experimental units. At the chosen site, *H. takanoi* is the dominating *Hemigrapsus* species while *H. takanoi* only occurs in comparatively low densities (Geburzi 2014). The experiment was conducted in two runs (subsequently referred to as 'run A' and 'run B') during the peak recruitment periods of *C. maenas* and *Hemigrapsus spp.*, as inferred from the survey data. The experiment runs were set up 3 July 2016 and 8 September 2016 for 53 and 49 days, respectively. During the experimental period the cages were controlled once a week to check the experimental set-up and to remove drifting macroalgae covering the mesh. At the end of the experiment, the cages were transported to the laboratory and their contents were sieved over a 0.5 mm mesh. All crabs that had recruited to the cages were determined to species level using a stereo microscope and measured to the nearest 0.01 mm with digital callipers. Additionally, the number of surviving predators in the inclusion cages was noted.

4.2.4. Predation experiment

To assess predation pressure and cannibalism exerted by subadults on newly settled crabs (recruits), a lab experiment was conducted. Cylindrical glass bowls (9.5 cm diameter, 8.0 cm height) were filled with 200 mL filtered seawater and equipped with a thin layer of fine sand and a mussel valve (approx. 20 mm length) as substrate and shelter. Each glass bowl was stocked with six recruits of either *C. maenas*, *H. takanoi* or *H. sanguineus* (CW 1.5–1.8 mm). They were collected in the field 1–4 d prior to the experiment and kept in flow-through aquaria until the experiment started. The bowls were randomly assigned to one out of four treatments, with six replicates each. Treatments were as follows (1) one subadult male *C. maenas* (CW 9.12 ± 0.65 mm), (2) one subadult male *H. takanoi* (CW 7.81 ± 0.85 mm) and (3) one subadult male *H. sanguineus* (CW 7.80 ± 0.36 mm) per bowl as predator, and (4) no predator as a control to assess natural background mortality during the course of the experiment. The difference in CW between *Carcinus* and *Hemigrapsus* predators was chosen to take into account the relatively smaller chelae of *C. maenas* compared to *Hemigrapsus spp.* (compare Payne & Kraemer 2013). All predators were starved for 24 h prior to the experiment to standardize hunger levels. The experiment was conducted in a climate chamber under constant temperature (15°C) and natural light/dark cycle. The experiment ran over 12 h and the number of surviving recruits was controlled hourly and noted for each bowl. As newly settled *Carcinus* and *Hemigrapsus* did not occur at the same time, the experiment had to be conducted in separate runs for *C. maenas* (25 June 2016) and *Hemigrapsus spp.* (24 October 2016) as prey, respectively. However, the results were considered comparable with each other since both runs were conducted under the same lab conditions.

4.2.5. Data analysis

For the analysis of the survey data, in a first step actual size classes representing moult stages were determined from the size-frequency distributions of each species. Recognition of the moult stages and estimation of mean size and standard deviation for each stage was performed using Bhattacharya's method (Bhattacharya 1967) implemented in FiSAT II software, version 1.2.2 (Gayanilo et al. 2005) (decomposition of size-frequency distributions is shown in Appendix 1). Size ranges for the stages were then determined by calculating the interception points of the probability density functions of neighbouring modal groups. The ranges were subsequently used to calculate size-class specific abundances (individuals m^{-2}) for each sampling date and site with the two samples per site pooled. Graphical output was produced with R, version 3.3.3 (R Core Team 2017) and the additional package 'ggplot2' (Wickham 2009).

The recruitment data were analysed by fitting a generalised linear model (GLM) with 'species' (*Carcinus* and Hemigrapsus) and 'treatment' (exclusion, open, *C. maenas* inclusion, *H. takanoi* inclusion and *H. sanguineus* inclusion) as predictor variables, and the counts of 0-year crabs as response variable. The model was fitted with a negative binomial error term and a log-link function, accounting for overdispersion and aggregation in the data as a source of unexplained variance in the model. The analysis was performed with R, version 3.3.3, using the function 'glm.nb' provided with the 'MASS' package (Venables & Ripley 2002), and the 'ggplot2' package was used for graphical output.

For the analysis of the predation experiment, a Kaplan-Meyer estimator of survival probability was calculated for each predator-prey combination. An asymptotic log-rank test was performed to detect overall differences of survival probabilities between species, and pairwise log-rank tests were subsequently performed for relevant predator-prey combinations. To correct for multiple testing in the pairwise comparisons, the p-values were adjusted using Holm's sequential Bonferroni correction. The survival analysis was performed with R, version 3.3.3, using the 'survival' package (Therneau 2015), and the 'survminer' package (Kassambara & Kosinski 2017) for plotting the survival curves.

4.3. Results

4.3.1. Survey

Over the two years of survey between December 2014 and December 2016, a total number of 2'073 *Carcinus maenas*, 4'566 *H. takanoi* and 4'193 *Hemigrapsus sanguineus* $\leq$ 10 mm CW were sampled and measured. From the size-frequency distributions, mean sizes and size ranges for the first five juvenile moult stages of each species were estimated (Tab. 4.1, subsequently referred to as C-1 to C-5. All individuals of each species from the 6th moult stage to 10 mm CW were pooled into a single size class (referred to as C-6), as individual numbers and

Tab. 4.1. Mean (estimated by Bhattacharya's method), minimum and maximum carapace width [mm] of juvenile *C. maenas*, *H. takanoi* and *H. sanguineus*. Size classes C-1 to C-5 represent actual moult stages, size class C-6 pools all individuals from the 6th moult stage to 10 mm CW.

Size class	*C. maenas*			*H. takanoi*			*H. sanguineus*		
	mean	min	max	mean	min	max	mean	min	max
C-1	1.41	1.22	1.62	1.68	1.34	1.87	1.89	1.43	2.10
C-2	1.88	1.62	2.13	2.10	1.87	2.34	2.32	2.10	2.54
C-3	2.49	2.13	2.85	2.58	2.34	2.84	2.80	2.54	3.12
C-4	3.11	2.85	3.35	3.10	2.84	3.36	3.40	3.12	3.75
C-5	3.61	3.35	3.91	3.76	3.36	4.30	4.33	3.75	4.76
C-6	–	3.91	10.00	–	4.30	10.00	–	4.76	10.00

separation indices were too low to define reliable size ranges for further moult stages. Mean CW of C-1 *C. maenas* (1.41 mm) is considerably smaller compared to *H. takanoi* (1.68 mm) and *H. sanguineus* (1.89 mm). The size difference between *C. maenas* and *H. takanoi* decreases with subsequent moults and approximately equals out from C-3 on, but it remains about the same between *C. maenas* and *H. sanguineus*.

The survey data revealed differing patterns of the three species in the occurrence and abundances of recruiting (C-1) and juvenile crabs at the two sampling sites. In general, lower densities of crabs were recorded on the oyster reef (site A) compared to the boulder groynes (site B) which is reflected in the total number of crabs in both habitats (3'632 at site A, 7'200 at site B). The recruitment of *C. maenas* at both sites started mid June and ended early September in 2015 and mid July in 2016, respectively (Figs. 2 and 3). The maximum density of C-1 *C. maenas* was 792 ± 104 individuals m^{-2} (mean ± SE), recorded at site B on 4 July 2016. Outside the well-defined recruitment period, no C-1 *C. maenas* were found in the samples. Density peaks of subsequent size classes of *C. maenas* were delayed by about two weeks compared to the preceding size class, and densities continuously decreased from C-1 to C-5, depicting growth and mortality between the moult stages. C-6 *C. maenas* were recorded year-round in low densities at both sites, with slightly higher densities observed from late summer to early winter.

Considering the spatial distribution patterns, *H. takanoi* dominates at site A (Fig. 4.2), while *H. sanguineus* dominates at site B, however densities of C-4 and

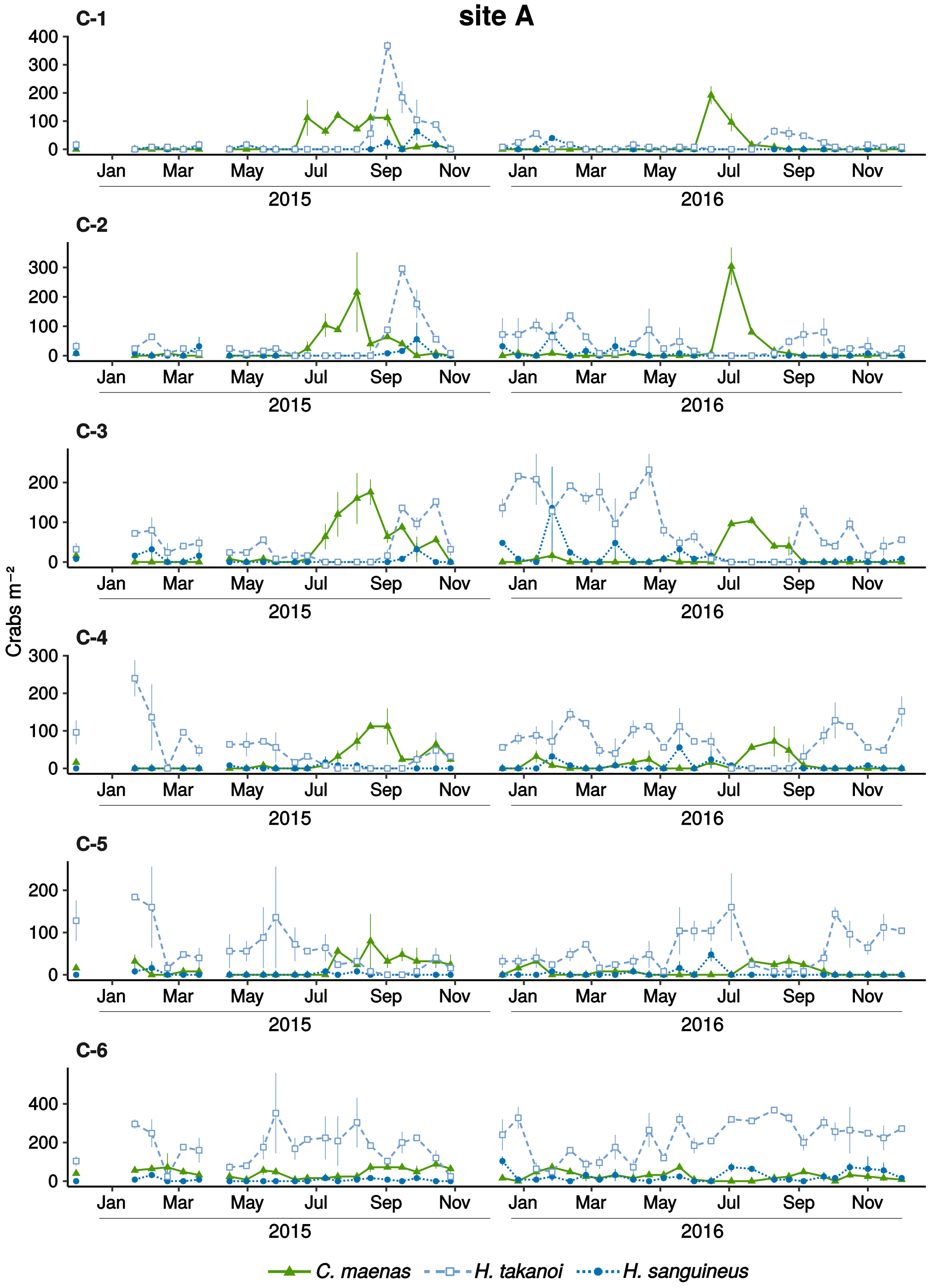


Fig. 4.2. Mean densities ± SE of juvenile crabs on an intertidal oyster-mussel-bed (site A) between Dec 2014 and Dec 2016, based on bi-weekly samplings. Numbers C1–C6 refer to size classes (see Tab. 4.1 for details). Green, solid: *C. maenas*, light blue, dashed: *H. takanoi*, dark blue, dotted: *H. sanguineus*. Gaps in the data are caused by samplings that had to be cancelled due to high water levels. Note different y-axis ranges.

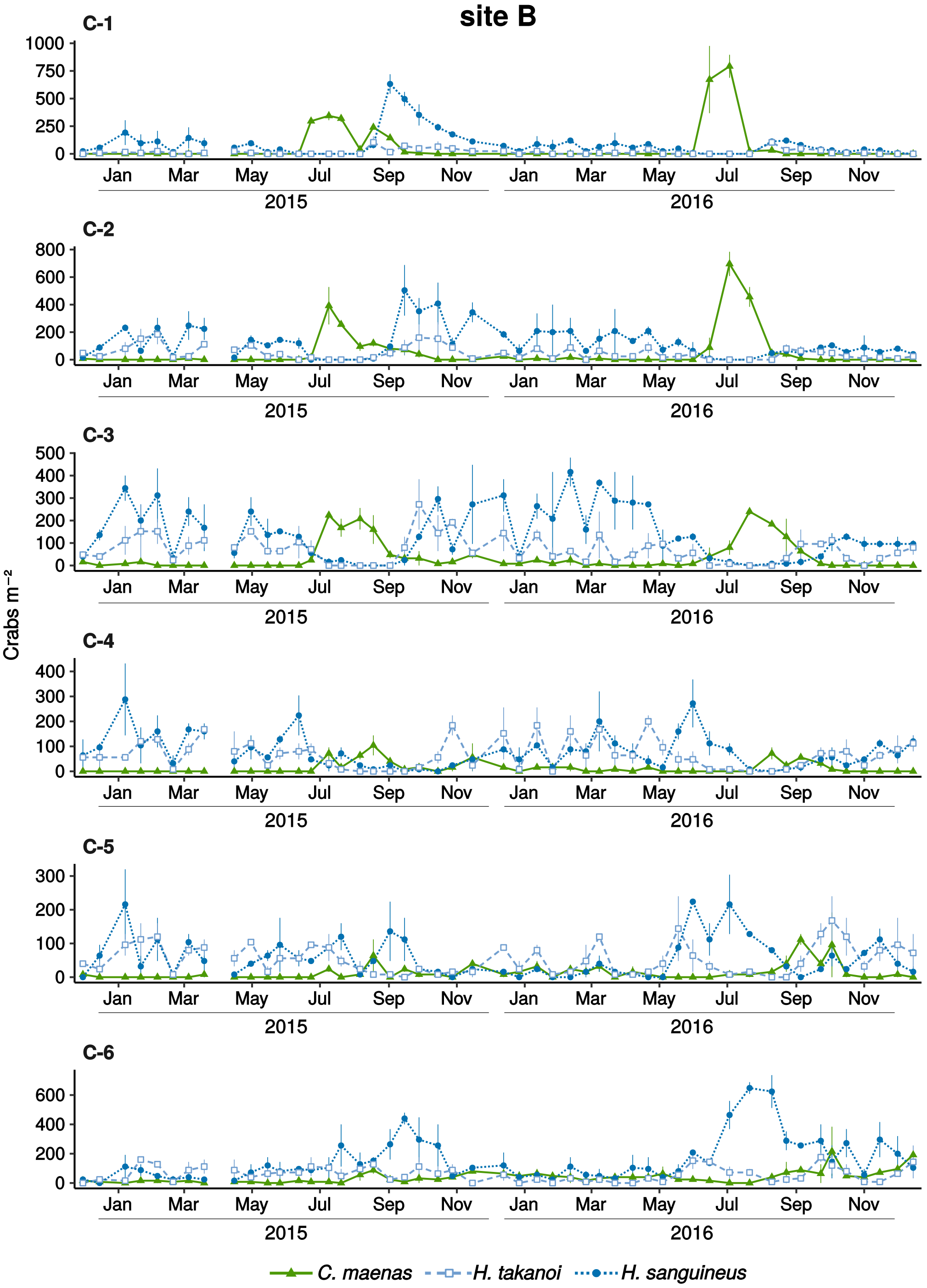


Fig. 4.3. Mean densities ± SE of juvenile crabs on intertidal boulder groynes (site B) between Dec 2014 and Dec 2016, based on bi-weekly samplings. Numbers C1–C6 refer to size classes (see Tab. 4.1 for details). Green, solid: *C. maenas*, light blue, dashed: *H. takanoi*, dark blue, dotted: *H. sanguineus*. Gaps in the data are caused by samplings that had to be cancelled due to high water levels. Note different y-axis ranges.

C-5 *H. takanoi* were similar to *H. sanguineus* from autumn to spring (Fig. 4.3). Recruitment of both *Hemigrapsus* species peaks at the same time in late August/early September. Maximum C-1 densities were 368 ± 16 individuals m^{-2} for *H. takanoi* and 632 ± 88 individuals m^{-2} for *H. sanguineus*, recorded on 3 September 2015, at site A and B, respectively. Intensity of recruitment of both *Hemigrapsus* species was much lower in 2016 compared to 2015, thus showing an opposing trend with *C. maenas*. Moulting stage C-1 of *Hemigrapsus spp.* also occurred throughout the winter months, with *H. sanguineus* regularly reaching densities around 100 individuals m^{-2} (Fig. 4.3). In both years however, the density of C-1 *Hemigrapsus spp.* dropped to zero at the beginning of the *C. maenas* recruitment period. Densities of subsequent size-classes of *Hemigrapsus spp.* increased as well time-delayed compared to the preceding size-class. Constantly high densities of C-2 and C-3 from October to May indicate a low mortality and slow growth of *Hemigrapsus* juveniles over the winter months. Densities of C-4 to C-6 increase towards the spring and summer, reaching a maximum coinciding with the recruitment period of *C. maenas*.

4.3.2. Recruitment experiment

Recruitment of *H. sanguineus* was as expected very low at the experimental site, because it is not the preferred habitat for this species (one and three crabs were found among all cages in run A and B, respectively). Therefore, only the recruitment of *H. takanoi* and *C. maenas* are compared in the following.

The results of the GLM revealed that recruitment of 0-year crabs differed significantly between the two species ($LRT_{species}$: df = 1, $\chi^2 = 29.68$, $p < 0.0001$). Recruitment of *C. maenas* during run A (July to early August 2016, main settlement period for *C. maenas*) was higher (17.0 ± 1.2 ind. $cage^{-1}$, all values given as mean ± SE) than of *H. takanoi* (8.9 ± 1.2 ind. $cage^{-1}$) during run B (September to mid October 2016, main settlement period for *Hemigrapsus spp.*), which may reflect the generally low ambient recruitment of *Hemigrapsus spp.* in 2016 (see section '3.1. Survey'). Furthermore, the recruitment of 0-year crabs varied for different species-treatment combinations ($LRT_{species\times treatment}$: df = 4, $\chi^2 = 22.86$, $p \leq 0.001$). This variation was mainly driven by contrasting recruitment patterns between the two *Hemigrapsus*-inclusion treatments and the three other treatments (Fig. 4.4). Recruitment of 0-year *C. maenas* was almost equal in the exclusion (18.8 ± 1.9 ind. $cage^{-1}$) and *C. maenas*-inclusion treatments (19.8 ± 3.3 ind./cage) and in the open cages (19.3 ± 2.1 ind. cage^{-1}). However, it was reduced in the *H. takanoi*- (15.7 ± 2.8 ind. cage^{-1}) and even more the *H. sanguineus*-inclusion treatments (11.1 ± 1.3 ind. cage^{-1}; Fig. 4.4 A). On the contrary, recruitment of 0-year *H. takanoi* was increased in the two *Hemigrapsus*-inclusion treatments (13.7 ± 2.8 ind. cage^{-1} for *H. takanoi* inclusion and 11.8 ± 3.1 ind. cage^{-1} for *H. sanguineus* inclusion) compared to the exclusion treatment (6.5 ± 1.6 ind. cage^{-1}) and open cages (8.2 ± 3.2 ind. cage^{-1}), while recruitment to *C. maenas*-inclusion cages was slightly lower compared to the exclusion control group (4.3 ± 1.4 ind. cage^{-1}; Fig. 4.4 B).

Colonisation of the open cages by crabs > 7.5 mm CW differed between species. It was 0.2 ± 0.4 ind. cage^{-1} for *H. sanguineus* and 3.6 ± 2.2 ind. cage^{-1} for *H. takanoi*. Both densities were consistent with their ambient densities at this site (pers. observation). Numbers of *C. maenas* in the open cages were 2.7 ± 2.9 ind. cage^{-1}. The cage design effectively prevented the colonisation of crabs > 7.5 mm CW to the exclusion and inclusion cages, and no additional crabs could be found at the end of the experiment. Mean survival of predator crabs in the inclusion cages ($n = 9$ at the beginning of the experiment) was 2.2/4.5 for *C. maenas* (run A and B, respectively), 5.5/7.5 for *H. takanoi* and 8.7/8.3 for *H. sanguineus*.

4.3.3. Predation experiment

The survival probability of newly settled crabs differed significantly between treatments (e.g. predator species) among species (asymptotic log-rank test: $df = 3$, $\chi^2 = 176.28$, $p < 0.0001$; Fig. 4.5). Newly settled *C. maenas* had a significantly lower survival probability when confronted to conspecific subadults compared to either *Hemigrapsus* species as predator (Log-rank tests: $df = 1$, $\chi^2 = 21.5$, adjusted $p \leq 0.0001$ for *H. takanoi* and $df = 1$, $\chi^2 = 14.7$, adjusted $p = 0.0014$ for *H. sanguineus*; Fig. 4.5 A). For *Carcinus* recruits, the effects of cannibalism are therefore higher than predation by both *Hemigrapsus* species.

Survival probabilities of newly settled *Hemigrapsus spp.* when confronted with a subadult conspecific were almost equal or only slightly reduced compared to the control treatments without conspecific predator (Fig. 4.5 B, C). This indicates a strong difference in the tendency to cannibalistic behaviour between *C. maenas* and *Hemigrapsus spp.* The survival probabilities of *Hemigrapsus* recruits were reduced to about 0.3 with *C. maenas* as predator, which

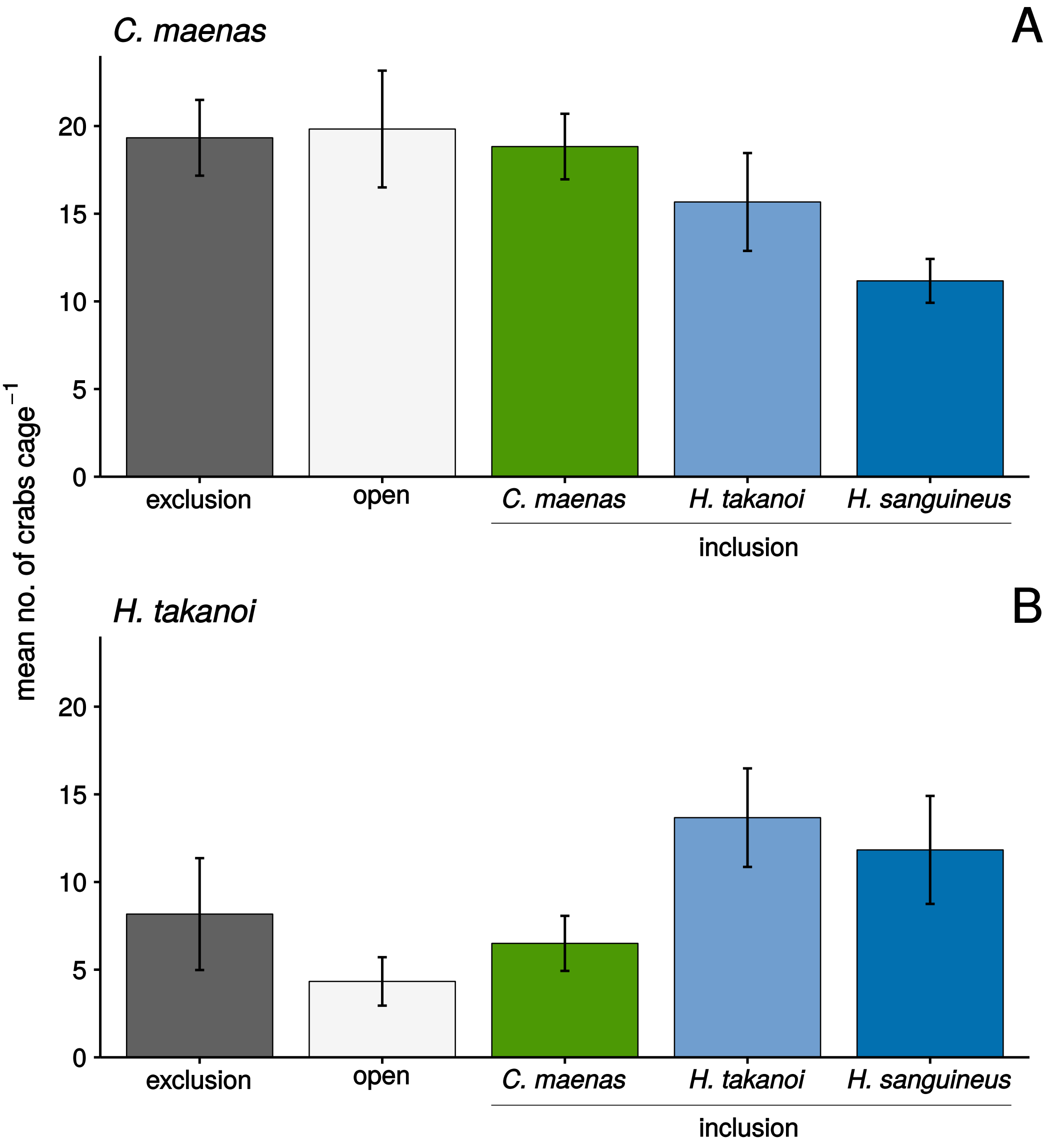


Fig. 4.4. Response of 0-year *C. maenas* (A) and *H. takanoi* (B) to the presence of adult crabs in the field experiments. Data are mean numbers ± SE of crabs $\leq$ 7.5 mm CW cage^{-1} for the different treatments. Number of cages per treatment n = 6. *Hemigrapsus sanguineus* was neglected in the analysis because of extremely low recruitment at the experimental site.

was significantly higher than those of *C. maenas* recruits (survival probability < 0.1) with *C. maenas* as predator (Log-rank tests: $df = 1$, $\chi^2 = 22.5$, adjusted $p \leq 0.0001$ for *H. takanoi* and $df = 1$, $\chi^2 = 23.5$, adjusted $p \leq 0.0001$ for *H. sanguineus*). The survival probabilities of *Hemigrapsus* recruits were, however, in the range of *C. maenas* survival probabilities with *Hemigrapsus spp.* as predators. Both *Hemigrapsus* species slightly reduced the survival of each other's juveniles, but survival probabilities of these predator-prey-combinations did not differ significantly from the same-species combinations (Fig. 4.5 B, C). Detailed statistical results for all pairwise comparisons are given in Appendix 2. Mortality of newly settled crabs in the control treatments was very low during the experiment, as only one *H. takanoi* was found dead at the last control. Thus, any differences in survival of newly settled crabs could be attributed to predation by subadults.

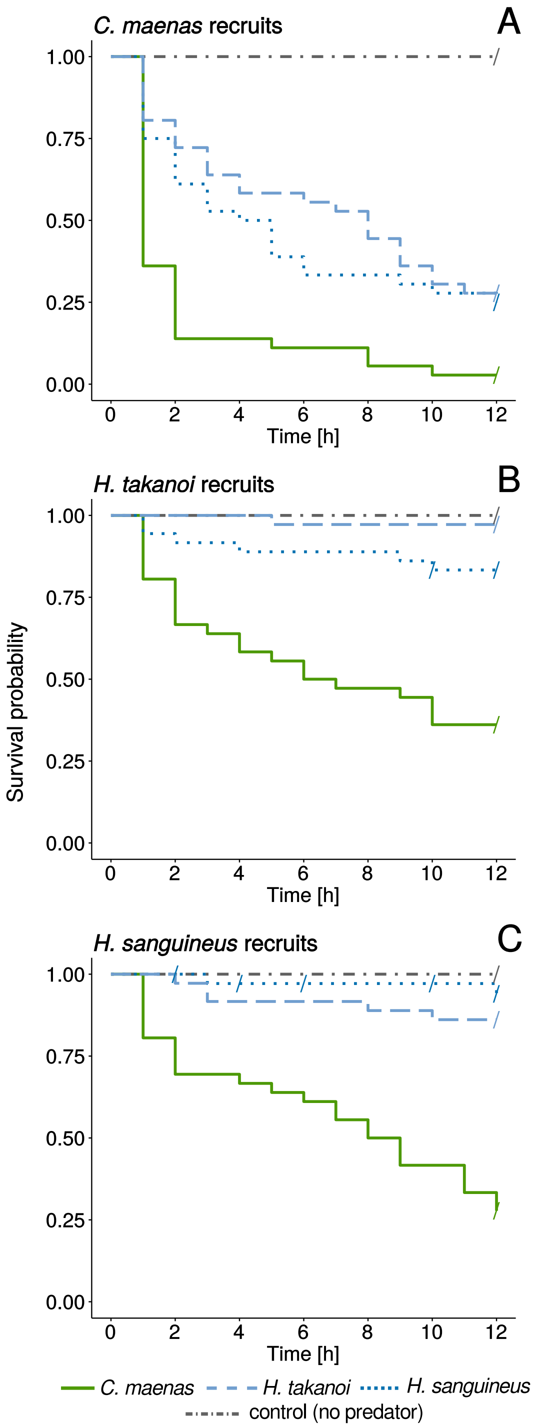


Fig. 4.5. Survival probabilities of recruits (1.5–1.8 mm CW) of *C. maenas* (A), *H. takanoi* (B) and *H. sanguineus* (C) (6 recruits per replicate [n = 6] per species) over 12 h, when paired with subadult (7.8–9.1 mm CW) *C. maenas* (green, solid lines), *H. takanoi* (light blue, dashed lines), *H. sanguineus* (dark blue, dotted lines) as predators, or no predator (grey, dot-dashed lines).

4.4. Discussion

This study revealed differential seasonal and spatial patterns of recruit and juvenile occurrence between invasive (*Hemigrapsus spp.*) and native (*Carcinus maenas*) crabs in the Wadden Sea. It furthermore revealed differential inter- and intraspecific effects of adult/subadult crabs on the recruitment of juveniles, particularly by differing levels of predation and cannibalism. The combined results describe important factors for the invasion success of *Hemigrapsus spp.*

4.4.1. Recruitment patterns of native *C. maenas* and introduced *Hemigrapsus spp.*

We detected a temporal shift of recruitment peaks between *C. maenas* and *Hemigrapsus spp.* The European shore crab *Carcinus maenas* shows a well defined recruitment period of 3 months at most with peak densities of C-1 individuals in early July, whereas no C-1 individuals were present at other times of the year. This is in accordance with earlier observations and the fact that *C. maenas* females only produce one brood per season in these latitudes (Naylor 1962, Crothers 1967, Dawirs 1985). The recruitment of *H. takanoi* and *H. sanguineus*, by contrast, begins in August and peaks late August to mid September. The onset of juvenile recruitment is in accordance with data for *H. sanguineus* and *H. penicillatus* from their native range (Pillay & Ono 1978, Fukui 1988). After the density peak in late summer, *Hemigrapsus* recruitment decreases, but C-1 individuals occur in the intertidal zone in lower densities until January (*H. takanoi*, site A) or even April/May of the following year (*H. sanguineus*, site B). It is, however, unlikely that *Hemigrapsus* actually recruits during the winter months, as ovarian, egg and larval development are strongly temperature-dependent and appear to be extremely prolonged at water temperatures below 15 °C (Epifanio 2013, van den Brink et al. 2013). The observed pattern might rather result from crabs which recruited in late autumn and overwintered in the intertidal at C-1 stage, with the long intermoult duration being as well a consequence of low temperatures (Leffler 1972).

The still considerably extended recruitment periods of *Hemigrapsus spp.* could be an advantageous strategy to compensate for short periods of unfavourable conditions in variable environments, or to avoid strong density-dependent intraspecific competition. On the other hand, the short period of massive recruitment in *C. maenas* might reflect a strategy to reduce the risk of predation for recruiting crabs. Interestingly, densities of C-1 *Hemigrapsus* drop to zero almost exactly at the time when *C. maenas* recruitment starts. Predation or expulsion by *C. maenas* is most likely not the cause of this decline, as in this case, at least some C-1 *Hemigrapsus* would be expected to survive/remain at the site. Whether the observed pattern is coincidentally caused by the natural rhythm of *Hemigrapsus* recruitment, or actually a reaction of *Hemigrapsus* to the high recruitment rates of *C. maenas*, needs further investigation. Besides a migration of juvenile *Hemigrapsus* to a different (micro-)habitat, also chemical cues affecting the timing and duration of larval release, larval development and metamorphosis may be responsible for the observed temporal pattern. For example, exudates released by conspecifics and other crab species play an important role in these processes, in particular on the 'time to moult' of the megalopa (Forward et al. 2001, Kopin et al. 2001, O'Connor 2007).

Overall, the shifted recruitment periods indicate temporal niche segregation between the early juvenile stages of *C. maenas* and *Hemigrapsus spp.*, by which direct competition for resources (e.g. food, shelter) is avoided. The differential timing of recruitment – and ultimately reproduction – may thus considerably facilitate the establishment of the non-native species, particularly in the initial phase of introduction, when *Hemigrapsus* abundances were still low. Similarly, differential timing of recruitment of *C. maenas* compared to native crabs is assumed to have facilitated its establishment as non-native species in Australia (Garside et al. 2015). As *C. maenas* has been shown to be inferior to same-sized *H. sanguineus* when competing for food and shelter (Jensen et al. 2002), the different recruitment periods can also be beneficial for the native species, especially when considering the size advantage of C-1 *Hemigrapsus spp.* over *C. maenas*. An earlier recruitment of *Hemigrapsus*, before *C. maenas*, can be assumed in this context to be even more advantageous for *Hemigrapsus*, and to potentially increase competitive pressure on *C. maenas* recruits. However, this could be prevented by too low water temperatures in spring which do not allow for earlier development of *Hemigrapsus* eggs and larvae. The apparent difference in recruitment site preference between *H. takanoi* (mussel-oyster-reef) and *H. sanguineus* (boulder groyne) coincides well their differing habitat preferences, with *H. sanguineus* dominating in rockier habitats with higher wave exposure, while *H. takanoi* dominates in muddier, more sheltered habitats (Mingkid et al. 2006, Dauvin et al. 2009, Landschoff et al. 2013). The fact that these differences in site preference are already pronounced at the C-1 stage, points to a pre-settlement process as the underlying mechanism. This spatial niche segregation into different microhabitats appears to be very effective, as it even occurred within a few meters distance between the windward and lee side of a longshore boulder groyne (Geburzi 2014). It likely plays an important role for the sympatrical spread of the two *Hemigrapsus* species, as it reduces competition for food and space, especially among recruits and early juvenile stages which occur in high abundances at the same time.

Growth patterns and temporal variation of densities of subsequent size classes C-2 to C-6 differ as well between *C. maenas* and *Hemigrapsus spp.* Density peaks of *C. maenas* C-2 to C-5 are delayed by about 2 weeks compared to the preceding class, reflecting regular moult intervals for about the first 2 months after recruitment. The occurrence of early juvenile *C. maenas* at the sampling sites is mostly restricted to 3 to 4 months following the recruitment peak and characterised by a sharp decline in density between consecutive size classes. Besides the possibility of high mortality due to strong predation pressure (Moksnes et al. 1998), these observations may also reflect size-dependent habitat shifts and migrations, as they were described by Thiel & Dernedde (1994). As they grow bigger, juvenile *C. maenas* emigrate from the mussel beds and clumps to adjacent sand flats and later to subtidal areas. Our survey data indicate emigration of juvenile *C. maenas* in early autumn, which is much earlier than before the arrival of *Hemigrapsus spp.* in the Wadden Sea, when it occurred only in late autumn (Beukema 1991, Thiel & Dernedde 1994). This could be a direct effect of the presence of *Hemigrapsus spp.* as a new competitor/predator, mitigating the beneficial effects of staying in the mussel beds (compare Griffen et al. 2015).

The data reflect well that *H. takanoi* and *H. sanguineus* are predominantly intertidal species in all stages after metamorphosis (Noël et al. 1997, Lohrer et al. 2000, van den Brink et al. 2012, Landschoff et al. 2013). Early *Hemigrapsus* juveniles (C-2 to C-5) occur year-round in higher densities than *C. maenas*, except for the peak periods of the respective *C. maenas* size class. Slowly decreasing densities of C-2 from September to May, while at the same time densities of C-3 and C-4 show an increasing trend, suggest that the youngest *Hemigrapsus* cohorts still moult during the winter months, though with very long moult intervals. With rising water temperatures in spring and summer, moult intervals apparently become shorter and moult synchronises, as rising temperatures accelerate growth, while the overall length of moulting intervals increases from stage to stage (Klein Breteler 1975, Dawirs 1985). This leads to pronounced density peaks of late juvenile *Hemigrapsus* (C-5 and C-6) between May and August, coinciding with the recruitment period of *C. maenas*. This pattern may have implications for *C. maenas*, as high densities of a new putative competitor/predator in an important recruitment habitat (Moksnes et al. 1998, Moksnes 2002) could negatively affect the recruitment success of the native species (see below).

4.4.2. Intra- and interspecific effects on crab recruitment

The results of the field caging experiment indicate differential intra- and interspecific influences of the presence of larger crabs on the recruitment of juvenile *C. maenas* and *H. takanoi*. No direct conclusions can be drawn about *H. sanguineus*, as they recruit only in very low numbers at the experimental site, but effects are assumed to be similar to *H. takanoi* considering their similar size and behaviour.

Both *Hemigrapsus* species substantially reduced the recruitment of *C. maenas* juveniles compared to the control treatment excluding larger crabs, in case of *H. sanguineus* by almost 50 %. However, no effect on native shore crab recruitment was detected in the *C. maenas*-inclusion treatment. A possible explanation for the slightly surprising lack of an effect in this treatment, which strongly contrasts the results of the corresponding treatment in the predation experiment (see below), might be the low survival of *C. maenas* predators in the cages. The low survival could be caused by cannibalism between the predators due to their very high initial density, which in turn reduced density-dependent effects of the larger *C. maenas* on conspecific recruits. The recruitment of *H. takanoi*, by contrast, was strongly enhanced by the presence of larger crabs of both *Hemigrapsus* species, and slightly, though not significantly, reduced by the presence of larger *C. maenas*. These findings are partly congruent with a similar experimental study at the U.S. east coast, also reporting a reduced recruitment of *C. maenas* when *H. sanguineus* was present (Lohrer & Whitlatch 2002). However, no positive effect of *H. sanguineus* on conspecific recruitment was observed in that study. The reduced *C. maenas* recruitment to *Hemigrapsus*-inclusion cages could be explained by either increased predation by *Hemigrapsus spp.* or an avoidance of *C. maenas* recruits based on chemical cues. The latter possibility is considered less likely as it would presume *C. maenas* to have 'learned' to distinguish and avoid chemical cues released by *Hemigrapsus spp.* within just a few years of coexistence. The enhanced recruitment of *H. takanoi* to *Hemigrapsus*-inclusion cages on the other hand indicates a positive response of *H. takanoi* to conspecific/congeneric cues. Such effects were already described for *H. sanguineus* (Kopin et al. 2001, O'Connor 2007), as well as several other crab species (reviewed in Forward et al. 2001). In summary, the results show that the presence of (sub)adult *Hemigrapsus spp.* enhances the recruitment of conspecifics/congeners while at the same time it impairs the recruitment of *C. maenas*. Thus, these processes reveal interactions between non-native and native crabs that may substantially contribute to the success of *Hemigrapsus spp.* in the southeastern North Sea.

The results of the predation experiment reveal different levels of predation and cannibalism on newly recruited crabs as an additional factor facilitating the establishment of the newly arrived *Hemigrapsus spp.* Subadults of *C. maenas* showed a generally higher tendency to prey on new recruits compared to subadult *Hemigrapsus*, and their predation pressure was highest on conspecifics (i.e. cannibalism). This distinct tendency to cannibalism is well described for *C. maenas* and considered an important factor for recruitment success and regulation of population density in this species (e.g. Moksnes et al. 1998, Moksnes 2004, Almeida et al. 2011). In contrast, subadult *Hemigrapsus* showed almost no cannibalism and only very low rates of intra-genus predation, while their predation pressure on *C. maneas* recruits was as high as vice versa. Griffen et al. (2015) already reported low rates of cannibalism in *H. sanguineus*, which is also the case for *H. takanoi*, as our results show. The establishment of *Hemigrapsus spp.* in the Wadden Sea intertidal thus added two new species preying on *C. maenas* recruits, while *C. maenas* is the only crustacean predator of *Hemigrapsus* recruits. The asymmetry of the predator-prey relationship between the three species might be even more pronounced in the field, as the density maxima of subadult *Hemigrapsus* on the oyster reef and boulder groynes occur around the peak periods of *C. maenas* recruitment. On the other hand, densities of subadult *C. maenas* only slightly increase during peak recruitment of *Hemigrapsus spp.*.

The experimental setup was of course a simplification of the natural conditions, as it did neither include variations in predator and prey densities, nor different levels of habitat complexity, nor alternative food sources, all of which influence the predation risk for crab recruits (Moksnes et al. 1998, Lohrer & Whitlatch 2002, Almeida et al. 2011). These factors might also explain why no reduction of *C. maenas* recruitment in *C. maenas* inclusion treatments was observed in the field experiment, probably along with the higher mortality of *C. maenas* predators (see above). Nevertheless, as the conditions were equal for all three species, the experiment provides comparable results for background levels of cannibalism and predation. The lack of juvenile cannibalism in *Hemigrapsus spp.* probably is of great importance for their invasion success, as it allows them to tolerate high densities of several cohorts in the same habitat. This result also complements the recently described high conspecific tolerance among similar-sized adult *H. sanguineus* in suitable habitats (Hobbs et al. 2017).

4.4.3. Conclusions

The data presented in this study show that timing of recruitment as well as direct (predation/cannibalism) and possibly also indirect (chemical cues) interactions of recruiting crabs contribute to the high invasion success of *H. takanoi* and *H. sanguineus* in the Wadden Sea. The presence of *Hemigrapsus* at a site enhances the recruitment of juveniles, leading to a positive feedback on crab densities in suitable habitats once they are occupied (Fig. 4.6). Low rates of cannibalism positively affect the maintenance of high population densities (but see Griffen et al. 2015, for effects of cannibalism in a dense *H. sanguineus* population). Among recruiting crabs, temporal niche segregation (between *C. maenas* and *Hemigrapsus spp.*, Fig. 6) and spatial niche segregation (between *H. takanoi* and *H. sanguineus*) may strongly reduce competition for resources. Increased predation pressure by subadult *Hemigrapsus* on recruiting *C. maenas* may further reduce competition for subsequently recruiting *Hemigrapsus spp.*

In the long run, it seems possible that *Hemigrapsus spp.* could take over the role as the dominant crab species in intertidal hard-bottom habitats in the Wadden Sea (Lohrer & Whitlatch 2002, van den Brink et al. 2012). However, the spread of *Hemigrapsus* will most likely not threaten the *C. maenas* population on a Wadden Sea-wide scale, as large parts of its adult population live subtidally and will provide a stable source of recruitment to the intertidal, where recruits

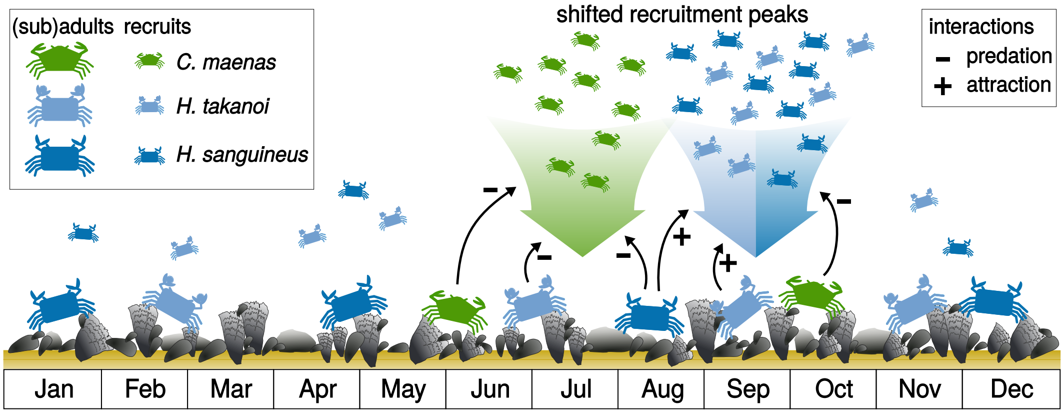


Fig. 4.6. Schematic illustration of processes and patterns shaping densities and interactions of native and invasive crabs in the Wadden Sea intertidal. Peak periods of juvenile recruitment are temporally shifted between *C. maenas* and *Hemigrapsus spp.* and no *Hemigrapsus* recruitment occurs during *C. maenas* recruitment peaks. From late autumn to early spring, only *Hemigrapsus spp.* are present in the intertidal. *C. maenas* (sub)adults prey on recruiting conspecifics as well as *Hemigrapsus spp.*, while *Hemigrapsus* (sub)adults prey only on *C. maenas* recruits but enhance *Hemigrapsus* recruitment (black arrows).

and juveniles also use other habitats, such as seagrass beds, as nursery ground (Moksnes et al. 1998, Landschoff et al. 2013), a habitat in which *Hemigrapsus* currently does not occur. Finally, the results of this study highlight the need to consider life-history traits in combination with interactions among juvenile stages in order to gain insight in the establishment and spread as well as potential impacts of marine non-native species.

Acknowledgements

We thank A. Cornelius, S. Lennartz and S. Anslinger for help in the field, A. Kornmann and K. Escobar-Wolf for technical support and valuable advice for the experiments, K.M. Wegner for great help with the statistical analysis of the settlement experiment, and G. Torres and L. Giménez for fruitful discussions about the larval and juvenile development of *Hemigrapsus*. We also thank the editor and two anonymous reviewers for the fast evaluation process, and the reviewers for their valuable comments. The project was made possible by financial support of the German Environmental Foundation (DBU), grant no. 20014/335.

References

Almeida, M.J., González-Gordillo, J.I., Flores, A.A.V. & Queiroga, H. (2011): Cannibalism, post-settlement growth rate and size refuge in a recruitment-limited population of the shore crab *Carcinus maenas*. *Journal of Experimental Marine Biology and Ecology* 410, 72–79, doi: 10.1016/j.jembe.2011.10.011.

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Ashelby, C.W., Sewell, J., Rostron, J., Shrubsole, R., Child, T. & Clark, P.F. (2017): Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90 (6), 695–708, doi: 10.1163/15685403-00003692.

Beukema, J.J. (1991): The abundance of shore crabs *Carcinus maenas* (L.) on a tidal flat in the Wadden Sea after cold and mild winters. *Journal of Experimental Marine Biology and Ecology* 153 (1), 97–113, doi: 10.1016/S0022-0981(05)80009-7.

Bhattacharya, C.G. (1967): A Simple Method of Resolution of a Distribution into Gaussian Components. *Biometrics* 23 (1), 115–135.

Bremner, J. (2008): Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology* 366, 37–47, doi: 10.1016/j.jembe.2008.07.007.

Breton, G., Faasse, M., Noël, P.Y. & Vincent, T. (2002): A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1), 184–189, doi: 10.1651/0278-0372(2002)022[0184:ANACIE]2.0.CO;2.

Buschbaum, C., Cornelius, A. & Goedknegt, M.A. (2016): Deeply hidden inside introduced biogenic structures – Pacific oyster reefs reduce detrimental barnacle overgrowth on native blue mussels. *Journal of Sea Research* 117, 20–26, doi: 10.1016/j.seares.2016.09.002.

Buschbaum, C., Lackschewitz, D. & Reise, K. (2012): Nonnative macrobenthos in the Wadden Sea ecosystem. *Ocean & Coastal Management* 68, 89–101, doi: 10.1016/j.ocecoaman.2011.12.011.

Carlton, J.T. & Cohen, A.N. (2003): Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs *Carcinus maenas* and *C. aestuarii*. *Journal of Biogeography* 30 (12), 1809–1820, doi: 10.1111/j.1365-2699.2003.00962.x.

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, doi: 10.1051/kmae/2011082.

Crothers, J.H. (1967): The biology of the Shore Crab *Carcinus maenas* (L.); 1. The background - anatomy, growth and life history. *Field Studies* 2, 407–437.

Dauvin, J.-C. & Dufossé, F. (2011): *Hemigrapsus sanguineus* (de Haan, 1835) (Crustacea: Brachyura: Grapsoidea) a new invasive species in European waters: the case of the French English Channel coast (2008–2010). *Aquatic Invasions* 6 (3), 329–338, doi: 10.3391/ai.2011.6.3.09.

Dauvin, J.-C., Tous Rius, A. & Ruellet, T. (2009): Recent expansion of two invasive crab species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions* 4 (3), 451–465, doi: 10.3391/ai.2009.4.3.3.

Dawirs, R.R. (1985): Temperature and larval development of *Carcinus maenas* (Decapoda) in the laboratory; predictions of larval dynamics in the sea. *Marine Ecology Progress Series* 24, 297–302.

De Haan, W. (1835): Crustacea. In: von Siebold, P.F. (ed.) *Fauna Japonica sive Descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batava imperium tenent, suscepto, annis 1823-1830 collegit, notis, observationibus et adumbrationibus illustravit P.F. de Siebold.* Leiden/Lugundi-Batavorum, pp. 25–64 Decas II, pls. 9–15, 17, C, D.

Edelist, D., Rilov, G., Golani, D., Carlton, J.T. & Spanier, E. (2013): Restructuring the Sea: Profound shifts in the world's most invaded marine ecosystem. *Diversity and Distributions* 19 (1), 69–77, doi: 10.1111/ddi.12002.

Epifanio, C.E. (2013): Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: A review. *Journal of Experimental Marine Biology and Ecology* 441, 33–49, doi: 10.1016/j.jembe.2013.01.010.

Forward, R.B.J., Tankersley, R.A. & Rittschof, D. (2001): Cues for Metamorphosis of Brachyuran Crabs: An Overview. *American Zoologist* 41 (5), 1108–1122.

Fukui, Y. (1988): Comparative Studies on the Life History of the Grapsid Crabs (Crustacea, Brachyura) Inhabiting Intertidal Cobble and Boulder Shores. *Publications of the Seto Marine Biological Laboratory* 33 (4/6), 120–162.

Garside, C.J., Glasby, T.M., Stone, L.J. & Bishop, M.J. (2015): The timing of *Carcinus maenas* recruitment to a south-east Australian estuary differs to that of native crabs. *Hydrobiologia* 762, 41–53, doi: 10.1007/s10750-015-2332-z.

Gayanilo, F.C., Sparre, F. & Pauly, D. (2005): FAO-ICLARM Stock Assessment Tools II (FiSAT II), revised version, user's guide. *FAO Computerized Information Series (Fisheries)* (8), 168.

Geburzi, J.C. (2014): Rekrutierungsmuster und interspezifische Wechselwirkungen von einheimischen (*Carcinus maenas*) und eingeschleppten (*Hemigrapsus spp.*) Krabbenarten im Wattenmeer. MSc thesis, Kiel University, Kiel, 58 pp.

Geburzi, J.C., Graumann, G., Köhnk, S. & Brandis, D. (2015): First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Records* 4 (2), 103–107, doi: 10.3391/bir.2015.4.2.06.

Gerlach, J.D.J. & Rice, K.J. (2003): Testing Life History Correlates of Invasiveness Using Congeneric Plant Species. *Ecological Applications* 13 (1), 167–179.

Gittenberger, A., Rensing, M., Stegenga, H. & Hoeksema, B. (2010): Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33, 21–76.

Gollasch, S. (1999): The Asian decapod *Hemigrapsus penicillatus* (De Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgoländer Meeresuntersuchungen* 52, 359–366, doi: 10.1007/BF02908909.

Gollasch, S. (2006): Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60 (2), 84–89, doi: 10.1007/s10152-006-0022-y.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

Gothland, M., Dauvin, J.-C., Denis, L., Jobert, S., Ovaert, J., Pezy, J.-P. & Spilmont, N. (2013): Additional records and distribution (2011-2012) of Hemigrapsus sanguineus (De Haan , 1835) along the French coast of the English Channel. *Management of Biological Invasions* 4 (4), 305–315, doi: 10.3391/mbi.2013.4.4.0.

Grabowski, M., Bacela, K. & Konopacka, A. (2007): How to be an invasive gammarid (Amphipoda: Gammaroidea) – comparison of life history traits. *Hydrobiologia* 590, 75–84, doi: 10.1007/s10750-007-0759-6.

Griffen, B.D., Vogel, M., Goulding, L. & Hartman, R. (2015): Energetic effects of diet choice by invasive Asian shore crabs: Implications for persistence when prey are scarce. *Marine Ecology Progress Series* 522, 181–192, doi: 10.3354/meps11160.

Grosholz, E.D., Ruiz, G.M., Dean, C.A., Shirley, K.A., Maron, J.L. & Connors, P.G. (2000): The Impacts of a Nonindigenous Marine Predator in a California Bay. *Ecology* 81 (5), 1206–1224.

Herborg, L.-M., Weetman, D., van Oosterhout, C. & Hänfling, B. (2007): Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology* 16 (2), 231–242, doi: 10.1111/j.1365-294X.2006.03133.x.

Hobbs, N.-V.S., Cobb, J.S. & Thornber, C.S. (2017): Conspecific tolerance and heterospecific competition as mechanisms for overcoming resistance to invasion by an intertidal crab. *Biological Invasions* 19, 765–772, doi: 10.1007/s10530-016-1290-3.

Jensen, G.C., McDonald, P.S. & Armstrong, D.A. (2002): East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp*. *Marine Ecology Progress Series* 225, 251–262, doi: 10.3354/meps225251.

Jungblut, S., Beermann, J., Boos, K., Saborowski, R. & Hagen, W. (2017): Population development of the invasive crab *Hemigrapsus sanguineus* (De Haan, 1853) and its potential native competitor *Carcinus maenas* (Linnaeus, 1758) at Helgoland (North Sea) between 2009 and 2014. *Aquatic Invasions* 12 (1), 85–96, doi: 10.3391/ai.2017.12.1.0.

Kassambara, A. & Kosinski, M. (2017): survminer: Drawing Survival Curves using 'ggplot2'. Version 0.3.1, https://cran.r-project.org/package=survminer.

Kerckhof, F., Haelters, J. & Gollasch, S. (2007): Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions* 2 (3), 243–257.

Klein Breteler, W.C.M. (1975): Laboratory experiments on the influence of environmental factors on the frequency of moulting and the increase in size at moulting of juvenile shore crabs, *Carcinus maenas*. *Netherlands Journal of Sea Research* 9 (1), 100–120, doi: 10.1016/0077-7579(75)90025-3.

Klein Breteler, W.C.M. (1976): Settlement, growth and production of the shore crab, *Carcinus maenas*, on tidal flats in the Dutch Wadden Sea. *Netherlands Journal of Sea Research* 10 (3), 354–376, doi: 10.1016/0077-7579(76)90011-9.

Kopin, C.Y., Epifanio, C.E., Nelson, S. & Stratton, M. (2001): Effects of chemical cues on metamorphosis of the Asian shore crab *Hemigrapsus sanguineus*, an invasive species on the Atlantic Coast of North America. *Journal of Experimental Marine Biology and Ecology* 265, 141–151.

Kraemer, G.P., Sellberg, M., Gordon, A. & Main, J. (2007): Eight-year Record of Hemigrapsus sanguineus (Asian Shore Crab) Invasion in Western Long Island Sound Estuary. *Northeastern Naturalist* 14 (2), 207–224, doi: 10.1656/1092-6194(2007)14[207:EROHSA]2.0.CO;2.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Leffler, C.W. (1972): Some effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. *Marine Biology* 14, 104–110.

Levin, P.S., Coyer, J.A., Petrik, R. & Good, T.G. (2002): Community-Wide Effects of Nonindigenous Species on Temperate Rocky Reefs. *Ecology* 83 (11), 3182–3193.

Lockwood, J.L., Cassey, P. & Blackburn, T.M. (2005): The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20 (5), 223–228, doi: 10.1016/j.tree.2005.02.004.

Lohrer, A.M., Fukui, Y., Wada, K. & Whitlatch, R.B. (2000): Structural complexity and vertical zonation of intertidal crabs, with focus on habitat requirements of the invasive Asian shore crab, *Hemigrapsus sanguineus* (de Haan). *Journal of Experimental Marine Biology and Ecology* 244 (2), 203–217.

Lohrer, A.M. & Whitlatch, R.B. (2002): Interactions Among Aliens: Apparent Replacement of One Exotic Species By Another. *Ecology* 83 (3), 719–732.

McDermott, J.J. (1998): The western Pacific Brachyuran *Hemigrapsus sanguineus* (Grapsidae) in its new habitat along the Atlantic coast of the United States: reproduction. *Journal of Crustacean Biology* 18 (2), 308–316.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Moksnes, P.-O. (2002): The relative importance of habitat-specific settlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs *Carcinus maenas* L. *Journal of Experimental Marine Biology and Ecology* 271, 41–73.

Moksnes, P.-O. (2004): Self-regulating mechanisms in cannibalistic populations of juvenile shore crabs *Carcinus maenas*. *Ecology* 85 (5), 1343–1354, doi: 10.1890/02-0750.

Moksnes, P.-O., Pihl, L. & van Montfrans, J. (1998): Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism. *Marine Ecology Progress Series* 166, 211–225.

Naylor, E. (1962): Seasonal Changes in a Population of *Carcinus maenas* (L.) in the Littoral Zone. *Journal of Animal Ecology* 31 (3), 601–609.

Noël, P.Y., Tardy, E. & D’Udekem d’Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l’Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Obert, B., Herly, M. & Grotjahn, M. (2007): First records of two crabs from the North West Pacific *Hemigrapsus sanguineus* and *H. takanoi* at the coast of Lower Saxony, Germany. *Wadden Sea Newsletter* 1, 21–22.

O’Connor, N.J. (2007): Stimulation of molting in megalopae of the Asian shore crab *Hemigrapsus sanguineus*: physical and chemical cues. *Marine Ecology Progress Series* 352, 1–8, doi: 10.3354/meps07315.

O’Connor, N.J. (2014): Invasion dynamics on a temperate rocky shore: from early invasion to establishment of a marine invader. *Biological Invasions* 16, 73–87, doi: 10.1007/s10530-013-0504-1.

Olden, J.D., Poff, N.L. & Bestgen, K.R. (2006): Life-History Strategies Predict Fish Invasions and Extirpations in the Colorado River Basin. *Ecological Monographs* 76 (1), 25–40.

Payne, A. & Kraemer, G.P. (2013): Morphometry and claw strength of the non-native Asian shore crab, *Hemigrapsus sanguineus*. *Northeastern Naturalist* 20 (3), 478–492, doi: 10.1656/045.020.0311.

Pillay, K.K. & Ono, Y. (1978): The breeding cycles of two species of grapsid crabs (Crustacea: Decapoda) from the North Coast of Kyushu, Japan. *Marine Biology* 45, 237–248.

Pöckl, M. (2009): Success of the invasive Ponto-caspian amphipod *Dikerogammarus villosus* by life history traits and reproductive capacity. *Biological Invasions* 11, 2021–2041, doi: 10.1007/s10530-009-9485-5.

R Core Team (2017): R: A language and environment for statistical computing. Version 3.4.2, https://www.r-project.org/.

Reise, K., Baptist, M., Burbridge, P., Dankers, N., Fischer, L., Flemming, B., Oost, A.P. & Smit, C. (2010): The Wadden Sea – A Universally Outstanding Tidal Wetland. *Wadden Sea Ecosystem* 29, 7–24.

Reise, K., Buschbaum, C., Büttger, H. & Wegner, K.M. (2017): Invading oysters and native mussels: From hostile takeover to compatible bedfellows. *Ecosphere* 8 (9), e01949, doi: 10.1002/ecs2.1949.

Rejmanek, M. & Richardson, D.M. (1996): What Attributes Make Some Plant Species More Invasive? *Ecology* 77 (6), 1655–1661.

Ruiz, G.M., Carlton, J.T., Grosholz, E.D. & Hines, A.H. (1997): Global Invasions of Marine and Estuarine Habitats by Non-Indigenous Species: Mechanisms, Extent, and Consequences. *American Zoologist* 37, 621–632, doi: 10.1093/icb/37.6.621.

Seeley, B., Sewell, J. & Clark, P.F. (2015): First GB records of the invasive Asian shore crab, Hemigrapsus sanguineus from Glamorgan, Wales and Kent, England. *Marine Biodiversity Records* 8, e102, doi: 10.1017/S1755267215000809.

Therneau, T.M. (2015): A package for survival analysis in S.

Thiel, M. & Dernedde, T. (1994): Recruitment of shore crabs *Carcinus maenas* on tidal flats: mussel clumps as an important refuge for juveniles. *Helgoländer Meeresuntersuchungen* 48, 321–332.

Townsend Peterson, A. (2003): Predicting the Geography of Species' Invasions via Ecological Niche Modeling. *The Quarterly Review of Biology* 78 (4), 419–433, doi: 10.1086/378926.

Van den Brink, A.M., Godschalk, M., Smaal, A.C., Lindeboom, H. & McLay, C.L. (2013): Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of the Marine Biological Association of the United Kingdom* 93 (1), 189–196, doi: 10.1017/S0025315412000446.

Van den Brink, A.M., Wijnhoven, S. & McLay, C.L. (2012): Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73, 126–136, doi: 10.1016/j.seares.2012.07.006.

Venables, W.N. & Ripley, B.D. (2002): *Modern Applied Statistics with S.* Fourth Edi Springer, New York, NY.

Wickham, H. (2009): *ggplot2: Elegant Graphics for Data Analysis.* Springer, New York, NY.

Williams, S.L. & Grosholz, E.D. (2008): The invasive species challenge in estuarine and coastal environments: Marrying management and science. *Estuaries and Coasts* 31 (1), 3–20, doi: 10.1007/s12237-007-9031-6.

Wolff, W.J. (2005): Non-indigenous marine and estuarine species in The Netherlands. *Zoologische Mededelingen Leiden* 79 (1), 1–116.

Wood, C.A., Bishop, J.D.D., Davies, C.J., Delduca, E.L., Hatton, J.C., Herbert, R.J.H. & Clark, P.F. (2015): *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4 (2), 109–113, doi: 10.3391/bir.2015.4.2.07.

Yamasaki, I., Doi, W., Mingkid, W.M., Yokota, M., Strüssmann, C.A. & Watanabe, S. (2011): Molecular-Based Method To Distinguish the Sibling Species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of Crustacean Biology* 31 (4), 577–581, doi: 10.1651/10-3366.1.

Appendix

Appendix 1

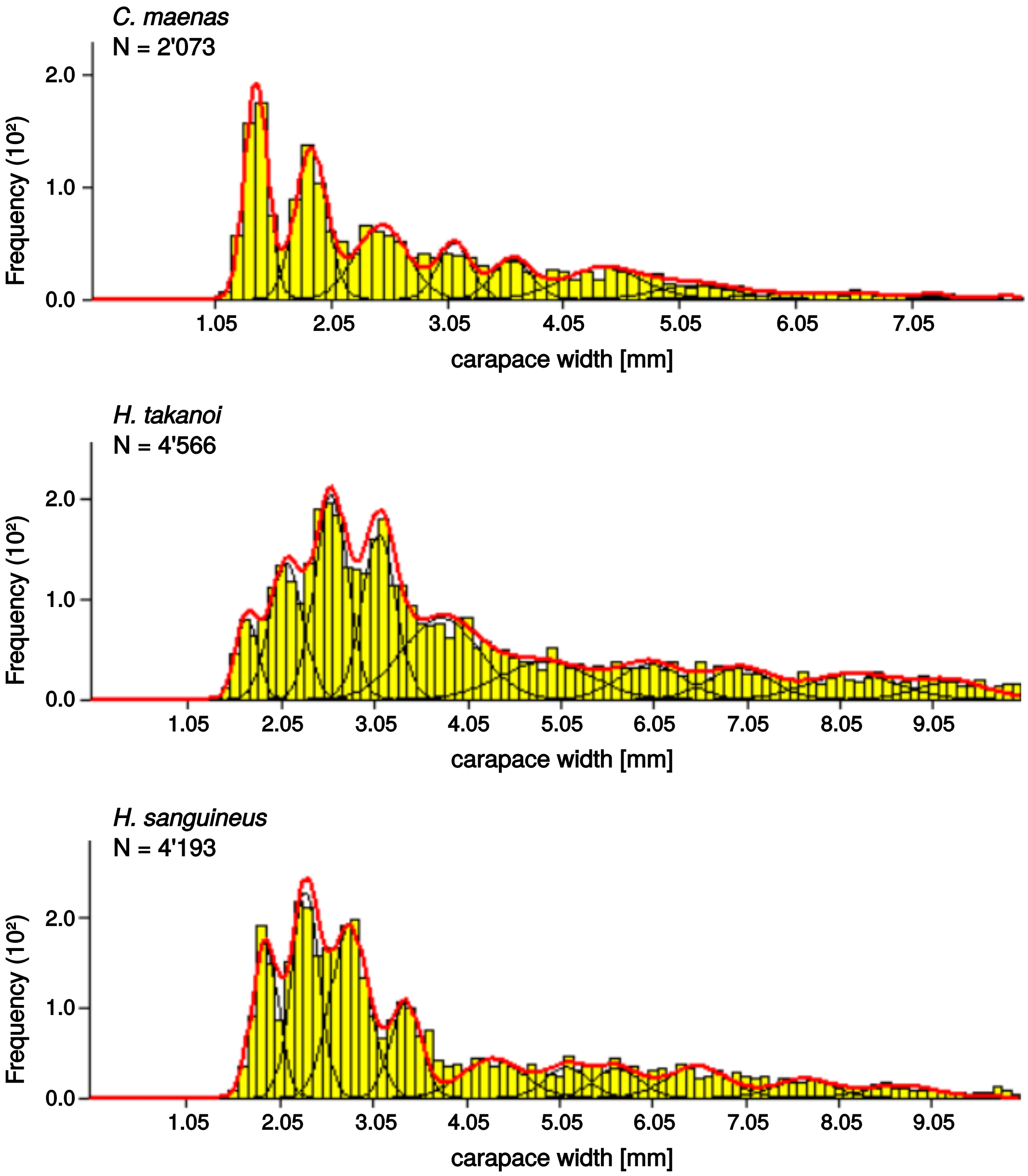


Fig. 4.7. Decomposition of the size-frequency distributions of *C. maenas* (top), H. takanoi (middle) and *H. sanguineus* (bottom) by Bhattacharya's method for the 2014–2016 survey data.

Appendix 2

Tab. 4.2. Detailed results of all pairwise Log-rank tests from the predation experiment. Provided are test statistics (χ^2), 'raw' p-values and adjusted p-values (Holm's sequential Bonferroni method) for combinations of recruit species and treatment (i.e. predator species) pairs as given in the first 4 columns of the table. For all tests df = 1. Treatment codes: C – subadult *C. maenas*, S – subadult *H. sanguineus*, T – subadult *H. takanoi*

Tested combination				χ^2	Raw p-value	Adj. p-value
Recruits	Treatment	Recruits	Treatment			
C. maenas	– C	*C. maenas*	– S	14.7	0.00012	0.0014***
			– T	21.5	< 0.0001	< 0.0001***
		H. sanguineus	– C	23.5	< 0.0001	< 0.0001***
			– S	74.6	< 0.0001	< 0.0001***
		H. takanoi	– C	22.5	< 0.0001	< 0.0001***
			– T	75.9	< 0.0001	< 0.0001***
C. maenas	– S	*C. maenas*	– T	0.5	0.48	1
		H. sanguineus	– C	0.8	0.36	1
			– S	37.9	< 0.0001	< 0.0001***
		H. takanoi	– S	25.8	< 0.0001	< 0.0001***
C. maenas	– T	*H. sanguineus*	– T	22.4	< 0.0001	< 0.0001***
		H. takanoi	– C	0.2	0.63	1
			– T	32.3	< 0.0001	< 0.0001***
H. sanguineus	– C	*H. sanguineus*	– S	33.5	< 0.0001	< 0.0001***
			– T	25.2	< 0.0001	< 0.0001***
		H. takanoi	– C	0.1	0.77	1
H. sanguineus	– S	*H. sanguineus*	– T	1.3	0.26	1
		H. takanoi	– S	2.1	0.14	1
			– T	0.4	0.52	1
H. sanguineus	– T	*H. takanoi*	– S	0.1	0.72	1
			– T	2.9	0.09	0.81
H. takanoi	– C	*H. takanoi*	– S	16.9	< 0.0001	0.0005***
			– T	31.1	< 0.0001	< 0.0001***
H. takanoi	– S	*H. takanoi*	– T	3.9	0.05	0.47

5. Genetic structure of range-extending Asian crabs *Hemigrapsus takanoi* (Decapoda, Brachyura, Varunidae) along European coasts

Jonas C. Geburzi[a, b], Christine Ewers-Saucedo[b] and Günther B. Hartl[a]

[a]*Kiel University, Zoological Institute, Population Genetics, Am Botanischen Garten 1-9, 24118 Kiel, Germany*
[b]*Kiel University, Zoological Museum, Hegewischstr. 3, 24105 Kiel, Germany*

In preparation for submission to:
Molecular Ecology

Abstract

Genetic studies of introduced non-native species are a valuable tool to investigate invasion history and pathways, source populations and multiple introductions of alien species, as well as evolutionary genetic changes following establishment in a new environment. In this study, we used a set of nine polymorphic microsatellites to analyse the population genetic structure of the introduced Asian shore crab *Hemigrapsus takanoi* along European coasts. Our dataset covers the complete known European range of the species, including the most recent (2014–2017) records from the southwestern Baltic Sea. The results show a similarly high genetic diversity of *H. takanoi* throughout Europe, and only weak population differentiation. Siginficant homozygote excess and prevalence of null alleles in all populations conincide with the

presumably high effective population size, recent expansion and population growth. Analyses for population structure along geographic regions give support for a separation between the Bay of Seine populations (northern France) and all other populations. Further, a surprisingly high (though still weak) differentiation between the neighbouring western and central Wadden Sea populations was detected. The Baltic Sea populations clustered within the Wadden Sea populations, suggesting a secondary introduction from the North Sea as the likely invasion pathway of *H. takanoi* to the Baltic Sea. The genetic data from the Baltic Sea populations in particular, depicting population status shortly after introduction, can furthermore serve as a 'baseline' data for future investigations of genetic changes during establishment and adaptation processes.

5.1. Introduction

Continuously rising numbers of human-mediated introductions of species into areas far beyond their native ranges receive ongoing scientific attention and are seen as an important dimension of global change (Ruiz et al. 2000, Simberloff et al. 2013, Chan & Briski 2017). The often rapid establishment and dispersal of such non-native species, which are then consequently termed 'invasive', can affect native as well as other non-native species and profoundly alter ecological communities in newly occupied ecosystems. Thus, new records of non-native species and sudden range extensions of established invaders are regularly cautiously monitored.

Investigations of invasion pathways and invasion history can deliver important information to understand dispersal and establishment processes, and are as well important in the context of risk assessment for range extensions and possible future invasion events (Kolar & Lodge 2001, Kulhanek et al. 2011). Genetic analyses have been proven a useful approach in this context. They can be used to detect multiple introductions of a species from different populations within the species' native range, which may lead to genetic admixture in invasive populations, counteracting loss of genetic diversity by usually small founder populations (Roman & Darling 2007, Rius et al. 2015, Wagner et al. 2017). In fact, genetic admixture following such 'kryptic' invasions may facilitate rapid adaptation to new environmental conditions and has been attributed to several cases of range extensions of non-native species (Herborg et al. 2007, Lejeusne et al. 2014, Wagner et al. 2017). Furthermore, genetic analyses can help to identify source populations and donor regions of non-native species and to reconstruct invasion pathways. In a more general context, invasion genetics also provide tools for investigating evolutionary adaptations of species to new or changing environments (Holland 2000, Geller et al. 2010, Cristescu 2015).

Ship traffic is widely seen as the most important vector for the transportation and introduction of marine non-native species (see Gollasch 2006, Katsanevakis et al. 2013). Uptake and discard of huge amounts of ballast water at origin and destination ports can lead to the release of hundreds of organisms by a single ship. Fouling communities at the outside of ship's hulls are further carriers of potential invaders. Consequently, the main pathways of primary species introductions and hot-spot regions of species' export and import reflect well the network of global shipping lines (Seebens et al. 2013). But also for secondary introductions (i.e. regional transport from the initial site of introduction), ship traffic, including recreational boating, plays an important role (Clarke Murray et al. 2011).

Decapod crustaceans, and especially the true crabs (Brachyura) are a particularly prominent group among marine invasive species, in both terms of species numbers and impacts. The long planktonic larval development of many species (often exceeding three weeks) makes them especially prone to be transported via ballast water, and their high reproductive and adaptive potential allows for rapid population growth and can lead to strong impacts on the invaded communities (Brockerhoff & McLay 2011, Hänfling et al. 2011).

Here, we investigate the population genetic structure of a recent Brachyuran invader to European coasts, the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe 2005. The native range of this species stretches along the coasts of the northwestern Pacific from southern Russia to China, including Japan and Taiwan (Asakura & Watanabe 2005, Yamasaki et al. 2011, Lee et al. 2013, Marin 2013), where it inhabits the intertidal and shallow subtidal zones and also occurs in estuaries (Asakura & Watanabe 2005, Mingkid et al. 2006).

Hemigrapsus takanoi was recorded in Europe for the first time in 1993 (Gollasch 1999), and a reproducing population was found at La Rochelle, France, in 1994 (Noël et al. 1997). In the following years, records from the Bay of Biscay coast (Noël et al. 1997, Noël & Gruet 2008), northern France (Breton et al. 2002, Dauvin 2009, Dauvin et al. 2009, Dauvin & Delhay 2010), the Dutch Delta (Nijland & Beekman 2000, Wolff 2005) and the Wadden Sea (Obert et al. 2007, Gittenberger et al. 2010, Landschoff et al. 2013) detailedly documented the rapid spread of *H. takanoi* along the European Atlantic and North Sea coasts. From northern France to the northern Wadden Sea it performed a remarkable 'tandem' invasion with the closely related *H. sanguineus*. Very recently, *H. takanoi* has also been reported from Great Britain (Wood et al. 2015, Ashelby et al. 2017) and the southwestern Baltic Sea (Geburzi et al. 2015), indicating a still dynamic range expansion of this species.

Markert et al. (2014) suggested three independent introductions of *H. takanoi* to Europe, to the Bay of Biscay, the French coast of the English Channel and the Dutch delta/southern North Sea. A recent study by Makino et al. (2017) found in fact evidence for multiple introductions of this species, with the population in the Bay of Seine (northern France) being a genetic admixture of

Chinese/Korean and Japanese origin, while the remaining European populations investigated were assigned exclusively to Japanese origin.

The aim of this study is to use polymorphic microsatellites for a regional-scale investigation of genetic structuring in the European *H. takanoi* populations, with a special focus on the current northern and eastern limits of its range, the Wadden and Baltic Seas. A main scope of our work is to shed light on the origin(s) of the Baltic Sea populations (Secondary introduction from elsewhere in Europe or independent new introduction from the native range?), especially in the light of the most recent range extensions of *H. takanoi* presented in this study. We further aim to detect possible migration/introduction routes as well as unrecognised introduction events in the southern North Sea, as several important international ports and high ship traffic in this region lead to an increased likelihood for introductions.

5.2. Materials and Methods

5.2.1. Sample collection and geographic information

A total of 395 specimen of *Hemigrapsus takanoi* were collected from 12 sites along the coasts of the Wadden Sea (Germany/Netherlands), the southwestern Baltic Sea (Germany), and the Bay of Biscay and Bay of Seine (France) between 2014 and 2017 (Tab. 5.1, Fig. 5.1). Sampling sites include the oldest known European population of *H. takanoi* in La Rochelle, France (site LRO), as well as the most recent reports from the Mecklenburg Bight in the German part of the Baltic Sea: Poel island near Wismar (site GOL, detected in autumn 2016, pers. comm. W. Wranik, University of Rostock), Neustadt/Holstein and the mouth of the Trave river near Lübeck (sites NEU and PRI, detected in summer 2017, this study).

Crabs were hand collected between cobbles or mussels at low tide or caught with a scoop from mussel clumps or underneath boulders in the shallow subtidal. All specimen were preserved as whole crabs in 96 % ethanol. For better preservation of muscle tissue, the chelae and largest walking legs were perforated with a thin needle.

Tab. 5.1. Geographic information, population codes and number of sampled individuals (N) for European *H. takanoi* populations included in this study; negative longitude values refer to locations west of the Greenwich meridian.

Sea	Region	Site/ Population	Locality code	Coordinates		Sampling date	N
				Lat	*Long*		
Wadden Sea	northern	List	LIS	55.0286	8.4349	12/2015	32
	central	Cuxhaven	CUX	53.8926	8.6864	02/2016	32
		Wilhelmshaven	WHV	53.5038	8.1030	12/2015	32
		Bensersiel	BEN	53.6815	7.5675	12/2015	36
	western	Mokbaai/Texel	TEX	53.0063	4.7667	04/2016	36
Baltic Sea	Kiel Bight	Kiel Fjord	KIE	54.3287	10.1481	04/2016	33
	Mecklenburg Bight	Neustadt	NEU	54.0964	10.8145	10/2017	7
		Priwall	PRI	53.9589	10.8836	10/2017	25
		Gollwitz/Poel	GOL	54.0241	11.4813	06 & 10/2017	38
English Channel	Bay of Seine	Ouistreham	OUI	49.2811	–0.2473	08/2017	47
		Honfleur	HON	49.4282	0.2298	08/2017	39
Atlantic Ocean	Bay of Biscay	La Rochelle	LRO	46.1385	–1.1742	04/2016 03/2017	38

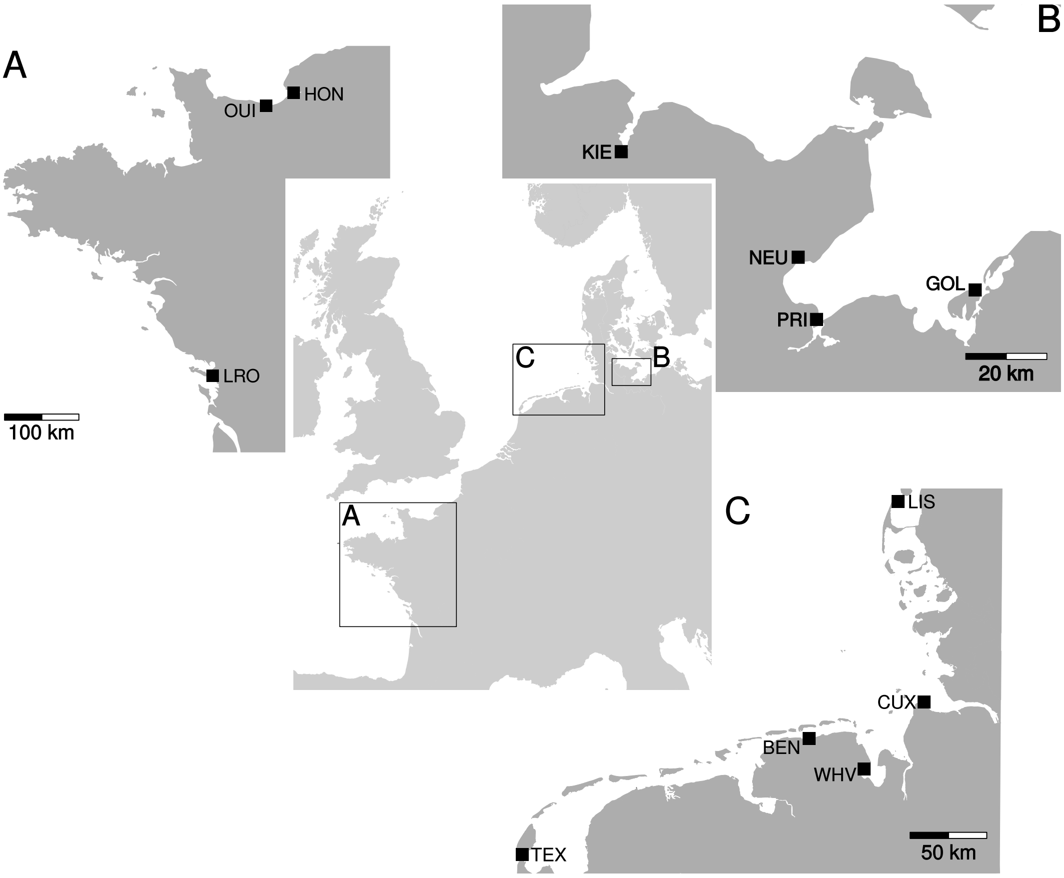


Fig. 5.1. Sampling sites of *H. takanoi* across Europe. For locality codes, refer to Tab. 5.1

5.2.2. DNA isolation, PCR amplification and microsatellite genotyping

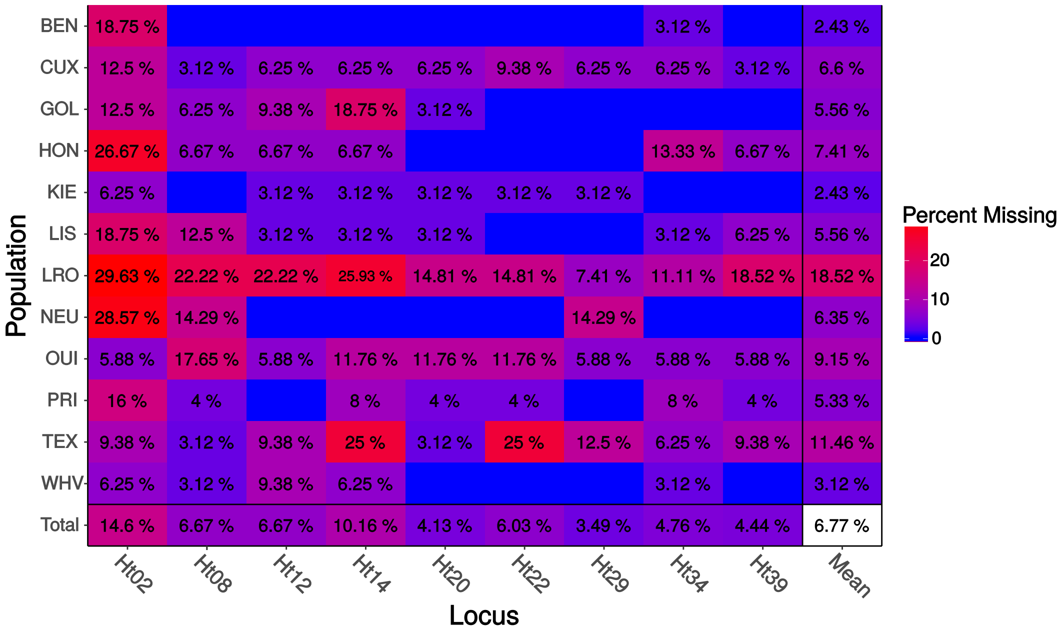
Genomic DNA was extracted from pereiopod or chela muscle tissue using the Invisorb Spin Tissue Mini or Invisorb Spin Forensic kit (Stratec Molecular, Berlin, Germany). Eleven tri-nucleotide microsatellite loci (Ht02, Ht08, Ht12, Ht14, Ht20, Ht22, Ht28, Ht29, Ht34, Ht39, Ht47; Poux et al. 2015) were amplified in three multiplex sets by PCR in 15 µL volumes containing final concentrations of 0.9 × PCR Buffer (Promega), 3.5 mM MgCl, 0.4 mM each dNTP (Roth), 0.3 µM each primer, 0.8 U GoTaq Polymerase (Promega) and 0.7–3 µL DNA template (depending on template concentration). PCR conditions were as follows: initial denaturation for 4 min at 95 °C, 30 cycles of denaturation for 30 s at 94 °C, annealing for 90 s at 57 °C, extension for 60 s at 72 °C, and a final extension of 20 min at 60 °C. PCR products were run on an AB 3730xl DNA Analyser (Applied Biosystems) with GeneScan 600 LIZ v2.0 size standard (Applied Biosystems) for fragment length analysis. Peaks were called and binned using the package 'Fragman' (Covarrubias-Pazaran et al. 2016) within the R environment, version 3.4.2 (R Core Team 2017).

5.2.3. Analysis of microsatellite genotypes

Genetic diversity in the samples was assessed by calculating the number of alleles per locus, observed and unbiased expected heterozygosity (H_{obs} and H_{exp}, Nei 1978), and allelic richness (A_r), the average number of alleles per population normalised to the smalles population size. A_r was calculated using the function 'allel.rich' of the R package 'PopGenReport' (Adamack & Gruber 2014). Deviations from Hardy-Weinberg-Equilibrium (HWE) were tested for each locus, both for each population separately and pooled over all populations, using the function 'hw.test' of the R package 'pegas' (version 0.10, Paradis 2010), with 1'999 Monte-Carlo permutations. To correct for multiple comparisons, p-values were corrected using Holm's sequential Bonferroni method (Holm 1979). Frequency of null-alleles for each locus was estimated based on the method of Brookfield (1996). Number of private alleles (i.e. alleles that occur only in one population) was calculated with the function 'private_alleles' implemented in the R package 'poppr' (version 2.5.0, Kamvar et al. 2014).

To assess possible genetic differentiation between the populations, we conducted an analysis of molecular variance (AMOVA, Excoffier et al. 1992), which is based on genetic distances between pairs of individual genotypes. Compared to the F-statistics measures of population differentiation (i.e. calculation of pairwise F_{ST} values), it has the advantage of not making assumptions about Hardy-Weinberg equilibrium and is thus also applicable for populations with significant homo- or heterozygote excess. The dependent variable in an AMOVA is a matrix containing genetic distance values for all possible pairs of genotypes (i.e. individuals) in the dataset. For the *H. takanoi* microsatellite data, three different distance matrices were calculated based on Nei's distance (Nei 1978), genetic distance based on relative dissimilarity (Prevosti et al. 1975), and the genetic distance of Cavalli-Sforza and Edwards (Cavalli-Sforza & Edwards 1967), the latter being the least sensitive to the presence of null alleles (Chapuis & Estoup 2007). Calculation of these distance matrices is implemented in the R package 'poppr' with the functions 'nei.dist', 'prevosti.dist' and 'edwards.dist'. As explanatory variables, we hierarchically used geographical groupings (population, region, sea; see Tab. 5.1). The analysis was performed using the function 'poppr.amova' in the R package 'poppr'. Significance of AMOVA results was assesed by randomising genotypes to populations in 999 Monte-Carlo permutations.

For further investigation of population structure, we conducted a discriminant analysis of principal components (DAPC, Jombart et al. 2010), implemented in the R package 'adegenet' (Jombart et al. 2008) with the function 'dapc'. The DAPC approach combines a principal component analysis (PCA) of the genotype data, which in a first step identifies alleles that best differentiate genotypes, and a discriminant analysis (DA), which in a second step uses the identified factors to maximise the variation between preassigned groupings. These groupings can either be *a priori* user-defined groups (e.g. sampled populations, regions), or clusters which are inferred directly from the data without any preliminary assumptions on population subdivision. The latter approach resembles a STRUCTURE analysis (Pritchard et al. 2000), but distinguishes from it by representing the maximal differentiation of the data with respect to the preassigned groupings, while STRUCTURE tries to identify groupings that fit the data best. The identification of the clusters is realised by the function 'find.clusters' in the R package adegenet. We conducted DAPCs following both approaches in order to assess if differences in the genotype data reflect geographic partinioning, and to detect possible genetic differentiation based on *a priori* unrecognised factors, respectively.



	Ht02	Ht08	Ht12	Ht14	Ht20	Ht22	Ht29	Ht34	Ht39	Mean
BEN	18.75 %							3.12 %		2.43 %
CUX	12.5 %	3.12 %	6.25 %	6.25 %	6.25 %	9.38 %	6.25 %	6.25 %	3.12 %	6.6 %
GOL	12.5 %	6.25 %	9.38 %	18.75 %	3.12 %					5.56 %
HON	26.67 %	6.67 %	6.67 %	6.67 %				13.33 %	6.67 %	7.41 %
KIE	6.25 %		3.12 %	3.12 %	3.12 %	3.12 %	3.12 %			2.43 %
LIS	18.75 %	12.5 %	3.12 %	3.12 %	3.12 %			3.12 %	6.25 %	5.56 %
LRO	29.63 %	22.22 %	22.22 %	25.93 %	14.81 %	14.81 %	7.41 %	11.11 %	18.52 %	18.52 %
NEU	28.57 %	14.29 %					14.29 %			6.35 %
OUI	5.88 %	17.65 %	5.88 %	11.76 %	11.76 %	11.76 %	5.88 %	5.88 %	5.88 %	9.15 %
PRI	16 %	4 %		8 %	4 %	4 %		8 %	4 %	5.33 %
TEX	9.38 %	3.12 %	9.38 %	25 %	3.12 %	25 %	12.5 %	6.25 %	9.38 %	11.46 %
WHV	6.25 %	3.12 %	9.38 %	6.25 %				3.12 %		3.12 %
Total	14.6 %	6.67 %	6.67 %	10.16 %	4.13 %	6.03 %	3.49 %	4.76 %	4.44 %	6.77 %

Fig. 5.2. Percentage of missing data (unsuccessful scoring) in the microsatellite dataset; rows: populations, columns: loci.

5.3. Results

5.3.1. Characterisation of the genotype data

We genotyped 315 out of the 395 initially collected individuals. As only very few (< 15) individuals were successfully scored on loci Ht28 and Ht47, these loci were removed from the dataset and not included in the analysis. All of the remaining nine loci were successfully amplified on 217 individuals, more than six loci on 292 individuals, and more than 3 loci on 312 individuals. Overall percentage of missing data was 6.77 %, these results being consistent with the observed prevalence of null alleles (Tab. 5.2). Missing data were not evenly distributed over the dataset, but were especially prevalent in the loci Ht02 and Ht14 and populations LRO and TEX with > 10 % missing data each (Fig. 5.2). Thus, all analyses were performed on three datasets with different amounts of missing data (including individuals that amplified on more than three, more than six, and on all nine loci, respectively) and results were quantitatively the same. The results reported in the following are based on the analyses including those 312 individuals that scored on at least four loci.

Tab. 5.2. Sample sizes (N) and measures for genetic diversity in European *H. takanoi* populations; H_{exp}: expected heterozygosity, H_{obs}: observed heterozygosity, A_r: allelic richness, HWE $p < 0.05$: no. of loci significantly deviating from HWE expectations (also indicated by bold H_{obs} values)

Population	N		Loci									Mean	HWE $p < 0.05$	private Alleles
			Ht02	Ht08	Ht12	Ht14	Ht20	Ht22	Ht29	Ht34	Ht39			
LIS	32	H_{exp}	0.88	0.90	0.86	0.87	0.93	0.88	0.67	0.86	0.83	0.85	2	1
		H_{obs}	0.65	**0.57**	0.77	0.65	0.84	**0.59**	0.59	0.61	0.63	0.66		
		A_r	6.45	6.78	6.10	6.25	7.67	6.34	3.94	5.87	5.45	6.09		
CUX	32	H_{exp}	0.91	0.89	0.89	0.88	0.91	0.88	0.60	0.88	0.82	0.85	3	0
		H_{obs}	**0.64**	**0.58**	0.87	**0.47**	0.93	0.72	0.37	**0.47**	0.55	0.62		
		A_r	7.19	6.53	6.57	6.41	7.19	6.43	3.18	6.56	5.18	6.14		
WHV	32	H_{exp}	0.86	0.89	0.88	0.88	0.91	0.91	0.60	0.90	0.86	0.86	3	2
		H_{obs}	**0.57**	0.74	0.79	**0.63**	0.81	0.88	0.53	0.87	**0.44**	0.70		
		A_r	6.21	6.60	6.50	6.38	7.14	7.13	4.04	6.98	5.84	6.31		
BEN	32	H_{exp}	0.88	0.90	0.90	0.86	0.93	0.87	0.72	0.90	0.77	0.86	3	0
		H_{obs}	**0.46**	**0.62**	0.75	0.62	0.94	0.66	0.53	**0.58**	0.53	0.63		
		A_r	6.51	6.78	6.94	5.95	7.60	6.20	4.05	6.83	5.18	6.23		
TEX	32	H_{exp}	0.92	0.91	0.91	0.90	0.93	0.84	0.77	0.89	0.88	0.88	6	7
		H_{obs}	**0.66**	**0.45**	0.83	**0.58**	**0.55**	0.58	**0.50**	0.80	**0.41**	0.60		
		A_r	7.44	7.07	7.18	5.78	6.71	6.56	3.51	7.59	6.38	6.68		
KIE	32	H_{exp}	0.89	0.88	0.89	0.92	0.92	0.90	0.79	0.88	0.86	0.88	5	1
		H_{obs}	**0.50**	**0.41**	0.87	**0.68**	0.84	**0.68**	0.65	0.72	**0.50**	0.65		
		A_r	6.67	6.45	6.77	7.40	7.39	6.93	4.73	6.47	5.98	6.53		
NEU	7	H_{exp}	0.80	0.82	0.87	0.82	0.88	0.89	0.51	0.79	0.82	0.80	0	0
		H_{obs}	0.40	0.50	0.57	0.57	1.00	0.57	0.33	0.71	0.29	0.55		
		A_r	5.20	5.54	6.48	5.42	6.82	6.97	3.25	5.18	5.15	5.56		
PRI	25	H_{exp}	0.87	0.89	0.91	0.82	0.89	0.88	0.70	0.93	0.88	0.86	4	7
		H_{obs}	**0.57**	**0.62**	0.80	**0.48**	**0.54**	0.67	0.64	0.87	0.71	0.66		
		A_r	6.33	6.52	7.18	6.86	7.67	6.01	4.72	6.86	6.33	6.28		
GOL	32	H_{exp}	0.88	0.89	0.90	0.90	0.92	0.88	0.69	0.92	0.85	0.87	5	1
		H_{obs}	**0.79**	**0.47**	0.76	**0.62**	0.84	**0.62**	**0.44**	0.78	**0.53**	0.65		
		A_r	6.47	6.58	6.97	7.01	7.47	6.53	3.97	7.49	5.86	6.48		
OUI	16	H_{exp}	0.81	0.83	0.88	0.71	0.90	0.83	0.65	0.89	0.78	0.81	0	2
		H_{obs}	0.75	0.86	0.88	0.40	0.80	0.60	0.80	0.81	0.69	0.73		
		A_r	5.28	5.73	6.59	4.03	6.98	5.38	2.94	6.75	5.13	5.42		
HON	15	H_{exp}	0.77	0.86	0.89	0.66	0.89	0.85	0.73	0.87	0.69	0.80	3	1
		H_{obs}	0.55	0.79	0.79	**0.29**	**0.40**	**0.53**	0.53	0.46	0.64	0.55		
		A_r	4.58	6.05	6.78	3.74	6.67	5.59	3.90	6.24	4.23	5.35		
LRO	25	H_{exp}	0.88	0.86	0.87	0.80	0.92	0.91	0.71	0.91	0.85	0.86	3	4
		H_{obs}	**0.39**	0.60	0.76	0.55	0.74	**0.52**	0.75	0.83	**0.59**	0.64		
		A_r	6.62	6.46	6.38	5.49	7.58	7.06	3.68	7.11	5.62	6.22		
		No. of alleles	26	23	28	20	35	26	12	32	17			
		HWE$p < 0.05$	8	8	0	7	3	5	2	5	5			
		A_r	6.25	6.42	6.70	5.89	7.24	6.46	3.82	6.66	5.53			
		Freq. null alleles	0.17	0.18	0.07	0.18	0.09	0.14	0.10	0.11	0.18			

This dataset contained 7 to 32 individuals per population (Tab. 5.2). The nine microsatellite loci were highly polymorphic, total numbers of alleles per locus were 12–35. Allelic richness was similar in all populations, ranging between 5.35 and 6.68, and mean expected heterozygosities varied between 0.80 and 0.88. Homozygote excess was prevalent, with significant deviations from HWE expectations on 3–6 loci in all populations except LIS, NEU and OUI. Estimated null allele frequencies ranged between 0.07 and 0.18. Conspiciously high numbers of private alleles were detected in the LRO (4), PRI and TEX (7 each) populations (Tab. 5.2).

5.3.2. Genetic differentiation

The AMOVA results indicated only weak genetic differentiation between populations, regions or seas. Results were similar for all three distance matrices. Variation between seas amounted to about 1.1 %, variation between regions to about 0.5 %, and variation between populations to about 1.6 % of total variation in the genotype data. Variation within populations consequently amounted to about 96 % of total variation, thus having the greatest contribution. However, the results of the randomisation tests indicated a significant structuring on the population level ($p < 0.01$) and also a marginally significant structuring on the level of seas ($p < 0.06$), despite their low contribution to total genetic variation.

The DAPC approach with cluster preassignments inferred from the genotype data by the ‘find.clusters’ function did not cluster individuals well by their population or geographical regions (Fig. 5.3), suggesting no strong geographic population structure. The proportion of preserved variance was 0.853.

The second DAPC approach, using the sampled populations as group preassignments, however, revealed some geographical structuring in the genetic data (Fig. 5.4 A). It clearly seperated the Bay of Seine populations (HON and OUI) from all other populations. The remaining populations form one big cluster, with the central Wadden Sea populations (CUX, WHV, BEN) and the northern Wadden Sea and Baltic Sea populations (LIS, KIE, NEU, PRI, GOL) forming two very closely related sub-groups. The TEX population from the western Wadden Sea marginally seperates from the cluster as a whole, but is surprisingly well seperated from the geographically closest central Wadden Sea populations. Assignment probabilities of individuals to their actual populations were however low (0.21 to 0.65). The proportion of preserved variance was 0.87. Only three alleles had a relative contribution greater than 0.05 to the observed clustering (Ht12_297, Ht22_165, Ht39_126; Fig. 5.4 B) and their allele frequencies differed maximally *c.* 0.3 between populations (Fig. 5.4 C). The observed population structure is thus the result of the little frequency differences in these few alleles.

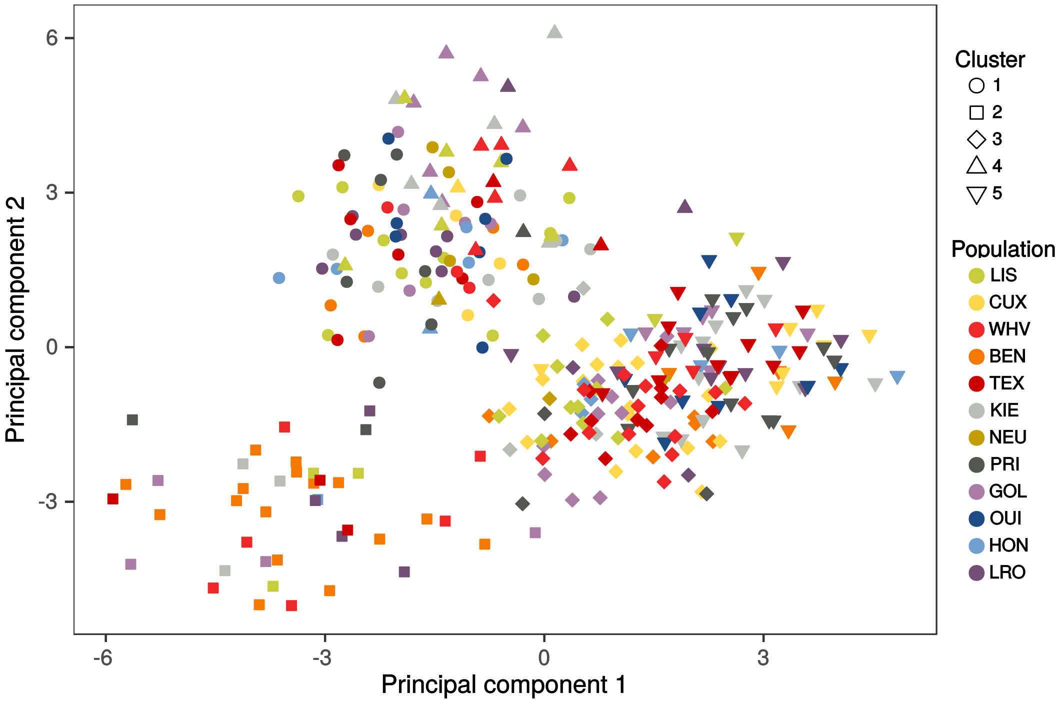


Fig. 5.3. Discriminant analysis of principal components (DAPC) using clusters inferred from the genotype data as preassigned groupings; plot of the first two principal components; point shape: cluster (1–5), point colour: actual population of individual.

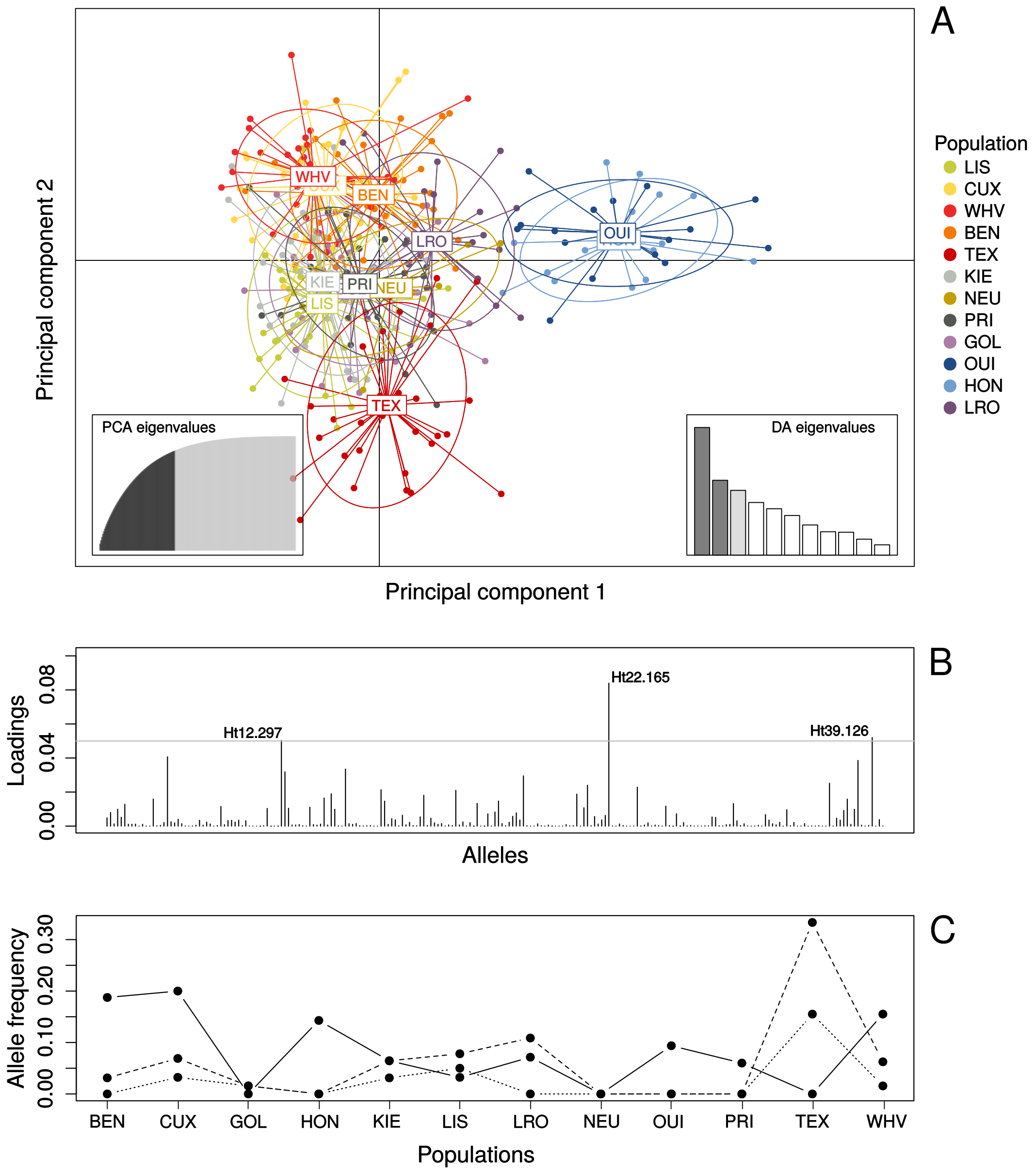


Fig. 5.4. Discriminant analysis of principal components (DAPC) using populations as preassigned groupings; (A) plot of the first two principal components of the DACP, colour: population; (B) bar graph of the relative contributions of alleles to the DACP principal components, alleles with a contribution > 0.05 (horizontal line) are indicated; (C) line plot of allele frequencies with significant contributions for each population.

5.4. Discussion

In the present study, we investigated the population genetics of the Asian shore crab *Hemigrapsus takanoi* in its invaded European range, including the most recently established populations in the Baltic Sea. It provides insights in the invasion history of this species and its dispersal pathways along European coasts, including the likely existence of multiple introductions. For the Baltic Sea populations in particular, a secondary introduction from within Europe appears the most likely introduction pathway. In the following, we further discuss the results of our analysis and their implications for our understanding of the establishment and ongoing range extension of *H. takanoi* in Europe.

5.4.1. Genetic diversity and structure of European *H. takanoi* populations

Genetic diversity, measured as allelic richness, was similarly high in all sampled populations, only the Bay of Seine populations had a slightly reduced A_r compared to the other populations. It was notably not reduced in the Baltic Sea populations, thus giving no indication of a genetic bottleneck during this most recent range extension. The microsatellite markers proved to be highly polymorphic, with 12–35 alleles per locus over all populations. Seperated by populations, numbers of alleles per locus were about 50 % lower, but in ranges of values reported by Poux et al. (2015) when characterising these markers, indicating a generally high genetic diversity of *H. takanoi*.

The analyses of genetic structure revealed some weak, however surprising, population differentiation of *H. takanoi* in Europe. As indicated by the DAPC using populations as preassigned groupings, the Bay of Seine populations are cleary seperated from all other European populations. This is in accordance with a recent study by Makino et al. (2017), who found the same pattern when using another set of microsatellites, and strongly supports the existence of at least two independent introductions of *H. takanoi* to Europe (see below). All the remaining samples from the Bay of Biscay, the Wadden Sea and Baltic Sea are less differentiated from each other. The DAPC results support two sub-groups of genetically very closely related populations, one in the central Wadden Sea, and one in the northern Wadden Sea/Baltic Sea. The latter indicates the geographically closest area of the North Sea to be the likely source of the *H. takanoi* introduction to the Baltic Sea. Interestingly, the population on Texel, in the westernmost part of the Wadden Sea, shows some tendency of separation from the other populations, and especially a surprisingly high degree of separation from the geographically closest central Wadden Sea. This conincides with the high number of private alleles found in the TEX population. AMOVA results support these findings of genetic structure by weak, but significant differences detected between populations and seas, driven by the contrast between the Bay of Seine and all other populations. It should however be noted that within-population genetic differences were by far the greatest source of variance. This is also illustrated by results of the DAPC without preassignments to *a priori* defined groups, which did not resolve the individuals by geographic population structure.

One factor potentially obscuring the results is the prevalence of null alleles on all loci (estimated frequency 0.7–0.18). Null alleles are caused by mutations in the microsatellite flanking regions, hampering the binding of primers during PCR amplification, thus leading to loci falsely scored as homozygous. Accordingly, we detected the strongest deviations from Hardy-Weinberg expectations on loci with the highest frequency of null alleles, providing a likely explanation for the observed homozygote excess. Null alleles are also a likely cause for the almost complete failure to amplify two loci (Ht28 and Ht47) from the initial set of microsatellites. Chapuis & Estoup (2007) showed that the frequency of null alleles increases with increasing effective population size, as the probability for mutations in microsatellite flanking regions rises. For *H. takanoi*, it seems legible to assume a large effective population size, considering the rapid population growth and high densities the species reaches (van den Brink et al. 2012, Gothland et al. 2014, Geburzi et al. 2018), in combination with early maturity and the possibility of interbreeding between subsequent cohorts (Gothland et al. 2014). In this context, one should also consider the fact that the microsatellite markers used in this study were characterised on individuals from only one population in Dunkerque, northern France (Poux et al. 2015), thus depicting only a fraction of the acutal genetic diversity in European *H. takanoi* populations, especially under the assumption of multiple introductions.

The overall weak statistical support of genetic structuring between *H. takanoi* populations in Europe might well be a consequence of the very high genetic variability between single individuals, thus inflating within-population variation compared to variation on higher geographic levels. A potential reason for this is a strong underestimation of actual genetic diversity, caused by small sample sizes in relation to population sizes. This may lead to an underestimation of rare, though possibly informative, genotypes. The surprisingly high number of private alleles found in the recently detected PRI population in the Baltic Sea, which did otherwise not differentiate from the other Baltic Sea and Wadden Sea populations, might be a hint for this.

5.4.2. Implications for invasion history and dispersal pathways

Our microsatellite data provide evidence for at least two independent introductions of *H. takanoi* to Europe, by clearly separating the populations from the Bay of Seine from all other populations. The very same pattern has recently been described by Makino et al. (2017), who, based on haplotypes derived from mitochondrial COI data, identified the Bay of Seine populations as a genetic admixture of source populations from Japan and the Yellow Sea between China and the Korean peninsula. The population in the Bay of Biscay, which was in 1994 the first reported European population of *H. takanoi* (Noël et al. 1997), is genetically very similar to the North and Baltic Sea populations, despite the long geographical distance between them. This finding supports the early hypothesis of Gollasch (1999) that the founders of the Bay of Biscay population originate from the hull fouling community of the car-carrying vessel *SPICA*, from which he collected the first specimen of *H. takanoi* (then identified as *H. penicillatus*) when it was docked at the port of Bremerhaven (central Wadden Sea, Germany) in 1993.

It remains unclear, if the *H. takanoi* individuals transported with *SPICA* also established a population at or near Bremerhaven already in the mid 1990's, which then remained undetected until the next records of *H. takanoi* from that area about 10 years later (Obert et al. 2007). Alternatively, the rapid spread of *H. takanoi* over the Wadden Sea between 2004 and 2008 could be the consequence of a secondary introduction from the Bay of Biscay, or another independent primary introduction from the same (likely Japanese) source population. A third, and perhaps the most likely possibility is a mixture of the above alternatives, i.e. that a small population existed in the central Wadden Sea since the mid 1990's, but was enabled to the observed rapid population growth and range extension only after it received new propagule supply by repeated introductions in the mid 2000's. Such events of – sometimes extreme – range extensions following repeated introductions of an invader have already been described for several species (e.g. Geller et al. 1997, Simon-Bouhet et al. 2006, Herborg et al. 2007, Facon et al. 2008, Rius et al. 2015), and are attributed to heterosis effects and a general increase in genetic diversity (Roman & Darling 2007, Wagner et al. 2017).

In general, the chances for multiple introductions of *H. takanoi* to the southern North Sea region can assumed to be high, as several international ports in that area are well connected with ports in the northwestern Pacific native range of *H. takanoi* via highly frequented cargo shipping lines (Seebens et al. 2013). Our results raise at least suspicion that another introduction event might account for the slight genetic differentiation between the population on Texel and the rest of the Wadden Sea populations. A possible initial invasion site would be the Dutch delta system with the big ports of Rotterdam and Antwerp, as it was already suggested by Markert et al. (2014). From there, *H. takanoi* could have reached the western Wadden Sea by secondary anthropogenic transport or natural dispersal of larval stages with the prevailing northeasterly longshore currents (compare Wolff 2005).

The source of the Baltic Sea populations of *H. takanoi* is most likely the northern Wadden Sea, considering the very close genetic relationship we detected between these two regions. This study thus provides further evidence that the recent establishment of *H. takanoi* in the southwestern Baltic Sea is the result of a short-range secondary introduction rather than an independent long-range introduction from the native range of the crab. The Kiel Canal, connecting the northern Wadden and southwestern Baltic Sea likely served as introduction pathway, as already the first Baltic Sea records of *H. takanoi* in Kiel Fjord, on the eastern entrance to the canal, suggested (Geburzi et al. 2015). This highly frequented artificial waterway is one of the major pathways for non-native species introductions into the Baltic Sea (Leppäkoski et al. 2002, Jazdzewski & Grabowski 2011, Katsanevakis et al. 2013). The presumed source population of *H. takanoi* and its subsequent spread in the Baltic Sea furthermore suggest that recreational private boat traffic may serve as an important vector in this invasion process. This is based on the fact that such traffic regularly occurs between the southern North and Baltic Seas via the Kiel canal, and the observation that until now all records of *H. takanoi* in the Baltic Sea were made in marinas or their vicinity, rather than bigger ports (own observations). Recreational boating has repeatedly been shown to have a high potential for carrying secondary invasions (Darbyson et al. 2009, Clarke Murray et al. 2011, Kelly et al. 2013), and the future spread of *H. takanoi* further into the Baltic Sea might likely rely on crabs occasionally 'hitch-hiking' on sailing boats or motor yachts.

5.4.3. Conclusions and directions for future research

Our results shed some light on the genetic structure and invasion history of *Hemigrapsus takanoi* in Europe, confirming the recently described existence of two independent introductions from its native range (Makino et al. 2017), characterising the recent invasion of the Baltic Sea as a secondary introduction from European populations and finding hints for a possible third, formerly unrecognised primary introduction.

However, the nine microsatellite loci used in this study were able to reveal population genetic structure with only weak significance, presumably due to the prevalence of null alleles and the likely little representation of actual genetic diversity, as discussed above. Bigger sample sizes per population and inclusion of more microsatellite loci could overcome these problems in future studies, but would also strongly increase working effort. More 'conservative' markers, like mitochondrial COI sequences, on the other hand, seem to be variable enough in *Hemigrapsus spp.* to depict also contemporary genetic structuring, and might thus be of productive use in further studies (compare e.g. Petersen 2007, Hong et al. 2012, Markert et al. 2014, Lord & Williams 2016, Blakeslee et al. 2017, Makino et al. 2017).

The possibility of a third introduction of *H. takanoi* to the Dutch delta, which we assumed as a potential cause for the observed differentiation of the western Wadden Sea populations could be clarified with the analysis of more samples from that region. Likewise, the recently detected populations in Great Britain (Wood et al. 2015, Ashelby et al. 2017) should be included in future studies for a more complete picture of the invasion and dispersal history of *H. takanoi* in Europe.

Finally, the recent expansion of *H. takanoi* into the comparatively isolated Baltic Sea, which was detected at a very early invasion stage, offers a unique possibility to study an establishment process from its very beginning from both, a genetical and ecological point of view. In this context, our data, depicting the genetic composition of the *H. takanoi* population at a very early stage of the establishment process, may be of value for future studies of genetic evolution and adaptation.

Acknowledgements

We thank A. Goedknegt, D. Thieltges, J.P. Pezy and B. Lebreton for their great efforts in collecting and sending crabs from the Netherlands and France, N. Weiler, S. Wagner and M. Bauer for their help with crab collections in Germany, and W. Wranik for sharing records and localities of the first *H. takanoi* in Wismar Bight. We thank J. Valqui for the introduction into the lab routines, and C. Schubart and his working group for invaluable help in optimising lab routines for crustacean population genetics and the very fruitful discussions during two stays at Regensburg University. Thanks also to T. Jombart for making the relaxing power of cat videos available during population genetics analyses via ‘showmekittens()’ in his great R package adegenet. We further thank the Institute of Clinical Molecular Biology in Kiel for providing Sanger sequencing as supported in part by the DFG Cluster of Excellence "Inflammation at Interfaces" and "Future Ocean", and especially the technicians S. Greve, T. Naujoks, S. Arndt and T. Henke for technical support. JCG gratefully received financial support by a PhD stipend from the German Environmental Foundation (DBU), project number 20014/335.

References

Adamack, A.T. & Gruber, B. (2014): PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5 (4), 384–387, doi: 10.1111/2041-210X.12158.

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Ashelby, C.W., Sewell, J., Rostron, J., Shrubsole, R., Child, T. & Clark, P.F. (2017): Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90 (6), 695–708, doi: 10.1163/15685403-00003692.

Blakeslee, A.M.H., Kamakura, Y., Onufrey, J., Makino, W., Urabe, J., Park, S., Keogh, C.L., Whitman Miller, A., Minton, M.S., Carlton, J.T. & Miura, O. (2017): Reconstructing the Invasion History of the Asian shorecrab, *Hemigrapsus sanguineus* (De Haan 1835) in the Western Atlantic. *Marine Biology* 164, 47, doi: 10.1007/s00227-017-3117-x.

Breton, G., Faasse, M., Noël, P.Y. & Vincent, T. (2002): A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1), 184–189, doi: 10.1651/0278-0372(2002)022[0184:ANACIE]2.0.CO;2.

Brockerhoff, A.M. & McLay, C.L. (2011): Human-Mediated Spread of Alien Crabs. In: Galil, B.S., Clark, P.F. & Carlton, J.T. (eds.) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts.* Invading Nature – Springer Series in Invasion Ecology 6, Springer Netherlands, pp. 27–106, doi: 10.1007/978-94-007-0591-3_2.

Brookfield, J.F.Y. (1996): A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5 (3), 453–455, doi: 10.1046/j.1365-294X.1996.00098.x.

Cavalli-Sforza, L.L. & Edwards, A.W.F. (1967): Phylogenetic Analysis: Models and Estimation Procedures. *American Journal of Human Genetics* 19 (3), 233–57, doi: 10.2307/2406616.

Chan, F.T. & Briski, E. (2017): An overview of recent research in marine biological invasions. *Marine Biology* 164, 121, doi: 10.1007/s00227-017-3155-4.

Chapuis, M.-P. & Estoup, A. (2007): Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24 (3), 621–631, doi: 10.1093/molbev/msl191.

Clarke Murray, C., Pakhomov, E.A. & Therriault, T.W. (2011): Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distributions* 17 (6), 1161–1172, doi: 10.1111/j.1472-4642.2011.00798.x.

Covarrubias-Pazaran, G., Diaz-Garcia, L., Schlautman, B., Salazar, W. & Zalapa, J. (2016): Fragman: an R package for fragment analysis. *BMC Genetics* 17 (1), 62, doi: 10.1186/s12863-016-0365-6.

Cristescu, M.E. (2015): Genetic reconstructions of invasion history. *Molecular Ecology* 24 (9), 2212–2225, doi: 10.1111/mec.13117.

Darbyson, E., Locke, A., Hanson, J.M. & Willison, J.H.M. (2009): Marine boating habits and the potential for spread of invasive species in the Gulf of St. Lawrence. *Aquatic Invasions* 4 (1), 87–94, doi: 10.3391/ai.2009.4.1.9.

Dauvin, J.-C. (2009): Asian Shore Crabs *Hemigrapsus spp.* (Crustacea: Brachyura: Grapsoidea) continue their invasion around the Cotentin Peninsula, Normandy, France: Status of the *Hemigrapsus* population in 2009. *Aquatic Invasions* 4 (4), 605–611, doi: 10.3391/ai.2009.4.4.6.

Dauvin, J.-C. & Delhay, J.-B. (2010): First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern Cotentin, Normandy, western English Channel. *Marine Biodiversity Records* 3, 1–3, doi: 10.1017/S1755267210000928.

Dauvin, J.-C., Tous Rius, A. & Ruellet, T. (2009): Recent expansion of two invasive crab species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions* 4 (3), 451–465, doi: 10.3391/ai.2009.4.3.3.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992): Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131 (2), 479–491, doi: 10.1007/s00424-009-0730-7.

Facon, B., Pointier, J.P., Jarne, P., Sarda, V. & David, P. (2008): High Genetic Variance in Life-History Strategies within Invasive Populations by Way of Multiple Introductions. *Current Biology* 18 (5), 363–367, doi: 10.1016/j.cub.2008.01.063.

Geburzi, J.C., Brandis, D. & Buschbaum, C. (2018): Recruitment patterns, low cannibalism and reduced interspecific predation contribute to high invasion success of two Pacific crabs in northwestern Europe. *Estuarine, Coastal and Shelf Science* 200, 460–472, doi: 10.1016/j.ecss.2017.11.032.

Geburzi, J.C., Graumann, G., Köhnk, S. & Brandis, D. (2015): First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Records* 4 (2), 103–107, doi: 10.3391/bir.2015.4.2.06.

Geller, J.B., Darling, J.A. & Carlton, J.T. (2010): Genetic perspectives on marine biological invasions. *Annual Review of Marine Science* 2, 367–93, doi: 10.1146/annurev.marine.010908.163745.

Geller, J.B., Walton, E.D., Grosholz, E.D. & Ruiz, G.M. (1997): Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* 6, 901–906, doi: 10.1046/j.1365-294X.1997.00256.x.

Gittenberger, A., Rensing, M., Stegenga, H. & Hoeksema, B. (2010): Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33, 21–76.

Gollasch, S. (1999): The Asian decapod *Hemigrapsus penicillatus* (De Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgoländer Meeresuntersuchungen* 52, 359–366, doi: 10.1007/BF02908909.

Gollasch, S. (2006): Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60 (2), 84–89, doi: 10.1007/s10152-006-0022-y.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

Hänfling, B., Edwards, F. & Gherardi, F. (2011): Invasive alien Crustacea: dispersal, establishment, impact and control. *BioControl* 56, 573–595, doi: 10.1007/s10526-011-9380-8.

Herborg, L.-M., Weetman, D., van Oosterhout, C. & Hänfling, B. (2007): Genetic population structure and contemporary dispersal patterns of a recent European invader, the Chinese mitten crab, *Eriocheir sinensis*. *Molecular Ecology* 16 (2), 231–242, doi: 10.1111/j.1365-294X.2006.03133.x.

Holland, B.S. (2000): Genetics of marine bioinvasions. *Hydrobiologia* 420, 63–71, doi: 10.1023/A:1003929519809.

Holm, S. (1979): A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics* 6 (2), 65–70.

Hong, S.-E., Kim, J.-K., Yu, J.-N., Kim, K.-Y., Lee, C.I., Hong, K.E., Park, K.Y. & Yoon, M. (2012): Genetic variation in the Asian shore crab *Hemigrapsus sanguineus* in Korean coastal waters as inferred from mitochondrial DNA sequences. *Fisheries and Aquatic Sciences* 15, 49–56, doi: 10.5657/FAS.2012.0049.

Jazdzewski, K. & Grabowski, M. (2011): Alien Crustaceans Along the Southern and Western Baltic Sea. In: Galil, B.S., Clark, P.F. & Carlton, J.T. (eds.) *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts*. Springer Netherlands, Dordrecht, pp. 323–344, doi: 10.1007/978-94-007-0591-3.

Jombart, T., Devillard, S. & Balloux, F. (2010): Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11, 94, doi: 10.1186/1471-2156-11-94.

Jombart, T., Devillard, S., Dufour, A.B. & Pontier, D. (2008): Revealing cryptic spatial patterns in genetic variability by a new multivariate method. *Heredity* 101, 92–103, doi: 10.1038/hdy.2008.34.

Kamvar, Z.N., Tabima, J.F. & Grünwald, N.J. (2014): Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2, e281, doi: 10.7717/peerj.281.

Katsanevakis, S., Zenetos, A., Belchior, C. & Cardoso, A.C. (2013): Invading European Seas: Assessing pathways of introduction of marine aliens. *Ocean & Coastal Management* 76, 64–74, doi: 10.1016/j.ocecoaman.2013.02.024.

Kelly, N.E., Wantola, K., Weisz, E. & Yan, N.D. (2013): Recreational boats as a vector of secondary spread for aquatic invasive species and native crustacean zooplankton. *Biological Invasions* 15 (3), 509–515, doi: 10.1007/s10530-012-0303-0.

Kolar, C.S. & Lodge, D.M. (2001): Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16 (4), 199–204.

Kulhanek, S.A., Ricciardi, A. & Leung, B. (2011): Is invasion history a useful tool for predicting the impacts of the world’s worst aquatic invasive species? *Ecological Applications* 21 (1), 189–202, doi: 10.2307/29779646.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Lee, S., Lee, S.-K., Rho, H.S. & Kim, W. (2013): New Report of the Varunid Crabs, *Hemigrapsus takanoi* and *Sestrostoma toriumii* (Crustacea: Decapoda: Varunidae) from Korea. *Animal Systematics, Evolution and Diversity* 29 (2), 152–159, doi: 10.5635/ASED.2013.29.2.152.

Lejeusne, C., Saunier, A., Petit, N., Béguer, M., Otani, M., Carlton, J.T., Rico, C. & Green, A.J. (2014): High genetic diversity and absence of founder effects in a worldwide aquatic invader. *Scientific Reports* 4, 5808, doi: 10.1038/srep05808.

Leppäkoski, E., Gollasch, S., Gruszka, P., Ojaveer, H., Olenin, S. & Panov, V.E. (2002): The Baltic – a sea of invaders. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1175–1188, doi: 10.1139/f02-089.

Lord, J.P. & Williams, L.M. (2016): Increase in density of genetically diverse invasive Asian shore crab (*Hemigrapsus sanguineus*) populations in the Gulf of Maine. *Biological Invasions* 19 (4), 1153–1168, doi: 10.1007/s10530-016-1304-1.

Makino, W., Miura, O., Kaiser, F., Geffray, M., Katsube, T. & Urabe, J. (2017): Evidence of multiple introductions and genetic admixture of the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae) along the Northern European coast. *Biological Invasions* 20 (4), 825–842, doi: 10.1007/s10530-017-1604-0.

Marin, I.N. (2013): New data on the distribution of hairy-clawed shore crabs of the genus *Hemigrapsus* (Decapoda: Varunidae) along the Russian mainland coast of the Sea of Japan. *Russian Journal of Marine Biology* 39 (4), 301–305, doi: 10.1134/S106307401304007X.

Markert, A., Raupach, M.J., Segelken-Voigt, A. & Wehrmann, A. (2014): Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatus* (De Haan, 1835) versus *Hemigrapsus takanoi* Asakura & Watanabe 2005. *Organisms Diversity & Evolution*, doi: 10.1007/s13127-014-0176-4.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89 (3), 583–590.

Nijland, R. & Beekman, J. (2000): *Hemigrapsus penicillatus* de Haan 1835 waargenomen in Nederland. *Het Zeepaard* 60 (3), 169–171.

Noël, P.Y. & Gruet, Y. (2008): Progression du crabe introduit *Hemigrapsus takanoi* Asukura & Watanabe 2005 (Crustacé, Décapode) vers le nord du Golfe de Gascogne. *Bulletin de la Société des Sciences Naturelles de l'Ouest de la France, Nouvelle Série* 30, 141–148.

Noël, P.Y., Tardy, E. & D'Udekem d'Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Obert, B., Herly, M. & Grotjahn, M. (2007): First records of two crabs from the North West Pacific *Hemigrapsus sanguineus* and *H. takanoi* at the coast of Lower Saxony, Germany. *Wadden Sea Newsletter* 1, 21–22.

Paradis, E. (2010): Pegas: An R package for population genetics with an integrated-modular approach. *Bioinformatics* 26 (3), 419–420, doi: 10.1093/bioinformatics/btp696.

Petersen, C.H. (2007): Historical demography and contemporary spatial genetic structure of an estuarine crab in the northeast Pacific (*Hemigrapsus oregonensis*). *Marine Biology* 150 (6), 1289–1300, doi: 10.1007/s00227-006-0442-x.

Poux, C., Gothland, M., Holl, A.C., Spilmont, N. & Arnaud, J.F. (2015): Highly polymorphic microsatellite markers in two species, the invasive shore crabs *Hemigrapsus sanguineus* and *Hemigrapsus takanoi* (Decapoda, Varunidae). *Conservation Genetics Resources* 7 (2), 569–572, doi: 10.1007/s12686-015-0426-6.

Prevosti, A., Ocaña, J. & Alonso, G. (1975): Distances between populations of Drosophila subobscura, based on chromosome arrangement frequencies. *Theoretical and Applied Genetics* 45 (6), 231–241, doi: 10.1007/BF00831894.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000): Inference of population structure using multilocus genotype data. *Genetics* 155 (2), 945–59.

R Core Team (2017): R: A language and environment for statistical computing. Version 3.4.2, https://www.r-project.org/.

Rius, M., Turon, X., Bernardi, G., Volckaert, F.A.M. & Viard, F. (2015): Marine invasion genetics: from spatio-temporal patterns to evolutionary outcomes. *Biological Invasions* 17, 869–885, doi: 10.1007/s10530-014-0792-0.

Roman, J. & Darling, J.A. (2007): Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology and Evolution* 22 (9), 454–464, doi: 10.1016/j.tree.2007.07.002.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J. & Hines, A.H. (2000): Invasion of Coastal Marine Communities in North America: Apparent Patterns, Processes, and Biases. *Annual Review of Ecology and Systematics* 31, 481–531, doi: 10.2307/annurev.ecolsys.37.091305.30000016.

Seebens, H., Gastner, M.T. & Blasius, B. (2013): The risk of marine bioinvasion caused by global shipping. *Ecology Letters* 16 (6), 782–790, doi: 10.1111/ele.12111.

Simberloff, D., Martin, J.L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B.S., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. & Vilà, M. (2013): Impacts of biological invasions: What's what and the way forward. *Trends in Ecology and Evolution* 28 (1), 58–66, doi: 10.1016/j.tree.2012.07.013.

Simon-Bouhet, B., Garcia-Meunier, P. & Viard, F. (2006): Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: Evidence from mitochondrial sequence data. *Molecular Ecology* 15, 1699–1711, doi: 10.1111/j.1365-294X.2006.02881.x.

Van den Brink, A.M., Wijnhoven, S. & McLay, C.L. (2012): Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73, 126–136, doi: 10.1016/j.seares.2012.07.006.

Wagner, N.K., Ochocki, B.M., Crawford, K.M., Compagnoni, A. & Miller, T.E.X. (2017): Genetic mixture of multiple source populations accelerates invasive range expansion. *Journal of Animal Ecology* 86 (1), 21–34, doi: 10.1111/1365-2656.12567.

Wolff, W.J. (2005): Non-indigenous marine and estuarine species in The Netherlands. *Zoologische Mededelingen Leiden* 79 (1), 1–116.

Wood, C.A., Bishop, J.D.D., Davies, C.J., Delduca, E.L., Hatton, J.C., Herbert, R.J.H. & Clark, P.F. (2015): *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4 (2), 109–113, doi: 10.3391/bir.2015.4.2.07.

Yamasaki, I., Doi, W., Mingkid, W.M., Yokota, M., Strüssmann, C.A. & Watanabe, S. (2011): Molecular-Based Method To Distinguish the Sibling Species *Hemigrapsus* *penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of Crustacean Biology* 31 (4), 577–581, doi: 10.1651/10-3366.1.

Part IV

General discussion

The current status of *Hemigrapsus* populations in Europe

About 20 years after being recorded in Europe for the first time, *Hemigrapsus takanoi* and *H. sanguineus* are distributed along large parts of northwestern European coastlines. In several regions, particularly in the Wadden Sea and southern North Sea, they even became the dominating intertidal crab species.

Recently observed range extensions indicate that *Hemigrapsus spp.* have not yet reached their ultimate distribution in Europe. This is best illustrated by the establishment of *H. takanoi* in the Baltic Sea, which I report in this thesis. Records from > 100 km away from initial introduction site within two years after first discovery show that *H. takanoi* spreads similarly rapidly in the Baltic than in the North Sea and French Atlantic. The confirmation of successful reproduction by reconstructing its larval cycle in my following study, shows the species' (pre-)adaptation to the brackish conditions of the Baltic Sea. The potential of *H. takanoi* to inhabit such extreme environments is also known from its native range, where it regularly occurs in estuaries and is found at salinities as low as 7 PSU (Asakura & Watanabe 2005, Mingkid et al. 2006). Even though these records consider only adult crabs, and assuming that *H. takanoi* larvae rely on higher salinities to undergo development (this thesis), it seems legible to expect a further spread of *H. takanoi* eastwards along the southern Baltic Sea coast, end eventually also along the southern coast of Sweden. Thus, the observed invasion of *H. takanoi* in the Baltic Sea might just have been a matter of time, chance and the proper vector.

The population genetic analysis of *H. takanoi* included in this thesis revealed the Wadden Sea area as the most likely source for the introduction to the Baltic Sea. Given the largely sympatrical occurrence of *H. takanoi* and *H. sanguineus* in the Wadden Sea, the latter species can be assumed of having a similar likelihood to be transferred to the Baltic Sea. However, no *H. sanguineus* were encountered in the Baltic Sea during my studies, its invasion most likely

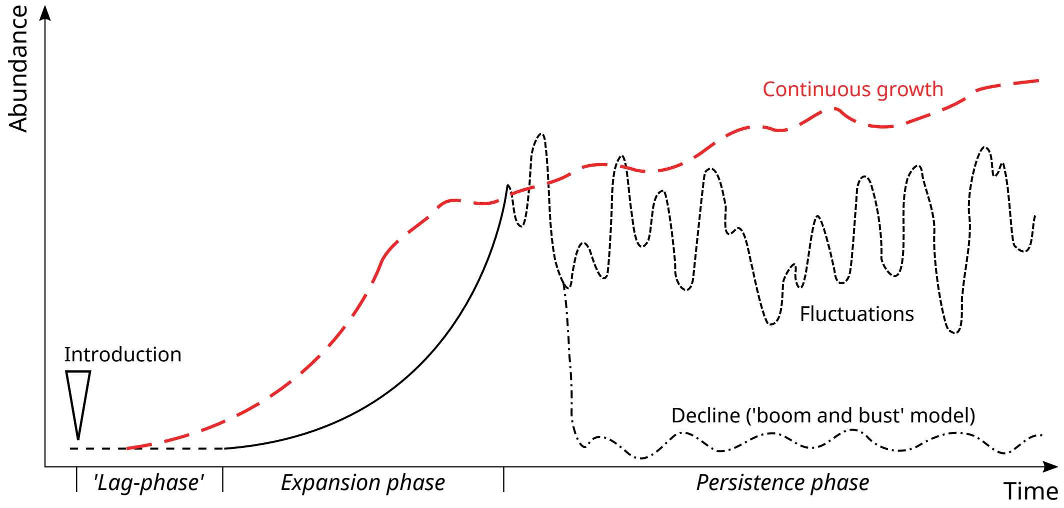


Fig. IV.5. Theoretical evolution of the abundances of introduced species. In the Wadden Sea, *Hemigrapsus spp.* (red line) seem to not pass into a persistance phase, but show continuous population growth (redrawn and modified after Boudouresque et al. 2005, Gothland et al. 2013)

being prevented by its higher salinity requirements (Epifanio et al. 1998). Nevertheless, the recent new records of *H. sanguineus* from Great Britain and the west coast of Sweden may be first indicators of a further northward spread around the British islands and Scandinavian west coast, both areas offering plenty of suitable habitat. Considering the wide latitudinal range over which *Hemigrapsus spp.* occur in their native range, both north- and southward future range extensions seem possible, as they were already hypothesised by Noël et al. (1997) and Breton et al. (2002) they for the first time reported populations of *Hemigrapsus spp.* in Europe.

Many invasion processes have been observed to follow a pattern of distinct phases from initial introduction to full establishment and spread. These include a lag time with small population sizes and very little or no population growth which may last up to several years or decades following the initial introduction (e.g. Crooks 2005, Lejeusne et al. 2014), before a phase of often exponential population growth. This expansion phase may then change over into a sharp decline to persistance at much lover density levels (‘boom and bust’ model), a relatively stable plateau on a high density level, or strong oscillations over time (Essink & Dekker 2002, Boudouresque et al. 2005, Gothland et al. 2013). This pattern does not seem to apply for *Hemigrapsus spp.* in the Wadden

Sea, as far as indicated by comparing my observations of their population development with earlier studies (e.g. Landschoff et al. 2013). Followed by only a very short lag time (if any at all), the populations of *Hemigrapsus spp.* rather appear to be continuously growing and spreading out, eventually culminating in the extremely high densities reported in this thesis (Fig. IV.5).

Several factors likely contribute to this conspicuous population development. Two questions I deem particularly interesting to follow. First, *Hemigrapsus spp.* might have developed ways of resource utilisation novel to the species community of the Wadden Sea, thus profiting from the high productivity of this ecosystem. Second, regular ‘supply’ with new propagules from outside the Wadden Sea (i.e. multiple invasions, either primary or secondary), which may lead to genetic admixture and in consequence higher potential for evolutionary adaptations (see below).

Irrespective of the underlying processes, the current state of *Hemigrapsus spp.* demonstrates that the Wadden Sea ecosystem is capable to sustain such extremely high densities of two new mid trophic level species without apparent detrimental effects on native or other non-native species. Ongoing studies are however needed to monitor future population development and to assess the long-term impacts of the presence of *Hemigrapsus spp.* in the Wadden Sea.

Factors of success

Several factors contributing to the invasion success of *Hemigrapsus spp.* are already known from earlier studies. These include high competitiveness on the level of adult crabs, high fecundity, and an overall strongly *r*-selected reproduction strategy (e.g. Jensen et al. 2002, van den Brink et al. 2012, Gothland et al. 2014). The results of my ecological investigations highlight the contributions of two formerly unrecognised factors to the success of *Hemigrapsus spp.* The first factor is the observed temporal shift of recruitment periods between *Hemigrapsus spp.* and the native *C. maenas*. The second factor are specific interaction patterns during early life history, notably the strong attractiveness of older conspecifics to recruiting crabs and the lack of cannibalism. Both factors together result in a powerful combination which enhances recruitment success and ultimately population growth by reducing inter- as well as intraspecific competition. The experimental results indicate that also mechanistic factors, like the observed interactions between invasive and native species belonging to the same ecological guild, should be considered in investigations of invasion processes, impacts, and risk assessments.

An interesting question which arose from the observed sharp temporal shift in recruitment periods between *Hemigrapsus spp.* and *C. maenas* is, whether this shift depicts the natural reproductive cycle or results from an adaptation of *Hemigrapsus spp.* in response to the presence of *C. maenas*. The latter case would be a striking case of rapid and profound evolutionary adaptation. Future research should be dedicated to reproductive cycles of *Hemigrapsus spp.* in their native ranges, as well as to comparative transcriptome analyses of genes involved in reproduction to clarify the background of this observation.

Invasion history, as inferred from population genetic studies, bears another set of success factors, notably multiple introductions and population admixture (e.g. Kulhanek et al. 2011, Cristescu 2015). The results of the microsatellite analysis of European *H. takanoi* populations included in this thesis reveal that both have occurred during this invasion process. My results confirmed the findings of the so far only other population genetic study on the invasion of *H. takanoi* with respect to the difference between the Bay of Seine population and all other European populations, suggesting an independent introduction to that area (compare Makino et al. 2017). Furthermore, they hint to a possible third independent introduction to the southern north sea and a generally more complex genetic structure of the North Sea populations, thus strongly encouraging further research.

Invasive species may profit from multiple introductions in several ways, some of which most likely also apply to *H. takanoi*. In particular, they increase propagule pressure, which can for example promote sudden expansion of formerly only small non-native populations, and they may lead to genetic admixture by bringing together individuals from different source populations. For *H. takanoi*, the latter appears to be the case in the Bay of Seine, and possibly also in the southern North Sea, and might have been one promoter for the species' rapid spread in this area in the mid 2000's.

Furthermore, genetic admixture in invasive populations can be directly linked to ecological traits. In this context, my observation of egg-carrying *H. sanguineus* females occurring almost year-round in the Wadden Sea, give rise to hypothesise an admixture effect, as such long periods of reproductive activity are neither described from the native, nor from other invaded ranges of the species. Thus, this observation might be the phenotypic expression of newly developed genotypes as a consequence of admixture from different source populations. To test this, a large scale comparative genetic study throughout the native range of *H. sanguineus* and the Wadden Sea populations would be needed.

Concluding remarks and outlook

Biological invasions are regularly seen negatively or at least sceptically by both, scientists and non-scientists. Especially the earlier phase of invasion biology research was dominated by alerting reports of solely negative impacts of invasive species, posing threats to biodiversity and economy, and culminating in the description of 'invasional meltdown' scenarios (e.g. Simberloff & Von Holle 1999, Bax et al. 2003, Grosholz 2005). Without neglecting that invasive species can in fact have extremely negative impacts, research in this field diversified during the last years, highlighting for example stabilising effects of invasive species in disturbed or species-poor ecosystems, 'neutral' outcomes of biological invasions or mutual adaptation and accommodation processes (e.g. Rodriguez 2006, Reise et al. 2017). This particularly applies to invasions in marine ecosystems, which, by their open nature, often seem to be both, highly vulnerable, but also highly integrative.

The establishment and dispersal of *Hemigrapsus takanoi* and *H. sanguineus* in German coastal waters, as judged by the results of this thesis and previous research, might be seen as an example for such a neutral or additive biological invasion. Nevertheless, this (and any new) invasion to the Wadden and Baltic Seas should be monitored intently, as impacts can change over time or vary strongly between ecosystems (Zenni & Nuñez 2013). However, given the rapid spread and massive population growth of the invading *Hemigrapsus spp.*, their case highlights a crucial problem when it comes to the management of invasive species in marine ecosystems: As soon as a species is fully established and begins to expand, attempts for eradication are usually in vain, especially in mobile species like crabs. Eradication attempts would also massively affect the whole community, eventually causing more harm than the invader they aim to eradicate (Zavaleta et al. 2001).

The results of this thesis still point out two aspects of rather 'indirect' invader management and prevention. First, the distribution of *H. sanguineus* in the Wadden Sea shows the importance of artificial structures, often literally serving as ‘stepping stones’ for invaders. Considering the observed strong preference of *H. sanguineus* for such artificial hard structures along the sedimentary Wadden Sea coast, a paradigm shift towards more ‘soft’ coastal defence, as suggested by Reise (2003), would most likely cause a strong decline of *H. sanguineus* and reduce the area of suitable habitat for this species. Second, the genetic data on *H. takanoi* indicate regional transfer, very likely by recreational boating, as introduction and dispersal vector into the Baltic Sea. Being highly underinvestigated, this vector however is believed to play an important role for the secondary spread of invasive species (Clarke Murray et al. 2011). The adoption of preventive measures in private and small-scale ship traffic might thus strongly reduce the number of regionally transferred organisms.

In summary, reproductive and early life-history traits, as well as invasion history and pathways have been identified in this thesis as important aspects for understanding the invasion success of *H. takanoi* and *H. sanguineus* in Europe. These aspects should therefore be considered in risk assessments dealing with possible further range extensions of these species. They might also be transferable to other ongoing, or potential future brachyuran invasions.

As further possible lines of research on the *Hemigrapsus* invasion in Europe, this thesis’ results suggest to broaden the scope of interaction and impact studies from native crabs to other species or the community level, and to study evolutionary and adaptive changes between different stages/timescales of invasion, especially in the light of the most recent range extensions. For both, the results of this study might serve as a valuable baseline.

References

Asakura, A. & Watanabe, S. (2005): *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25 (2), 279–292, doi: 10.1651/C-2514.

Bax, N.J., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. (2003): Marine invasive alien species: a threat to global biodiversity. *Marine Policy* 27 (4), 313–323.

Boudouresque, C.-F., Ruitton, S. & Verlaque, M. (2005): Large-Scale Disturbances , Regime Shift and Recovery in Littoral Systems Subject To Biological Invasions. In: *UNESCO-ROSTE/BAS workshop on regime shifts*. Ed. by V. Velikova & N. Chipev Varna, Bulgaria, pp. 85–101.

Breton, G., Faasse, M., Noël, P.Y. & Vincent, T. (2002): A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1), 184–189, doi: 10.1651/0278-0372(2002)022[0184:ANACIE]2.0.CO;2.

Clarke Murray, C., Pakhomov, E.A. & Therriault, T.W. (2011): Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distributions* 17 (6), 1161–1172, doi: 10.1111/j.1472-4642.2011.00798.x.

Cristescu, M.E. (2015): Genetic reconstructions of invasion history. *Molecular Ecology* 24 (9), 2212–2225, doi: 10.1111/mec.13117.

Crooks, J.A. (2005): Lag times and exotic species : The ecology and management of biological invasions. *Ecoscience* 12 (3), 316–329.

Epifanio, C.E., Dittel, A.I., Park, S., Schwalm, S. & Fouts, A. (1998): Early life history of *Hemigrapsus sanguineus*, a non-indigenous crab in the Middle Atlantic Bight (USA). *Marine Ecology Progress Series* 170, 231–238, doi: 10.3354/meps170231.

Essink, K. & Dekker, R. (2002): General patterns in invasion ecology tested in the Dutch Wadden Sea: The case of a brackish-marine polychaetus worm. *Biological Invasions* 4, 359–368, doi: 10.1023/a:1023692825663.

Gothland, M., Dauvin, J.-C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.-P., Tous Rius, A. & Spilmont, N. (2014): Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142, 41–49, doi: 10.1016/j.ecss.2014.03.012.

Gothland, M., Dauvin, J.-C., Denis, L., Jobert, S., Ovaert, J., Pezy, J.-P. & Spilmont, N. (2013): Additional records and distribution (2011-2012) of Hemigrapsus sanguineus (De Haan , 1835) along the French coast of the English Channel. *Management of Biological Invasions* 4 (4), 305–315, doi: 10.3391/mbi.2013.4.4.0.

Grosholz, E.D. (2005): Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences* 102 (4), 1088–1091, doi: 10.1073/pnas.0308547102.

Jensen, G.C., McDonald, P.S. & Armstrong, D.A. (2002): East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp. Marine Ecology Progress Series* 225, 251–262, doi: 10.3354/meps225251.

Kulhanek, S.A., Ricciardi, A. & Leung, B. (2011): Is invasion history a useful tool for predicting the impacts of the world's worst aquatic invasive species? *Ecological Applications* 21 (1), 189–202, doi: 10.2307/29779646.

Landschoff, J., Lackschewitz, D., Kesy, K. & Reise, K. (2013): Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions* 8 (1), 77–87, doi: 10.3391/ai.2013.8.1.09.

Lejeusne, C., Saunier, A., Petit, N., Béguer, M., Otani, M., Carlton, J.T., Rico, C. & Green, A.J. (2014): High genetic diversity and absence of founder effects in a worldwide aquatic invader. *Scientific Reports* 4, 5808, doi: 10.1038/srep05808.

Makino, W., Miura, O., Kaiser, F., Geffray, M., Katsube, T. & Urabe, J. (2017): Evidence of multiple introductions and genetic admixture of the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae) along the Northern European coast. *Biological Invasions* 20 (4), 825–842, doi: 10.1007/s10530-017-1604-0.

Mingkid, W.M., Akiwa, S. & Watanabe, S. (2006): Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo Bay. *Crustaceana* 79 (9), 1107–1121.

Noël, P.Y., Tardy, E. & D'Udekem d'Acoz, C. (1997): Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Sciences* 320 (9), 741–745, doi: 10.1016/S0764-4469(97)84823-8.

Reise, K. (2003): More Sand to the Shorelines of the Wadden Sea: Harmonizing Coastal Defense with Habitat Dynamics. In: Wefer, G., Lamy, F. & F, M. (eds.) *Marine Science Frontiers for Europe.* Springer, Berlin, Heidelberg, pp. 203–216, doi: 10.1007/978-3-642-55862-7_13.

Reise, K., Buschbaum, C., Büttger, H. & Wegner, K.M. (2017): Invading oysters and native mussels: From hostile takeover to compatible bedfellows. *Ecosphere* 8 (9), e01949, doi: 10.1002/ecs2.1949.

Rodriguez, L.F. (2006): Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions* 8 (4), 927–939, doi: 10.1007/s10530-005-5103-3.

Simberloff, D. & Von Holle, B. (1999): Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1, 21–32.

Van den Brink, A.M., Wijnhoven, S. & McLay, C.L. (2012): Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73, 126–136, doi: 10.1016/j.seares.2012.07.006.

Zavaleta, E.S., Hobbs, R.J. & Mooney, H.A. (2001): Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution* 16 (8), 454–459, doi: 10.1016/S0169-5347(01)02194-2.

Zenni, R.D. & Nuñez, M.A. (2013): The elephant in the room: The role of failed invasions in understanding invasion biology. *Oikos* 122, 801–815, doi: 10.1111/j.1600-0706.2012.00254.x.

Eidesstattliche Erklärung

Hiermit erkläre ich,

Jonas C. Geburzi,

dass ich diese Doktorarbeit selbstständig verfasst sowie alle wörtlichen und inhaltlichen Zitate als solche gekennzeichnet habe.

Die Arbeit wurde unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft verfasst.

Sie hat weder ganz noch in Teilen einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen.

Kiel, den 09.01.2018