Ecology and ecophysiology of invasive and native decapod crabs in the southern North Sea

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Dissertation

in fulfilment of the requirements for the
doctoral degree in Natural Sciences (Dr. rer. nat.)

at the Faculty 02 - Biology and Chemistry
of the University of Bremen, Germany

September 2017

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Date of colloquium	01 December 2017, 3 pm
Room	NW2 C0300, University of Bremen

Dedicated to Nadine, Alex, and Dominik

Contents

Summary

The Asian shore crab *Hemigrapsus sanguineus* is native to the coasts of China, Korea and Japan. It first invaded the U.S. east coast in the late 1980s and its present range extends from North Carolina to Maine. It was detected in France and the Netherlands in 1999. Nowadays, *H. sanguineus* is fully established at the European Atlantic coast. It can be found from the Contentin Peninsula, France to the German Wadden Sea, including the rocky island of Helgoland. In the U.S. and in Europe, *H. sanguineus* shares habitats with the native European green crab *Carcinus maenas* and competitive interactions between both are suggested. Thus, the main aims of this thesis were 1) to evaluate the status of *H. sanguineus* and *C. maenas* around Helgoland in relation to wave action, 2) to compare the respiratory metabolism and energy demand of both species, and 3) to assess their energy storage capacities and dietary preferences.

Four sites around the island of Helgoland were surveyed for the abundances of *C. maenas* and *H. sanguineus* in 2009 and 2014. Numbers of *H. sanguineus* rose from 2009 to 2014, overall resembling those of *C. maenas* in 2014. While *C. maenas* clearly outnumbered *H. sanguineus* at the most wave-exposed sites, the opposite case was found at the most sheltered locations. At this sheltered site, up to 144 specimens of *H. sanguineus* were found per square meter - the highest density found in Europe so far. The dominance of the invader at this site was partially explained by the ‘gregarious settlement’ behavior of *H. sanguineus* megalopae. This species is known to settle preferentially, where the larvae recognize waterborne cues of adult conspecifics. Compared to their North American invasion history, *H. sanguineus* around Helgoland were in their ‘middle phase’ of invasion and the abundance is likely to continue to increase in the future.

The respiratory performance of *C. maenas* and *H. sanguineus* was compared at different temperatures. At higher temperatures, small and medium-sized *H. sanguineus* consumed significantly more oxygen than *C. maenas* of comparable masses. Species-specific numerical models for the calculation of respiration rates using individual mass and the water temperature were established and applied to ultimately calculate the carbon and energy demands of both species. Abundance and biomass of *H. sanguineus* around Helgoland was about 21% and 59% of the values for *C. maenas*, respectively. The annual energy demand, however, was about 76 to 119%, depending on the degree of presumed herbivory or carnivory of both species. *C. maenas* retreats to the subtidal or burrows in the winter months, whereas *H. sanguineus* is present in the intertidal throughout the winter. *H. sanguineus*, thus represents a winter predation pressure that was not present in the intertidal areas prior to its invasion.

Facing increasing abundances in the future, the overall energetic impact of the *H. sanguineus* population will increase.

The energy storing capacities of *C. maenas* and *H. sanguineus* were examined by extracting lipids from their midgut glands. Total lipid contents of *H. sanguineus* were higher than for *C. maenas* and showed a strong seasonality, whereas *C. maenas* values did not reveal this pattern. These results were explained by the much higher lipid demand of *H. sanguineus* for reproduction. *H. sanguineus* produces more eggs annually, which are better equipped with lipids compared to *C. maenas*. The midgut gland samples were subsequently analyzed using fatty acid trophic biomarker indices. The indices indicating the consumption of Bacillariophyceae, Chlorophyta, and especially Phaeophyceae were higher in *H. sanguineus*, suggesting an overall higher degree of herbivory. In contrast, indices for Rhodophyta and carnivory were higher in *C. maenas*. Thus, competition for food might be low in a habitat rich in macroalgae but is probably stronger in habitats with low macroalgae abundances. Utilizing mainly energy-poor algae material to synthetize energy-rich lipid stores might be a beneficial adaptation of *H. sanguineus* to successfully establish as an invading species.

In conclusion, *H. sanguineus* is a successful invader around the island of Helgoland and will most likely continue to increase in abundance in the future. A key feature of being successful may be the ability to produce many high-quality offspring by utilizing low-quality, but virtually unlimited food resources. *H. sanguineus* thereby shows a high energy demand which it needs to cover via food intake. In habitats where competitive interactions for food are strong, effects on the native community may be expected.

Zusammenfassung

Die Asiatische Felsenkrabbe *Hemigrapsus sanguineus* ist an den Küsten Chinas, Koreas und Japans einheimisch. Ab 1980 wanderte sie an der US-Ostküste ein, wo die Verbreitung nun von North Carolina im Süden bis Maine im Norden reicht. *H. sanguineus* wurde schließlich 1999 in Frankreich und den Niederlanden entdeckt. Heutzutage ist *H. sanguineus* an der europäischen Atlantikküste voll etabliert. Sie kann von der Contentin-Halbinsel Frankreichs bis zum deutschen Wattenmeer und auch auf der Felseninsel Helgoland gefunden werden. In den USA und in Europa teilt sich *H. sanguineus* das Habitat mit der einheimischen Europäischen Strandkrabbe *Carcinus maenas*. Kompetitive Interaktionen zwischen den beiden Arten sind bereits beschrieben worden. Die Hauptziele dieser Doktorarbeit waren daher: 1) den Status von *H. sanguineus* und *C. maenas* um Helgoland herum in Bezug auf Wellen-Exposition zu erheben, 2) den respiratorischen Metabolismus und den Energiebedarf beider Arten zu vergleichen und 3) die Fähigkeiten zur Energiespeicherung und die Nahrungspräferenzen zu erheben.

Vier Stellen um Helgoland herum wurden 2009 und 2014 in Bezug auf Abundanz von *C. maenas* und *H. sanguineus* hin untersucht. Die Zahlen von *H. sanguineus* stiegen von 2009 nach 2014. In 2014 war *H. sanguineus* ähnlich häufig wie *C. maenas*. *C. maenas* übertraf *H. sanguineus* zahlenmäßig an der Stelle mit der höchsten Wellenexposition, während der gegenteilige Fall an der geschütztesten Stelle auftrat. Dort wurden bis zu 144 Tiere pro Quadratmeter gefunden - die höchste bislang gemessene Dichte in Europa. Die Dominanz von *H. sanguineus* im geschützten Bereich wurde teilweise durch die Ansiedlungsstrategie der Megalopa-Larven von *H. sanguineus* erklärt. Die Larven siedeln sich bevorzugt dort an, wo sie wasserlösliche Geruchsstoffe von Adulten wahrnehmen können. Im Vergleich zu Nordamerika befindet sich *H. sanguineus* um Helgoland herum in der „mittleren“ Invasionsphase und es wird erwartet, dass die Abundanz in der Zukunft weiter ansteigt.

Die respiratorische Leistungsfähigkeit von *C. maenas* und *H. sanguineus* wurde bei verschiedenen Temperaturen experimentell untersucht. Kleine und mittlere *H. sanguineus* haben bei höheren Temperaturen mehr Sauerstoff verbraucht als vergleichbar schwere *C. maenas*. Artspezifische Regressionsmodelle wurden für die Berechnung des Sauerstoffverbrauchs erstellt, auf Basis von individueller Körpermasse und der Wassertemperatur. Mit Hilfe dieser Modelle wurde der Kohlenstoff- und Energieverbrauch von beiden Arten ermittelt. Abundanz und Biomasse der *H. sanguineus*-Population um Helgoland herum betrug jeweils 21% und 59% des Wertes für *C. maenas*. Der jährliche Energiebedarf betrug jedoch zwischen 76 und 119%, je nach Grad der Herbivorie, bzw.

Karnivorie der beiden Arten. *C. maenas* zieht sich im Winter in subtidale Bereiche zurück oder vergräbt sich im Sand, während *H. sanguineus* das ganze Jahr über im Gezeitenbereich vorkommt. *H. sanguineus* übt demnach im Winter einen Fraßdruck aus, der vor der Einwanderung dieser Art im Gezeitenbereich nicht vorhanden war. Wenn die Abundanz von *H. sanguineus* in der Zukunft weiter steigt, kann also auch erwartet werden, dass der energetische Gesamteinfluss dieser Art auf den Gezeitenbereich weiter zunimmt.

Die Fähigkeit von *C. maenas* und *H. sanguineus* Energie zu speichern wurde über die Extraktion von Lipiden aus der Mitteldarmdrüse beider Arten ermittelt. Lipidgehalte von *H. sanguineus* waren höher als bei *C. maenas* und einer starken Saisonalität unterworfen, was bei *C. maenas* nicht der Fall war. Für *H. sanguineus* wurden diese Ergebnisse mit dem sehr hohen Lipidbedarf für die Reproduktion erklärt. Diese Art produziert jährlich deutlich mehr und lipidreichere Eier als *C. maenas*. Im Anschluss wurden die Lipidproben der Mitteldarmdrüsen anhand von trophischen Fettsäuremarker-Indices untersucht. Die Indices, die auf den Verzehr von Diatomeen, Grünalgen und besonders von Braunalgen hinweisen, waren höher in *H. sanguineus*. Dies lässt auf einen insgesamt höheren Grad an Herbivorie schließen. Im Gegensatz dazu waren die Werte des Rotalgen- und des Karnivorie-Index in *C. maenas* höher. Die Konkurrenz um Futter scheint somit gering zu sein, wenn das Habitat reich an Makroalgen ist. In Gebieten mit wenigen Makroalgen ist die Konkurrenz wahrscheinlich höher. Die Verwendung von energiearmem Algenmaterial zur Synthese von energiereichen Lipidspeichern ist möglicherweise ein Vorteil von *H. sanguineus*, der zum Invasionserfolg dieser Art beiträgt.

Zusammenfassend betrachtet ist *H. sanguineus* im Bereich von Helgoland eine erfolgreiche invasive Art und es ist wahrscheinlich, dass ihre Abundanz in der Zukunft weiter zunimmt. Ein entscheidender Vorteil scheint dabei zu sein, dass diese Art in der Lage ist, energiearme, aber unlimitierte Nahrung zu nutzen, um zahlreiche sowie energiereiche Nachkommen zu erzeugen. Dabei weist *H. sanguineus* einen hohen Energiebedarf auf, der über die Nahrungsaufnahme gedeckt werden muss. In Gebieten, wo die Nahrungskonkurrenz zu anderen Arten hoch ist, könnten somit Effekte auf die einheimische Artengemeinschaft erwartet werden.

1 Introduction

1.1 Biological invasions: Definitions and importance

‘Non-indigenous’ or ‘alien’ species are species that occur in habitats, which they did not inhabit in their original, ‘native’ distribution and which are geographically separated from their native habitat. In contrast, so called ‘range-expanding’ species colonize new habitats, which are geographically in direct vicinity to their native areas. The transport into a new, geographically distant habitat is often facilitated by human activity such as shipping in ballast water or accidental co-introduction with species of aquaculture purpose (Hellmann et al. 2008, Prentis et al. 2008).

In cases, where a non-indigenous species was proven to have any adverse effect on biodiversity or ecosystem services in its new habitat, it may be regarded as an ‘invasive’ species (Sakai et al. 2001 and references therein, Colautti and MacIsaac 2004 and references therein, EU 2014). Thus, by far not all non-indigenous species are necessarily invasive and non-indigenous species can be invasive in some regions but not in others. However, it is possible that a non-indigenous species sooner or later becomes invasive in its new habitat (Sakai et al. 2001 and references therein).

Biological invasions are seen as a major driver of ecological change worldwide (Didham et al. 2005). Thus, they have enormous impacts on a variety of ecosystems (Vitousek et al. 1997) and were identified as one of the major threats to marine biodiversity (e.g. Grosholz 2002). Generally, non-indigenous species potentially alter community structures in invaded habitats and may negatively influence ecosystem functioning (Ruiz et al. 2000). In habitats, where the impact of an invader is high, the impact of invasive species may i) drive native competitors to local extinction and threat ii) ecosystem services, iii) human economic interest and iv) human health (Ruiz et al. 2000, Gurevitch and Padilla 2004, Simberloff et al. 2013).

Of the four threats listed above, local extinction caused by invasive species is the only threat that does not seem to apply for marine habitats. None of the 21 described modern extinctions in the marine realm, which were reported by the IUCN Red List, were attributed to the impact of invasive species alone. However, 11.8% of the native marine species are considered critically endangered due to the impact of alien species, among other causes. (Gurevich and Padilla 2004). For the three other threats mentioned above, many examples from the marine realm underline the importance of scientific research on biological invasions. Overall, due to these adverse effects on socio-economics and the almost exponential increase in reported introductions in aquatic and terrestrial habitats over the last 200 years, the body of available literature grew at almost the same rate (Kennedy et al. 2002).

1.2 Invasive crustaceans

Crustaceans comprise the most non-native species in the majority of aquatic habitats (e.g. Ruiz et al. 2000, Leuven et al. 2009). One explanation for this may be that most aquatic introduction vectors have the ability to carry crustaceans. The large spectrum of life styles, physiological abilities, and reproductive strategies enables crustaceans to withstand many adverse conditions they may be subjected to during the transition (Carlton 2011). In fact, any crustacean group is able to enter a new habitat via several different vectors. Many crustacean taxa, including Cirripedia, Copepoda and Decapoda, can be transported by all seven categories of human-mediated vectors as classified by Carlton (2011): vessels and other watercraft; maritime activities (equipment, sea planes, commerce); movement, holding and release of living organisms including associated biota; contaminated gear and footwear; marsh restoration; floating plastic debris; and canals. Even the least successful invading crustacean taxa were proven to be transported by three of the seven vector categories: e.g. Euphausiacea, Branchiura and Cephalocarida.

The current knowledge identifies the groups Decapoda, Amphipoda and barnacle-like Cirripedia as the major crustacean groups, which frequently invade new ecosystems. However, small and/or cryptogenic groups such as Copepoda or Mysidacea are most likely underestimated and underreported invaders (Carlton 2011). In particular, brachyuran and crab-like anomuran decapod crabs are commonly found invasive species all around the globe (Ruiz et al. 2000, Brockerhoff and McLay 2011). In total, 73 decapod species (as of 2011) are considered non-native and established a stable population in their new habitats. Of these, two brachyuran crabs, the European green crab *Carcinus maenas* (Linnaeus 1758) and the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards 1853, are members of the global list of the 100 "worst" invading species (from the Global Invasive Species Database, Brockerhoff and McLay 2011).

This thesis focuses on two brachyuran decapod species, the native European green crab *C. maenas* and the invasive Asian shore crab *Hemigrapsus sanguineus* (De Haan 1835) in the North Sea. Both species are sharing similar habitats in European waters since the introduction and spread of *H. sanguineus* in 1999.

1.3 *Carcinus maenas*

The European green crab *Carcinus maenas* (Fig. 1.1) has its native range in north-east Atlantic waters and the adjacent seas: north-west Europe (up to 70° N), northern Africa, North Sea, and Baltic Sea (Carlton and Cohen 2003). *C. maenas* is a well-known and highly invasive species, which made it on the list of the 100 "worst" invaders worldwide (Global Invasive Species Database, Brockerhoff and McLay 2011). In fact, *C. maenas* represents a case, where presumably many vectors transported larvae or juveniles into new habitats (Klassen and Locke 2007).

Fig. 1.1: The European green crab *Carcinus maenas*, dorsal view, photo: S. Jungblut.

C. maenas was introduced already in 1817 to the southern coast of Massachusetts, USA, from where it spread further north and south (Fig. 1.2, Carlton and Cohen 2003). It invaded the tip of Cape Cod in 1872, Boston and Maine by 1893 and Nova Scotia, Canada, by 1953 (Carlton and Cohen 2003). With increasing latitude, the northward invasion seemed to slow down. In 1998, *C. maenas* was detected at the shores of the Gulf of St Lawrence in Canada (Carlton and Cohen 2003). South of the initial introduction site, *C. maenas* is known to be established as far as Delaware Bay, USA. Chesapeake Bay is not infested (Carlton and Cohen 2003).

The first specimen of *C. maenas* on the US west coast was found in 1989 in San Francisco Bay, California (Fig. 1.2, Cohen et al. 1995, Brockerhoff and McLay 2011). By now, *C. maenas* is well established in this area and also further north up to Vancouver Island, Canada (from 1999 on, Carlton and Cohen 2003, Behrens Yamada and Gillespie 2008). South of San Francisco Bay, *C. maenas* is established at shores as south as Monterey Bay (Grosholz and Ruiz 1995, Carlton and Cohen 2003).

Established populations of *C. maenas* can also be found in Australia. First records were made in the area of Melbourne, Victoria, in 1877. Now, the invader can be found from Adelaide, South Australia, through Victoria, to Narooma in New South Wales (Carlton and Cohen 2003).

Carlton and Cohen (2003 and references therein) summarized records of *C. maenas* in areas, where the species arrived but subsequently did not establish successfully: the Red Sea around 1817, the Bay of Panama and Sri Lanka in 1866, the area of Rio de Janeiro in 1857, the eastern tip of Brazil (Pernambuco) around 1899, Madagascar in 1922, Myanmar in 1933, the western tip of Australia (Perth) in 1965, in Pakistan in 1971, in South Africa in 1983 and in Japan in 1984 (Fig. 1.2). The latter two introductions, however, were managed by hybrids of *C. maenas* and its sibling species *C. aestuarii* Nardo 1847, which is native in the Mediterranean Sea.

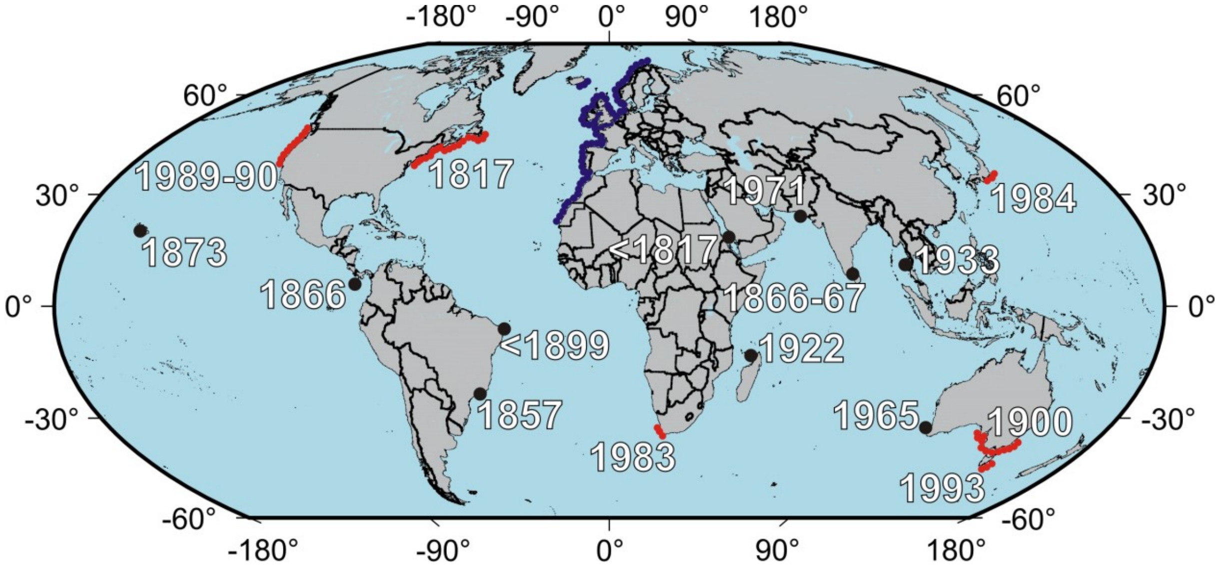

Fig. 1.2: Worldwide distribution of *Carcinus maenas*. Native (blue bands) and invaded (red bands) areas as well as one-time collections (black circles), including the reported years (after Carlton and Cohen 2003, Blakeslee et al. 2010). This map was produced and adapted using the software GMT 5.3.1 and CorelDRAW X4.

1.4 *Hemigrapsus sanguineus*

The Asian shore crab *Hemigrapsus sanguineus* (Fig. 1.3) is native to the coasts of southeastern China, Korea, Japan and the Russian Sakhalin Island (Fig. 1.4, Fukui 1988, Stephenson et al. 2009). It invaded the US east coast from the late 1980s on and is currently found from Cape Hatteras in North Carolina to the Schoodic Peninsula in Maine (Williams and McDermott 1990, Delaney et al. 2008, Epifanio 2013). The first specimens were found in Europe at the French and Dutch Atlantic coasts in the late 1990s (Breton et al. 2002).

Populations are nowadays established from the Contentin Peninsula, France to the German Wadden Sea (Obert et al. 2007, Gothland et al. 2013, Landschoff et al. 2013). Both major invasions, western and eastern Atlantic, are relatively well documented, because the first specimens were found soon after the hypothetical introductions.

Fig. 1.3: The Asian shore crab *Hemigrapsus sanguineus*, dorsal view, photo: S. Jungblut

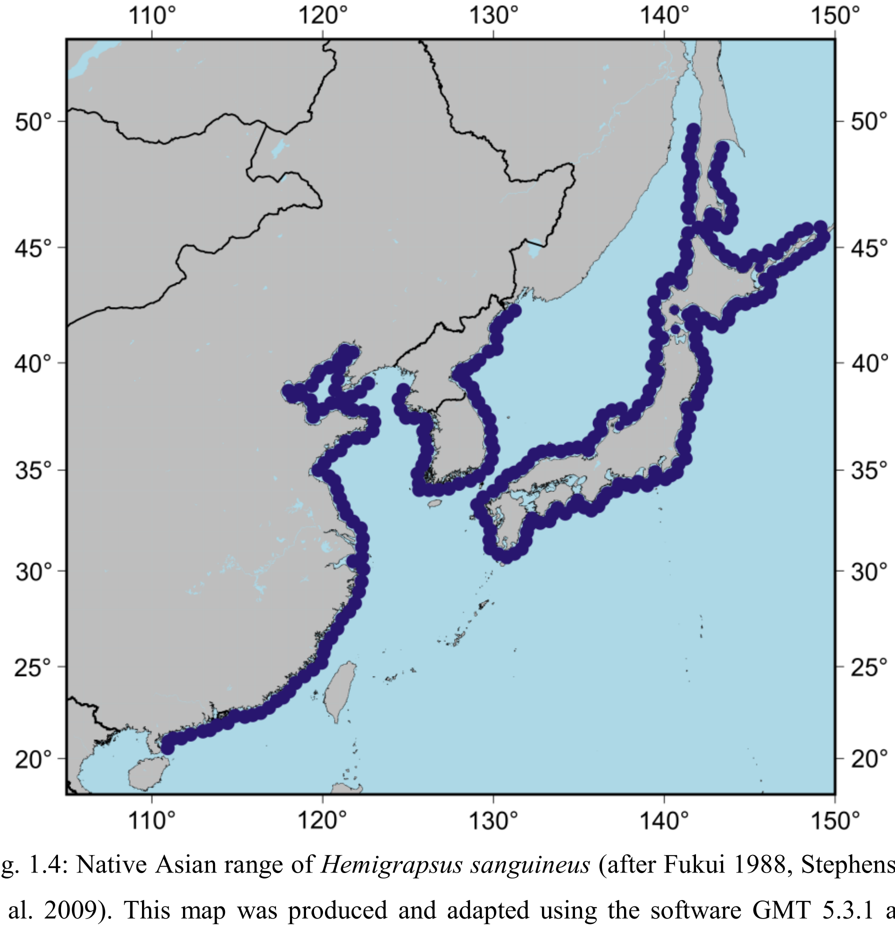

Fig. 1.4: Native Asian range of *Hemigrapsus sanguineus* (after Fukui 1988, Stephenson et al. 2009). This map was produced and adapted using the software GMT 5.3.1 and CorelDRAW X4.

1.4.1 *H. sanguineus* in the United States of America

In the USA, *H. sanguineus* was initially found north of the mouth of Delaware Bay, at Townsends Inlet, New Jersey, in 1988 (Williams and McDermott 1990). Six years later, in 1994, the invader already reached Long Island Sound, Connecticut (McDermott 1998). In 1992, *H. sanguineus* was already found even further north, at Woods Hole, Massachusetts, but the abundances were still low until 1994 (O'Connor 2014) (Fig. 1.5).

At a site close to Townsends Inlet, *H. sanguineus* established rapidly and comprised over 75% of the local crab abundance and biomass in 2001. In the years 2011 and 2012, *H. sanguineus* was found to have decreased at this site and the native Atlantic mud crab *Panopeus herbstii* became dominant, comprising about 80% of the total crab abundance (Schab et al. 2013). For the time being, this is the only available report on a decreasing *H. sanguineus* population. This case may provide some local evidence for the rarely observed 'boom and bust' model (Williamson 1996). Apart from this, *H. sanguineus* remained to be the dominant crab species at Townsends Inlet, where coarse sediments could probably have hindered mud crabs to re-establish (Schab et al. 2013).

In Long Island Sound, the population of *H. sanguineus* was monitored from 1998 to 2005 (Kraemer et al. 2007). After two years of similar abundances of *H. sanguineus* and the co-occurring flat back mud crab *Eurypanopeus depressus* (together 99% of crab abundance), the population of the latter declined dramatically and did not reach values higher than 1% for the last years. This study also reports the highest abundances of *H. sanguineus* ever recorded in the U.S. range with about 305 ind. m^{-2} (Kraemer et al. 2007).

O'Connor (2014) observed *H. sanguineus* populations in Massachusetts and Rhode Island over a period of 12 years (1998 to 2010). Thereby, the invasion of *H. sanguineus* was divided in three major phases: 'early', 'middle' and 'late'. In 1998 and 1999, the 'early' phase, the abundances of *H. sanguineus* were lower than those of resident mud crabs of the Panopeidae-family and the European green crab *Carcinus maenas*. This phase was followed by similar abundances in the year 2000 ('middle' phase) and finally remarkably higher abundances of *H. sanguineus* between 2001 and 2010 ('late' phase). In the late phase, *H. sanguineus* showed abundances close to 200 ind. m^{-2}, whereas mud crabs remained lower than 5 ind. m^{-2} (O'Connor 2014).

North of Cape Cod, *H. sanguineus* numbers decreased with increasing latitude. This decline was attributed to temperature limitations of the larval development (Delaney et al. 2008, Stephenson et al. 2009). However, latest research revealed overall increases in abundance also in this area and suggested increasing temperatures to be a main driver of *H. sanguineus*' spread further north (Lord and Williams 2017).

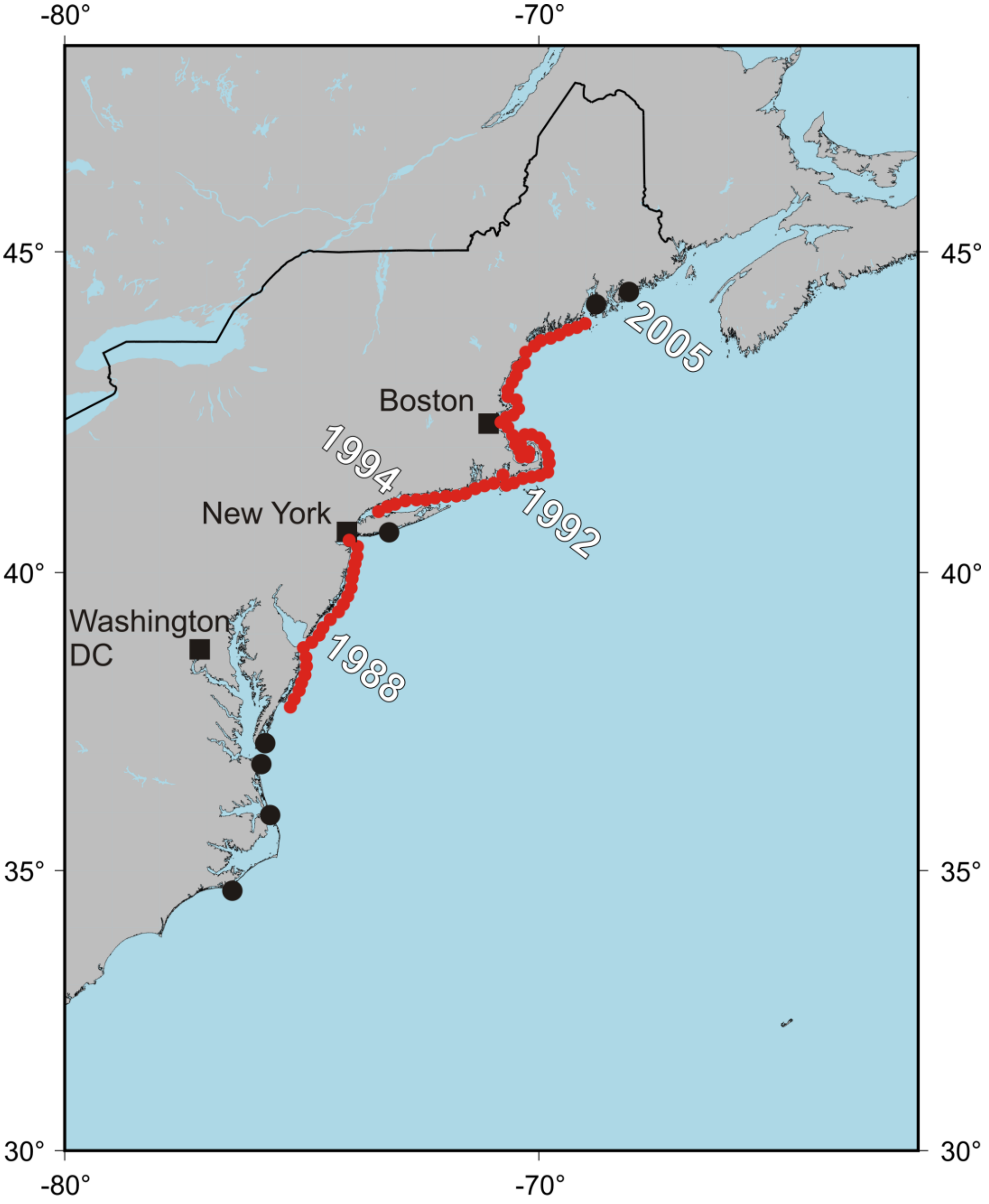

Fig. 1.5: Distribution of *Hemigrapsus sanguineus* in the invaded U.S. east coast range (red band). Black circles mark one-time findings. For references see text. This map was produced and adapted using the software GMT 5.3.1 and CorelDRAW X4.

1.4.2 *H. sanguineus* in Europe

In August 1999, *H. sanguineus* was found in the harbor of Le Havre, France and in the Oosterschelde estuary, The Netherlands (Fig. 1.6, Breton et al. 2002). The Dutch Wadden Sea was reached in 2004 (Gittenberger et al. 2010). The 'gap' between Le Havre and the

Oosterschelde was closed with records at the French Opal coast and the Belgian coast in 2005 and 2006, respectively (d´Udekem d´Acoz 2006, Dauvin et al. 2009). German waters were reached in 2007. A single male *H. sanguineus* was found in October 2007 on the rocky offshore island of Helgoland, Germany (H. Auel, pers. comm.). Again, some specimens were recorded around Helgoland in July 2008 (Scrosati et al. 2011, M. Molis, pers. comm.). Also in 2007, the first specimens were detected in the German Wadden Sea, close to the island of Norderney (Obert et al. 2007). From 2008 to 2010, the French Contentin peninsula, east of Le Havre, was infested (Dauvin 2009a, b, Dauvin and Dufossé 2011). In 2008, *H. sanguineus* also reached the northern border of the German Wadden Sea, when it was found on the island of Sylt (Landschoff et al. 2013).

The populations of *H. sanguineus* in the areas mentioned above can be regarded as fully established. Apart from these, single to few individuals were found in the Adriatic Sea in 2003 (Schubart 2003), in the Black Sea in 2008 (Micu et al. 2010), at the Skagerrak coast of Sweden (M. Berggren, pers. comm.) and in southern Great Britain (Seeley et al. 2015) (Fig. 1.6).

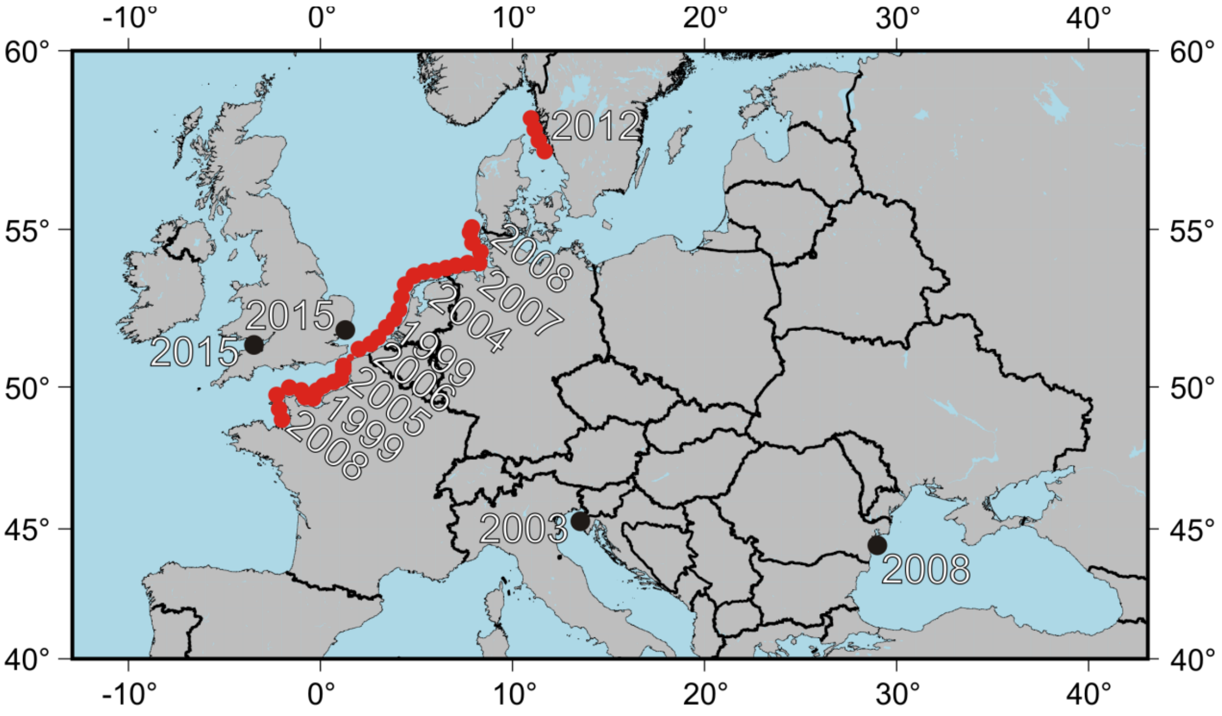

Fig. 1.6: Distribution of *Hemigrapsus sanguineus* in the invaded European range (red band), including the reported years. Black circles mark one-time findings. For references see text. This map was produced and adapted using the software GMT 5.3.1 and CorelDRAW X4.

1.5 Interactions between *C. maenas* and *H. sanguineus*

Both species, *C. maenas* and *H. sanguineus*, are invasive at the U.S. east coast. In Europe the first species is native, while the latter is the invader. In both areas, *C. maenas* mainly dominated the intertidal zone but numbers of *H. sanguineus* increased rapidly after its arrival. The development of both populations was documented in a number of studies. At the U.S. east coast, the increase of the *H. sanguineus* population was generally accompanied by a massive decrease of *C. maenas* abundances (Lohrer and Whitlatch 2002, Kraemer et al. 2007, O´Connor 2014). However, Schab et al. (2013) found a decreasing population of *H. sanguineus* and a recovering population of native mud crabs *Panopeus herbstii* H. Milne Edwards, 1834 in two investigations, which were 10 years apart in an area, where *C. maenas* is not abundant. Along the European coast, also *H. takanoi* Asakura and Watanabe 2005, a sibling species of *H. sanguineus*, can be found in relatively high abundances together with *H. sanguineus* and *C. maenas* (van den Brink et al. 2012, Landschoff et al. 2013, Gothland et al. 2014). It was concluded that, on a population level, *Hemigrapsus* spp. do not have a negative influence on *C. maenas* in the German Wadden Sea yet (Landschoff et al. 2013). In the Dutch delta, the *Hemigrapsus* spp. invasion profited from already low *C. maenas* abundances. However, *H. sanguineus* was not identified to be the cause but a facilitating factor of the decline (van den Brink et al. 2012). Generally, the European *C. maenas* populations do not seem to suffer severely from the invasions of *H. sanguineus* and *H. takanoi*. This may partly be due to an enormous subtidal *C. maenas* population but also due to still relatively low *Hemigrapsus* spp. densities in Europe compared to the U.S. (Kraemer et al. 2007, O´Connor 2014).

The population developments and the attempt to estimate the competitive strength of *H. sanguineus* versus *C. maenas* led to a number of studies comparing both species and their interactions on a community or individual level. For instance, North American *H. sanguineus* were able to dominate *C. maenas* in direct competition for food. Furthermore, *H. sanguineus* was able to force *C. maenas* out of shelter (Jensen et al. 2002). While *C. maenas* was superior for food in only some occasions, it was usually faster in finding food items compared to *H. sanguineus* (MacDonald et al. 2007). When foraging together with a conspecific, *H. sanguineus* was found to consume remarkably less food per individual compared to foraging alone or together with one *C. maenas* (Griffen 2006). The individual consumption was also less compared to two *C. maenas* foraging together. The author stated that, in an unpublished laboratory study, he found conspecific pairs of crabs spending 68% more time on aggressive behavior against each other than heterospecific pairs. Consequently, the effect of intraspecific aggression reducing prey consumption is stronger in *H. sanguineus* than in *C. maenas* and generally increases with crab density (Griffen 2006, Griffen and Williamson 2008). Furthermore, *C. maenas* consumes less food when *H. sanguineus* is present, which is not the case vice versa (Griffen and Byers 2006a, Griffen et al. 2008). This was partly explained by the fact that *H. sanguineus* predates on *C. maenas* to a higher degree than vice versa but does not do so on conspecifics (Lohrer and Whitlatch 2002, Griffen 2006, Griffen and Byers 2006a, b, Griffen and Williamson 2008). Generally, both species can be regarded as opportunistic omnivores. According to gut content analysis, their dietary spectrum largely overlaps, although *H. sanguineus* showed a tendency towards an algae dominated diet (e.g. Ropes 1968, Tyrell and Harris 1999, Lohrer et al. 2000b, Griffen et al. 2012).

Most ecological comparisons were studies from the U.S. east coast, where both *C. maenas* and *H. sanguineus* are invasive. In Europe, however, the native *C. maenas* population may have a competitive advantage, as it is presumably better adapted to local conditions (Dauvin 2009a). At least for the German Wadden Sea, the impact of *H. sanguineus* on the *C. maenas* population, if at all, seems to be confined to juveniles (Landschoff et al. 2013). If the European populations of *H. sanguineus* increase and reach numbers as observed in the US (Kraemer et al. 2007, O'Connor 2014) or higher, an effect on the native *C. maenas* population is expected.

1.6 Objectives

Summarizing the previous subchapters, the invasion and establishment of *H. sanguineus* at the U.S. east and the European costs has been highly successful. At only one site in the U.S., decreasing abundances were reported so far. Anywhere else in the U.S. and in Europe, the abundances of *H. sanguineus* are generally increasing. The available literature suggested competitive advantages of *H. sanguineus* over *C. maenas*. However, mostly ecological comparisons of the two species are available. Physiological data of *H. sanguineus*, however, are almost absent but needed i) to explore the physiological basis and consequences of its competitive superiority, ii) to estimate the overall ecological effects of this species on its habitat.

Considering this background, the aims of this PhD project were to

1) assess the status (abundance, biomass, densities) of *H. sanguineus* and *C. maenas* around the rocky island of Helgoland, Germany (Publication I).
2) evaluate *H. sanguineus*' invasion success in relation to wave exposure (Publication I).

3) investigate species-specific differences of the respiratory metabolism and energy demand in relation to temperature, seasons, sexes, and biomass (Publication II).
4) calculate the annual energy demand of both species' populations around Helgoland (Publication II) on the basis of their densities (Publication I).
5) compare the ability of *C. maenas* and *H. sanguineus* to store energy in form of lipids in relation to season, biomass, and sex (Publication III).
6) apply the fatty acid trophic marker concept to both crab species and reveal species specific dietary differences in relation to season, biomass, and sex (Publication III).

2 Materials and methods

This section gives a brief summary about the sampled area and methodologies used for conducting the research, which is finally presented in the single publications. For more details on the materials and methods, please refer to the individual articles. All measured or sampled organisms for the three publications were collected in the Helgoland intertidal area (Fig. 2.1).

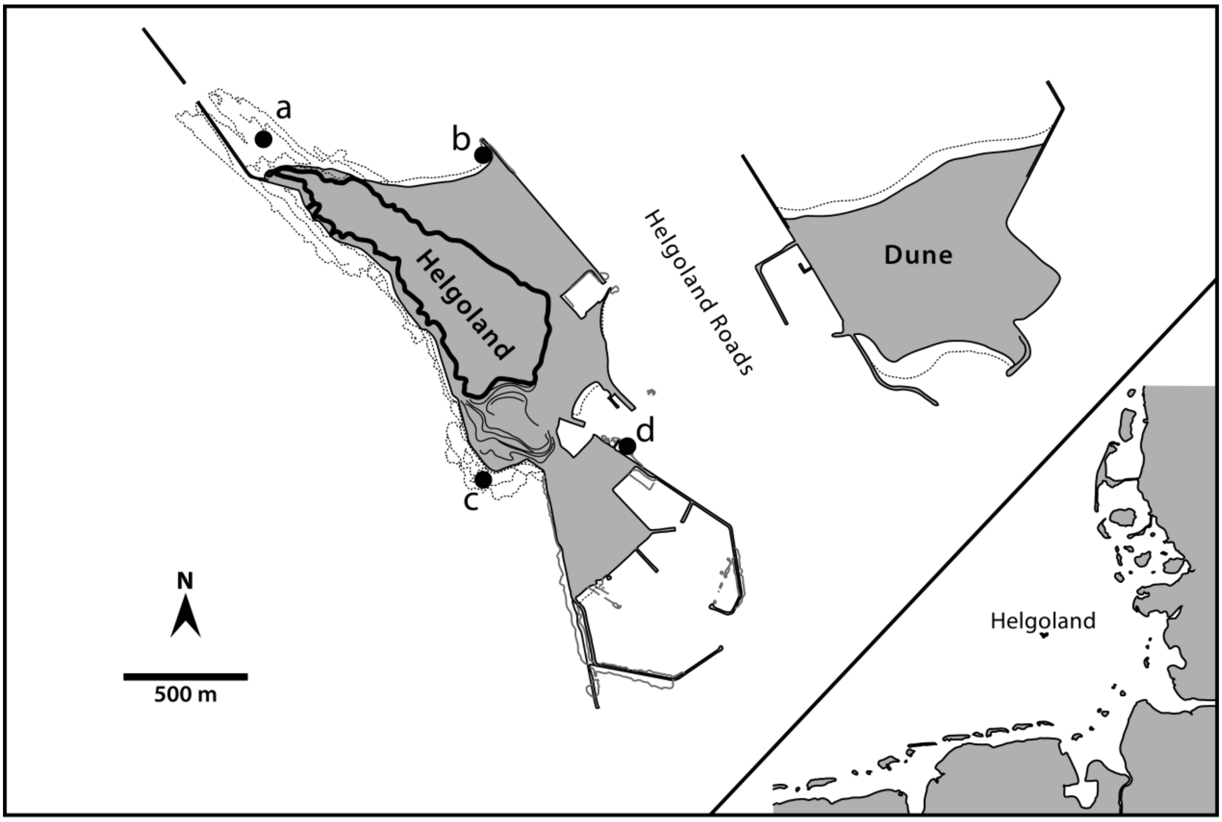

Fig. 2.1: The island of Helgoland and its location in the German Bight. Samples for publication I were collected at all four indicated sites (a, b, c, d). Samples for the publications II and III were collected exclusively at site c. Map from publication I.

2.1 Publication I

In August 2009 and August 2014, a crab inventory survey was conducted in the intertidal areas of Helgoland. At four different sites around the island (Fig. 2.1), three tidal heights were sampled each. In each tidal height, four quadrats of 0.25 m^{-2} each were placed on the bottom. All crabs in the quadrat, which were larger than 5 mm in carapace width, were collected. Canopy and underlying sediment were carefully examined for small crabs. After taken back to the laboratories of the Helgoland Marine Station, all crabs were sorted by species and sex, females were examined for eggs and carapace width was measured. Based on carapace width to wet biomass equations, crab biomass from the two inventory surveys was calculated. Abundance and biomass data were correlated to the wave exposition of a respective site. As data on wave action were not available, wind force and the openness of a site to the sea were used as an indicator for exposition. Wind data were downloaded from the webpage of the German Weather Service DWD (www.dwd.de).

2.2 Publication II

The respiratory metabolism of *C. maenas* and *H. sanguineus* was investigated by using a custom-made flow-through respiration measurement system (Fig. 2.2). Crabs were collected in an intertidal area at the southwestern tip of Helgoland in April, June, August, and October 2015 (site c in Fig. 2.1) and the measurements took place in temperature controlled rooms of the Helgoland Marine Station. The October measurements had unfortunately to be omitted since the hose pump, which created the water flow through the respiration chambers, broke and the supplement pump could not be trusted to have a constant flow rate.

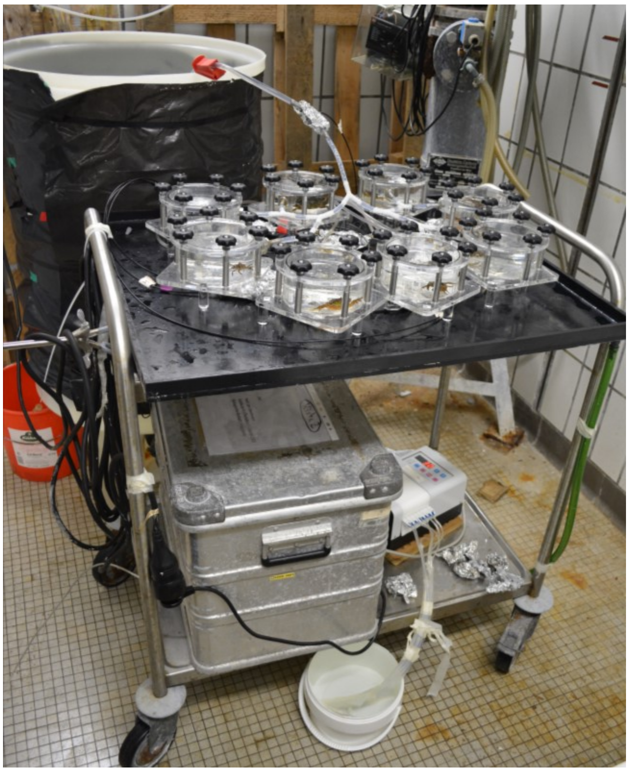

Fig. 2.2: Setup of the respiration measurement device. The water in the tank in the background was pre-tempered in the respective temperature-controlled room. The box on the lower left was holding the actual respiration measuring device, which was connected to the laptop outside in the hallway. Photo: S. Jungblut

After the collection, crabs were maintained for 24 h in darkness at 10°C to standardize hunger levels. Right before starting a set of measurements, seven crabs were selected and carapace width and fresh mass were obtained. Seven crabs could be measured simultaneously per set of measurements, while one respiration chamber acted as control. Each set of crabs was measured subsequently at four temperature levels (5, 10, 15, and 20°C) for eight hours each. For this procedure, the whole measurement setup was moved from one temperature controlled room to the other. From the available eight hours of measurement per temperature level, the first three hours were omitted to ensure the temperature acclimation of water inside the respiration chambers to the new temperature. During the running measurement one oxygen concentration reading was taken per minute. The frequency distribution of the about 300 oxygen consumption values per crab and temperature were mostly normally distributed with a pronounced peak. The median of these data represented the peak well and was the basis of all further calculations. We used these data to examine respiratory differences between the two species according to season, sex, temperature and biomass. We calculated and compared the respiration rates of 0.5, 5, and 10 g individuals at different temperatures. Additionally, we used the statistical results to construct species-specific models to calculate individual respiration with the animals' biomass and the ambient temperature. With the help of these equations and the population densities from publication I, we calculated the metabolic energy demand of both species' populations in 2014.

2.3 Publication III

Total lipid and fatty acid analyses were conducted with *C. maenas* and *H. sanguineus* collected in an intertidal area of the southwestern tip of the island of Helgoland in April, June, August, and September 2015 (site c in Fig. 2.1). Macroalgae were sampled in August 2015 exclusively. All samples were taken to the laboratories of the Marine Station Helgoland. Crabs were separated by species and sex. Carapace widths and fresh masses were measured, then the animals were dissected to obtain their midgut glands. These were immediately weighed and frozen at -80°C. Algae samples were rinsed with distilled water and also frozen. At the end of each field campaign, the samples were transported on dry ice to the laboratories of the University of Bremen. Each sample was lyophilized for 48 h, dry mass was obtained, and the total lipid content was extracted with a mixture of dichloromethane and methanol. Lipid extracts were then transmethylated in methanol containing 3% concentrated sulphuric acid and fatty acid components were determined in a gas chromatograph.

The data analysis included seven models, which were built to examine differences in species, sex, season and fresh mass: two condition indices, the hepatosomatic index and the total lipid content as well as five different newly developed fatty acid-based dietary indices (carnivory, Chlorophyta (green algae), Phaeophyceae (brown algae), Rhodophyta (red algae), and Bacillariophyceae (diatoms)). How these parameters were calculated is listed in Table 2.1. Additionally, the whole fatty acid compositions of the crab and algae species were compared with Principal Component Analyses.

Table 2.1: Equations for the calculations of condition parameters and trophic marker indices (publication III).

Parameter	**Equation**
Hepatosomatic Index	$\text{HSI} = \text{wet mass}_{\text{hepatopancreas}} / (\text{fresh mass}_{\text{crab}} - \text{wet mass}_{\text{hepatopancreas}})$
Total lipid content ($\%_{\text{DM}}$)	$\text{TL} = \text{mass}_{\text{extracted lipids}} / \text{dry mass}_{\text{sample}} * 100$
Carnivory Index	I_{Ca} = 18:1(n-9) / [16:1(n-7) + 16:4(n-3) + 16:4(n-1) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) + 18:4(n-3) + 20:4(n-6) + 20:5(n-3)]
Chlorophyta Index	I_{Ch} = 16:4(n-3) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3)
Phaeophyceae Index	I_{P} = 18:4(n-3)
Rhodophyta Index	I_{R} = 20:5(n-3) / [16:0 + 18:0 + 22:6(n-3)]
Bacillariophyceae Index	I_{B} = 16:1(n-7) + 16:4(n-1)

3 Outline and contributions

This overview outlines the three first author papers, which are included in this PhD thesis and describes the contribution of each author and the contribution of the first author in even more detail.

3.1 Publication I

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

Simon Jungblut, Jan Beermann, Karin Boos, Reinhard Saborowski, Wilhelm Hagen

I conducted the second sampling campaign in 2014 and wrote the manuscript with scientific advice of Dr Saborowski and Prof Dr Hagen. Dr Beermann conducted the first sampling campaign in 2009 and gave advice on draft versions of the manuscript. Dr Boos contributed the statistical analysis and editorial comments on the manuscript. This article was published in the journal *Aquatic Invasions*, 12 (1): 85 – 96, DOI: https://doi.org/10.3391/ai.2017.12.1.09.

Contribution of the first author in % of the total work load (100% for each of the following categories):

Experimental concept and design: ca. 10%
Acquisition of (experimental) data: ca. 50%
Data analysis and interpretation: ca. 70%
Preparation of Figures and Tables: ca. 85%
Drafting of the manuscript: ca. 90%

3.2 Publication II

Native versus invasive brachyuran crabs in a European rocky intertidal: respiratory performance and energy demand with implications on their habitat

Simon Jungblut, Karin Boos, Morgan Lee McCarthy, Reinhard Saborowski, Wilhelm Hagen

I designed the respiration chambers, developed the experimental design, conducted measurements, processed the data and drafted the manuscript. Morgan McCarthy assisted in two of the three measurement campaigns and revised the manuscript. Dr Boos conducted the statistical analysis and gave editorial comments on the manuscript. Dr Saborowski and Prof Dr Hagen gave scientific advice on the respiration chambers, the experimental design as well as advice and editorial comments on the draft versions of the manuscript. This article is under review in the journal *Marine Biology*.

Contribution of the first author in % of the total work load (100% for each of the following categories):

Experimental concept and design: ca. 90%
Acquisition of (experimental) data: ca. 80%
Data analysis and interpretation: ca. 70%
Preparation of Figures and Tables: ca. 95%
Drafting of the manuscript: ca. 90%

3.3 Publication III

Seasonal lipid storage and dietary preferences of native European vs. invasive Asian shore crabs

Simon Jungblut, Morgan L McCarthy, Karin Boos, Reinhard Saborowski, Wilhelm Hagen

I developed the experimental design, conducted the collection, procession and analysis of samples and drafted large parts of the manuscript. Morgan McCarthy assisted in two of the four field campaigns, in the procession and analysis of the samples. He also revised draft versions of the manuscript. Dr Boos provided the statistical analysis as well as editorial comments on the manuscript. Dr Saborowski and Prof Dr Hagen gave scientific advice during the whole project and editorial comments on the manuscript. This article is submitted to the journal *Marine Ecology Progress Series*.

Contribution of the first author in % of the total work load (100% for each of the following categories):

Experimental concept and design: ca. 90%
Acquisition of (experimental) data: ca. 80%
Data analysis and interpretation: ca. 75%
Preparation of Figures and Tables: ca. 95%
Drafting of the manuscript: ca. 85%

4 Publication I

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

Simon Jungblut, Jan Beermann, Karin Boos, Reinhard Saborowski, Wilhelm Hagen

published in *Aquatic Invasions*, 2017, 12 (1): 85 – 96

DOI: https://doi.org/10.3391/ai.2017.12.1.09

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.

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4.1 Abstract

The Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1853) has recently established populations in the North Sea and now occurs within the range native to the green crab *Carcinus maenas* (Linnaeus, 1758). To determine potential competitive effects and to assess the progress of the invasion, species-specific population characteristics (numerical abundances, biomasses, and size distributions) of the two species around the island of Helgoland (German Bight, southern North Sea) were compared for surveys conducted in 2009 and 2014. Sampling sites were chosen based on accessibility and differed in their topography and wave exposure, which allowed testing for the influence of these factors on the establishment success of *H. sanguineus*. The numerical abundance and biomass of *H. sanguineus* increased markedly and approached those of *C. maenas* in 2014. At a sheltered site, *H. sanguineus* even outnumbered *C. maenas*, whereas the opposite was observed at a site exposed to strong winds and waves. Although such contrasting abundance patterns between the native and the introduced shore crab may be the result of direct interference, the dominance of *H. sanguineus* at the sheltered site may also be explained by enhanced larval settling rates caused by odors of conspecifics. The results suggest that the invasion of *H. sanguineus* has not yet reached its equilibrium, and population abundances in the North Sea are expected to continue increasing.

Keywords

Biological invasion, alien species, Asian shore crab**,** European green crab**,** wind and wave exposure, rocky shore, intertidal ecology, German Bight

4.2 Introduction

Non-indigenous species can affect ecosystem structure and functioning in many ways, sometimes drastically. Drastic alterations may ultimately affect human economic interest, ecosystem services, and even human health (Riuz et al. 2000, Simberloff et al. 2013). Biological invasions are therefore regarded as one of the major threats to biodiversity in the marine realm (Grosholz 2002). Non-native species may compete with native residents for food or space (or both) and often largely displace resident species by spreading into all available habitats at high population densities (Bax et al. 2003). While biotic interactions are crucial in determining invasion success, environmental characteristics such as temperature regime and wave exposure have repeatedly been shown to limit the spread of non-native species in their new habitats (Pörtner 2002; Hampton and Griffiths 2007; Russel et al. 2008).

On a global scale, brachyuran crabs frequently invade estuarine and marine coastal ecosystems (Brockerhoff and McLay 2011). The European green crab *Carcinus maenas* (Linnaeus 1758), for example, successfully invaded the Atlantic coast of North America by 1817. Thereafter, it appeared at many other locations around the world including the Pacific coast of North America and the shores of South Africa and Australia (Carlton and Cohen 2003).

The invasion of the Asian shore crab *Hemigrapsus sanguineus* (De Haan 1835) along the Atlantic shore of North America in the early 1990´s is well documented (McDermott 1998; Ledesma and O'Connor 2011; Lohrer and Whitlatch 2002). After the first specimens were detected in 1988 at Townsend´s Inlet, north of the mouth of the Delaware Bay, *H. sanguineus* rapidly spread along much of the North American east coast. Its present distribution in the USA ranges from Cape Hatteras in North Carolina to the Schoodic Peninsula in Maine (Williams and McDermott 1990; Delaney et al. 2008; Epifanio 2013). In Europe, *H. sanguineus* was first detected in August 1999 in the harbor of Le Havre, France, and shortly afterwards in the Oosterschelde, The Netherlands (Breton et al. 2002). Today, it is distributed from the Contentin Peninsula in France to the German Wadden Sea (Obert et al. 2007; Gothland et al 2013; Landschoff et al. 2013). Recently, it was reported from the English and western Swedish coasts (Seeley et al. 2015; M. Berggren, Sven Lovén Centre for Marine Sciences – Kristineberg, Fiskebäckskil, Sweden, pers. comm.).

The island of Helgoland is located in the German Bight and represents the only natural hard-bottom habitat in the south-eastern North Sea. The island is small (1 km^2), relatively isolated, and is surrounded by an intertidal area of about 0.7 km^2. As a result, Helgoland provides a unique location to study the dispersal of an invading species and potential competitive processes between an invader and a native ecological equivalent. The intertidal areas around Helgoland are characterized by high biological diversity and the European green crab dominates the crustacean fauna (Reichert and Buchholz 2006). In October 2007, a single male *H. sanguineus* was found on Helgoland (H. Auel, Bremen Marine Ecology, University of Bremen, Bremen, Germany, pers. comm.). In July 2008, *H. sanguineus* was found in low numbers at one site (Scrosati et al. 2011; M. Molis, Alfred Wegener Institute, Bremerhaven, Germany, pers. comm.) and quickly spread over the entire rocky intertidal zone around the island. Currently, *H. sanguineus* is the only non-indigenous brachyuran species with the potential to compete with the native shore crab, *C. maenas*, in the intertidal areas around Helgoland. The Asian shore crab, *Hemigrapsus takanoi* Asakura and Wanatabe, 2005 can also be observed in Helgoland's intertidal area, but has not yet succeeded in establishing a sustainable population (J. Beermann and S. Jungblut, pers. obs.).

On the Atlantic coast of North America, where both species are non-indigenous, several studies have indicated strong competitive interactions between them (e.g. Jensen et al. 2002; Lohrer and Whitlatch 2002; O'Connor 2014). North American *H. sanguineus* were dominant over *C. maenas* in direct competition for food (Jensen et al. 2002). On Helgoland, however, *H. sanguineus* faces an abundant population of *C. maenas* in its native habitat. Being the native species, this may give *C. maenas* a competitive advantage due to enhanced adaptations to local conditions (Dauvin 2009). In the German Wadden Sea the impact of the *Hemigrapsus* species on *C. maenas* seems to be negligible or, if present, confined to juvenile *C. maenas* (Landschoff et al. 2013). However, populations of *H. sanguineus* may continue to grow, as has been observed at the Atlantic coast of North America (Kraemer et al. 2007; O'Connor 2014).

The present study tested for changes in the population abundances and sizes of the alien *H. sanguineus* and the native *C. maenas* between the early phase of its invasion in August 2009 and five years later in August 2014. The unique topography of Helgoland also allowed evaluation of the possible influence of key environmental parameters (e.g., exposure to wind and waves) on the abundance and population structure of the two species.

4.3 Methods

4.3.1 Sampling sites

Four intertidal sampling sites were selected around the island of Helgoland: a northwestern site ('Felswatt'), a northeastern site ('Nordstrand'), a southwestern site ('Kringel'), and a southeastern site ('Augusta Mole') (Figure 4.1). The northwestern site consisted of a flat rock plateau with few loose stones that were largely covered by the macroalgae *Fucus serratus* Linnaeus, 1753*, Fucus vesiculosus* Linnaeus, 1753 and *Sargassum muticum* Fensholt, 1955. The other three sites were comprised of many small rocks to large immovable boulders lying on top of patches of coarse sand. These hard structures were patchily covered with small to medium-sized macroalgae such as: *Ulva* spp. Linnaeus, 1753; *Enteromorpha* spp. Link 1820; *Chondrus crispus* Stackhouse, 1797; and *Mastocarpu*s *stellatus* Guiry, 1984 (Bartsch and Tittley 2004). The northeastern and southwestern sites exhibited a similar slope between the high and low water line. The southeastern site was characterized by a relatively steep slope. For the present study, we quantified the site parameter 'wave exposure' on the basis of openness and shape of the respective site to the sea in combination with local wind data from 2009 to 2014 (Burrows et al. 2008 and references therein; wind data derived from the webpage of the German Weather Service DWD, www.dwd.de).

4.3.2 Sampling procedure

Each of the four sites was sampled at low tide around noon on four consecutive days in August 2009 and in August 2014. Samples were taken along defined transects from the low- to the high-water line at three shore levels: close to the high-tide line (high-level), in the middle of the *Fucus* zone (mid-level), and close to the low-tide line in the lower *Fucus* zone (low-level) (Reichert and Buchholz 2006). In each zone, four quadrats of 0.25 m² were randomly deployed (3 x 4 = 12 samples per site in total) and all decapod crabs with carapace widths ≥ 5 mm were collected. If necessary, the algal cover was removed, all stones in the quadrat were lifted, and any crabs captured. Within each zone, the replicated quadrats were placed 2 m to 5 m apart. As the intertidal area of Helgoland is dominated by large immovable boulders, the chosen quadrat size allowed for sampling in the narrow areas between the boulders. Additionally, a small quadrat ensured that all crabs present could be caught by one person.

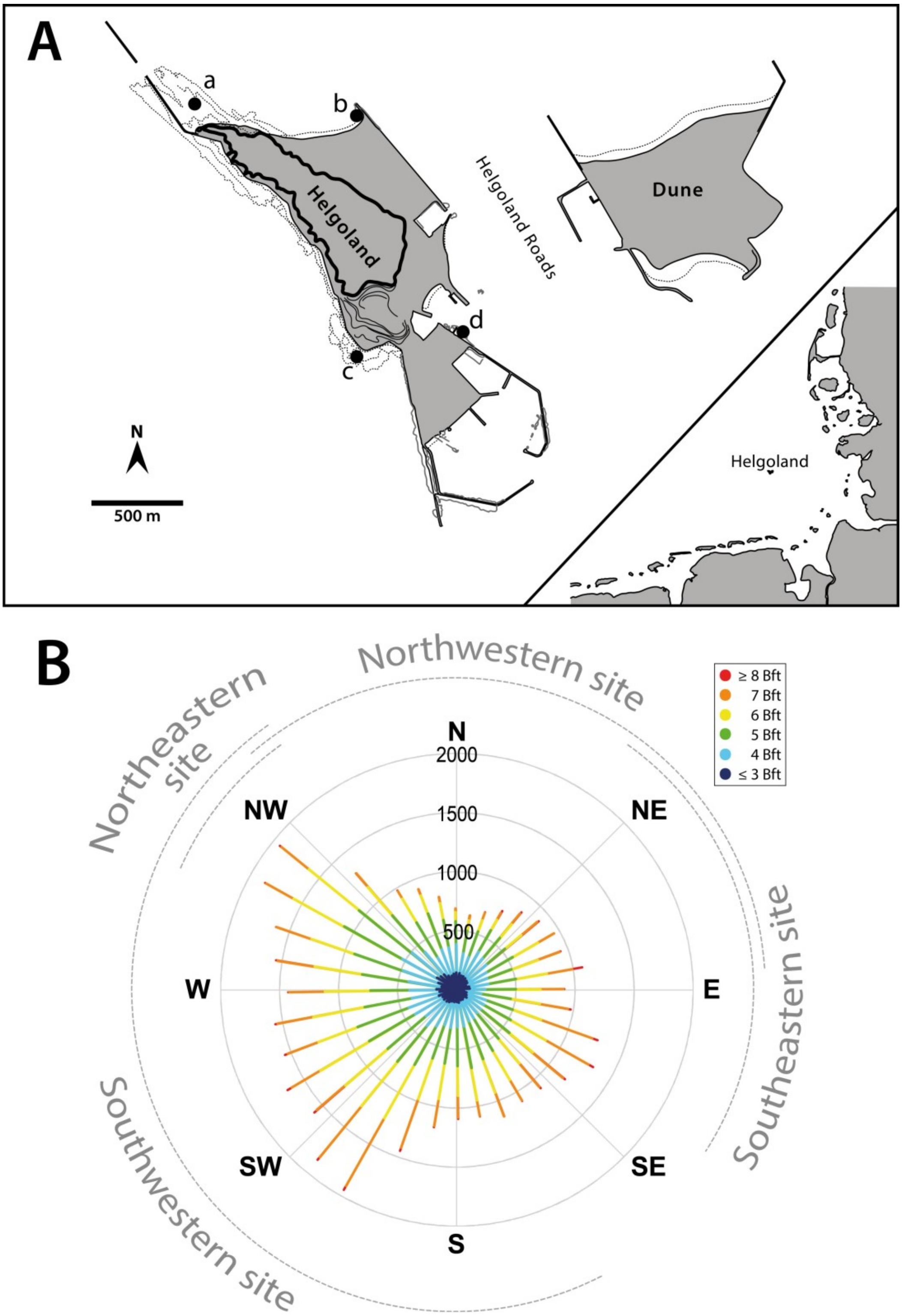

Figure 4.1: A) The main island of Helgoland with its neighbouring island Dune and their location in the German Bight (southeastern North Sea). Sampling sites are indicated by black circles: a) northwestern site, b) northeastern site, c) southwestern site, d) southeastern site. Finer lines indicate the intertidal areas. B) Wind exposure of sampling sites around the island of Helgoland. The upright central scale shows the cumulative number of hours with certain mean wind forces per 10° wind direction interval (specified in Beaufort (Bft) and different colours). Outer dashed lines indicate the assigned wind directions of the sampled locations according to their openness to the sea. Classified ranges therefore represent the sites' exposure to multi-directed exposure to wind and waves.

In the laboratory, all collected crabs were counted, sex determined, females were checked for eggs, and carapace widths (CW) were measured (using Vernier calipers). Individuals of *H. takanoi*, the edible crab *Cancer pagurus* Linnaeus, 1758 and the bristly crab *Pilumnus hirtellus* (Linnaeus, 1761) were recorded in very low abundances. These species were not considered in any further analyses because they were of minor importance for the focus of the present study.

4.3.3 Statistical analyses

Abundance and biomass

To calculate biomass (fresh mass) for *C. maenas* and *H. sanguineus*, we established carapace width to biomass regression relationships using animals collected in October 2014. Carapace width was measured to the nearest 0.5 mm. Individuals were then blotted for approximately 10 s with tissue paper and weighed (nearest mg) on an electronic scale (n = 86 for *C. maenas* and n = 102 for *H. sanguineus*). The best-fitting statistical relationships were the polynomial functions:

$y = -0.01172x + 0.002300x^2 + 0.0001759x^3$ ($R^2 = 0.995$) for *C. maenas*;

$y = -0.07253x + 0.009637x^2 + 0.0001775x^3$ ($R^2 = 0.981$) for *H. sanguineus*.

Where y is wet mass in mg and x is carapace width in mm. From these relationships, crab biomass per species and site was calculated.

To test for the effects of ‘species’, ‘year’, ‘site’ and ‘shore level’ on numerical abundance and biomass of the crabs, linear models were applied. Two sequential model approaches were conducted to avoid over-parameterization in a single model containing all the factors listed above. In the first approach (for abundance and biomass separately), a model was run that included the fully crossed (fixed) effects factors: ‘species’ (*H. sanguineus* and *C. maenas*), ‘year’ (2009 and 2014) and ‘site’ (northwestern, northeastern, southwestern and southeastern). In the second set of models, we selected only those sites for which the previous analyses revealed significant results. The new analyses were then run separately for these locations and included the factor ‘shore level’ (low, mid and high) instead of ‘site’.

The abundance data were tested using a generalized linear model (GLM) with Poisson distributed error term and log-link function. A quasi-Poisson correction was applied to account for unexplained variance in the model (i.e. overdispersion in the data). Biomass data were analyzed by applying linear models (LM) with a Gaussian error term. Prior to the latter analyses, the data were log-transformed to meet the assumptions of normal distribution and homogeneous variances of the residuals.

All models were fitted in R, version 3.2.0 (R Development Core Team 2015), using the generic functions ‘lm’ and ‘glm’. Graphs were produced with the software GraphPad Prism (version 5.03). For all models, the following diagnostics of model stability were examined: Cook’s distance, leverage (Quinn and Keough 2002), and dfbetas (Cohen and Cohen 2008). For the latter, data points were excluded one by one from the data sets and the derived estimates were compared with those obtained from the models based on all data points. Overall, the checks (Cooks distance and leverage) confirmed that no influential cases were present. When checking the dfbetas, however, some influential deviations in each of the models were detected. This instability was due to the low numbers of replicates and was therefore assumed to be negligible. Nonetheless, interpretation and discussion of the results were performed with caution.

For the first-approach models, the significances of the interaction terms and main factors were established with likelihood ratio tests (LRT) using the R function ‘anova’ with the argument ‘test’ set to ‘Chisq’ (for abundance data) and ‘F’ (for biomass data), respectively. We compared the deviances of the respective full models with those of the corresponding reduced models not comprising the respective factor and/or term of interest. Pairwise post-hoc comparisons were run to test for individual differences between factor levels. We accounted for multiple testing with a Bonferroni correction of the alpha level. In fact, for the abundance and biomass analyses, we corrected for 15 analyses that were re-ran with relevelled intercepts for pairwise comparisons in the outputs. This resulted in a reduced alpha level of $\alpha = 0.0033$.

For all second-approach models, we established the significances of the full models by testing them against the respective null models (with LRTs). Because of low numbers of replicates in these analyses ($n = 4$), we refrained from going into further statistical analyses and chose to interpret the result on a descriptive basis, depending on the significance of the full models.

Size distribution

To test for differences between the distributions of size classes of the two crab species at the respective sites between 2009 and 2014, individual Pearson´s Chi-squared tests were conducted. Where counts of crabs for a certain size class were ≤ 5, the p-values were estimated based on 50,000 replicated simulations. Size classes were defined in 5 mm intervals, ranging from 5-9.5 mm CW as the smallest size class and > 35 mm CW as the largest size class. To identify the size classes responsible for a significant difference between the two distributions, confidence intervals (CI) for each size class per year were calculated (Crow and Gardner 1959). Where confidence intervals for a specific size class did not overlap, the difference between the respective years was considered significant. All χ^2 tests were calculated using R, version 3.2.0 (R Development Core Team 2015). Images were produced with the Software Graph Pad Prism (version 5.03).

4.4 Results

4.4.1 Exposure

Wind exposure data for each of the sites (Figure 4.1B) indicated the southwestern site experienced remarkably higher wind exposure, resulting in higher wave action and turbulence.

4.4.2 Abundance

Female *H. sanguineus,* including those carrying eggs, were present in both years (Table 4.1). In 2014, the percentage of ovigerous females were higher at three of the four sites in 2014 compared to 2009.

The results of the 'first approach model' revealed that the numerical abundances of *C. maenas* and *H. sanguineus* varied between the two years for sampling sites around the island of Helgoland ($LRT_{species*year*location}$: df = 3, χ^2 = 35.475, p = 0.018). In the northwestern intertidal (Figure 4.2A), the abundances of both crab species did not differ significantly between the two years. In both years, however, *C. maenas* was more abundant than *H. sanguineus* (9.6 and 6.5 ind./0.25 m^2 for *C. maenas* and 0.1 and 0.8 ind./0.25 m^2 for *H. sanguineus* in 2009 and 2014, respectively). At the northeastern site (Figure 4.2B), a decrease in abundance of *C. maenas* from 2009 (6.9 ind./0.25 m^2) to 2014 (3.6 ind./0.25 m^2) was observed, but this was not statistically significant, whereas a pronounced increase in abundance from 3.8 to 10.1 ind./0.25 m^2 was found for *H. sanguineus*. Accordingly, in 2009 the abundances of *C. maenas* were slightly higher than those of *H. sanguineus*, however in 2014 the inverse was observed with abundances of *H. sanguineus* being significantly higher than those of *C. maenas* (10.1 vs. 3.6 ind./0.25 m^2). The abundance of *C. maenas* at the southwestern site (Figure 4.2C) nearly doubled from 7.9 ind./0.25 m^2 in 2009 to 14.8 ind./0.25 m^2 in 2014. No clear difference was detected for *H. sanguineus* (3.4 and 4.8 ind./0.25 m^2 in 2009 and 2014, respectively). Abundances of *C. maenas* were generally higher than those of *H. sanguineus* in both years. This pattern was even more pronounced in 2014 than in 2009. At the southeastern site (Figure 4.2D) no significant differences between the abundances of *C.*

maenas or *H. sanguineus* were detected between species and years. These abundances ranged between 1.9 and 4.3 ind./0.25 m².

Table 4.1: Numbers of *Hemigrapsus sanguineus* females, numbers of ovigerous (ovig.) females, and percentage of ovigerous females per site around the island of Helgoland, German Bight (southern North Sea) in 2009 and 2014.

site	**2009**			**2014**		
	total	**ovig.**	**ovig. (%)**	**total**	**ovig.**	**ovig. (%)**
Northwest	0	0	0	4	1	25.0
Northeast	12	0	0	59	26	44.1
Southewest	14	2	14.3	27	10	37.0
Southeast	3	1	33.3	15	4	26.6

4.4.3 Biomass

Similar to the abundance data, the analyses revealed a significant three-way interaction in the ‘first approach model’ for the biomass data (LRT: $F_{176,3} = 6.4786$, $p < 0.001$), indicating that the biomass data of the two species differed between sites and years. At the northwestern site, no significant differences were detected between the years (Figure 4.2E). Changes in biomass were most pronounced for the northeastern site (Figure 4.2F), where *C. maenas* clearly decreased in biomass (from 13.6 in 2009 to 5.8 g/0.25 m^2 in 2014) while the biomass of *H. sanguineus* significantly increased (from 0.9 to 22.2 g/0.25 m^2). This increase of *H. sanguineus* was also evident at the southwestern site, although it was not significant (2.9 to 9.0 g/0.25 m^2; Figure 4.2G), and no between-year difference was detected for *C. maenas*. At the southeastern site (Figure 4.2H), *H. sanguineus* showed a significant increase in biomass from 1.0 g/0.25 m^2 in 2009 to 6.0 g/0.25 m^2 in 2014, while *C. maenas* biomass did not differ between years.

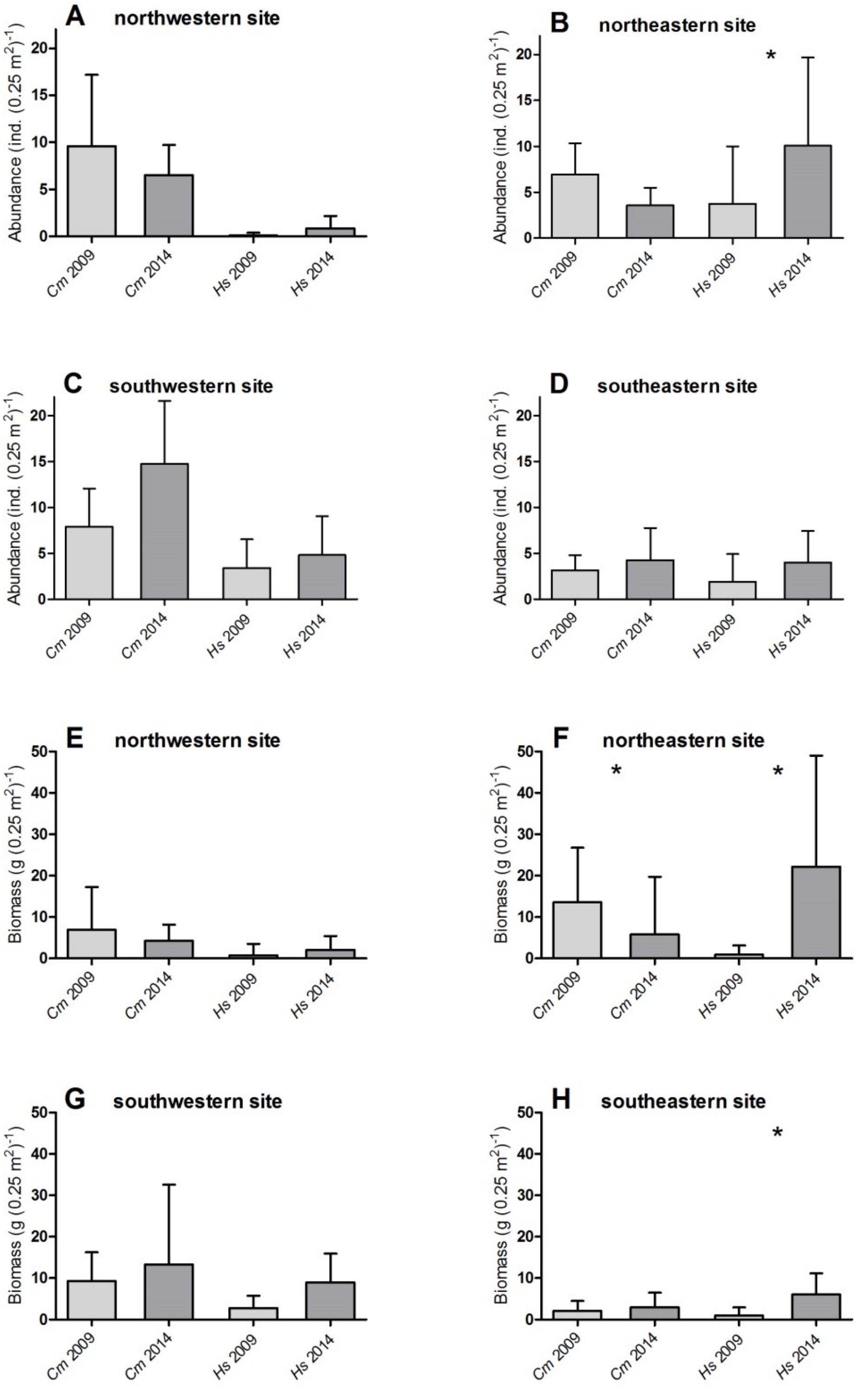

Figure 4.2: Abundances (A to D) and biomasses (E to H) of *Carcinus maenas* (*Cm*) and *Hemigrapsus sanguineus* (*Hs*) in 2009 (light grey bars) and 2014 (dark grey bars) at the sampling sites around the island of Helgoland, German Bight (southern North Sea) (means ± SD are presented). Asterisks indicate significant inter-annual differences per species.

4.4.4 Size distributions

The size distribution of *C. maenas* differed significantly between years at three of four sites (Figure 4.3A to D). At the northeastern site, individuals of the size classes ‘10 to 14.5 mm CW’ and ‘15 to 19.5 mm CW’ were most numerous in 2009 (Figure 4.3B), while in 2014 most individuals belonged to the smallest size class ‘5 to 9.5 mm CW’. At the two southern sites, no size class was dominant in 2009, but in 2014 most of the individuals belonged to the size class of ‘5 to 9.5 mm CW’ (Figure 4.3C and D).

Similar to *C. maenas*, the size distribution of *H. sanguineus* differed significantly between the two years (Figure 4.3E to H). At the northeastern site significantly more individuals occurred in the size classes between 10 and 24.5 mm CW in 2014 than in 2009. Similar patterns were observed at the southwestern site for the size class ‘15 to 19.5 mm CW’ and at the southeastern site for the size classes between 10 and 19.5 mm CW (Figure 4.3F, G and H).

4.4.5 Abundance and biomass at different shore levels

The southwestern and the northeastern sites exhibited significant differences in the ‘first approach models’ for crab abundances and biomasses. When evaluated possible effects of the shore levels (‘second approach models’), the abundance of *C. maenas* increased in all three shore levels from 2009 (range of 5.5 to 12.5 ind./0.25 m^2) to 2014 (range of 10.5 to 21.0 ind./0.25 m^2) at the southwestern site ($LRT_{\text{full model}}$: df = 11, $\chi^2 = 181.71$, $p < 0.001$, Figure 4.4A). In contrast, *H. sanguineus* showed only minor increases in abundance at all shore levels, being less abundant than *C. maenas* in all zones and in both years (Figure 4.4A). At the northeastern site ($LRT_{\text{full model}}$: df = 11, $\chi^2 = 178.36$, $p < 0.001$, Figure 4.4B), *H. sanguineus* clearly increased in abundance at the high shore level from 0.8 ind./0.25 m^2 in 2009 to 3.5 ind./0.25 m^2 in 2014. At the mid-level, no changes in abundance were observed. In both years, *H. sanguineus* was significantly more abundant than *C. maenas* (4.5 and 3.3 ind./0.25 m^2 for *C. maenas* and 10.3 and 8.5 ind./0.25 m^2 for *H. sanguineus*). The lower shore level was subject to the largest changes in abundance. From 2009 to 2014, numbers of *C. maenas* decreased significantly from 9.8 to 2.5 ind./0.25 m^2, whereas those of *H. sanguineus* markedly increased from 0.3 to 18.3 ind./0.25 m^2.

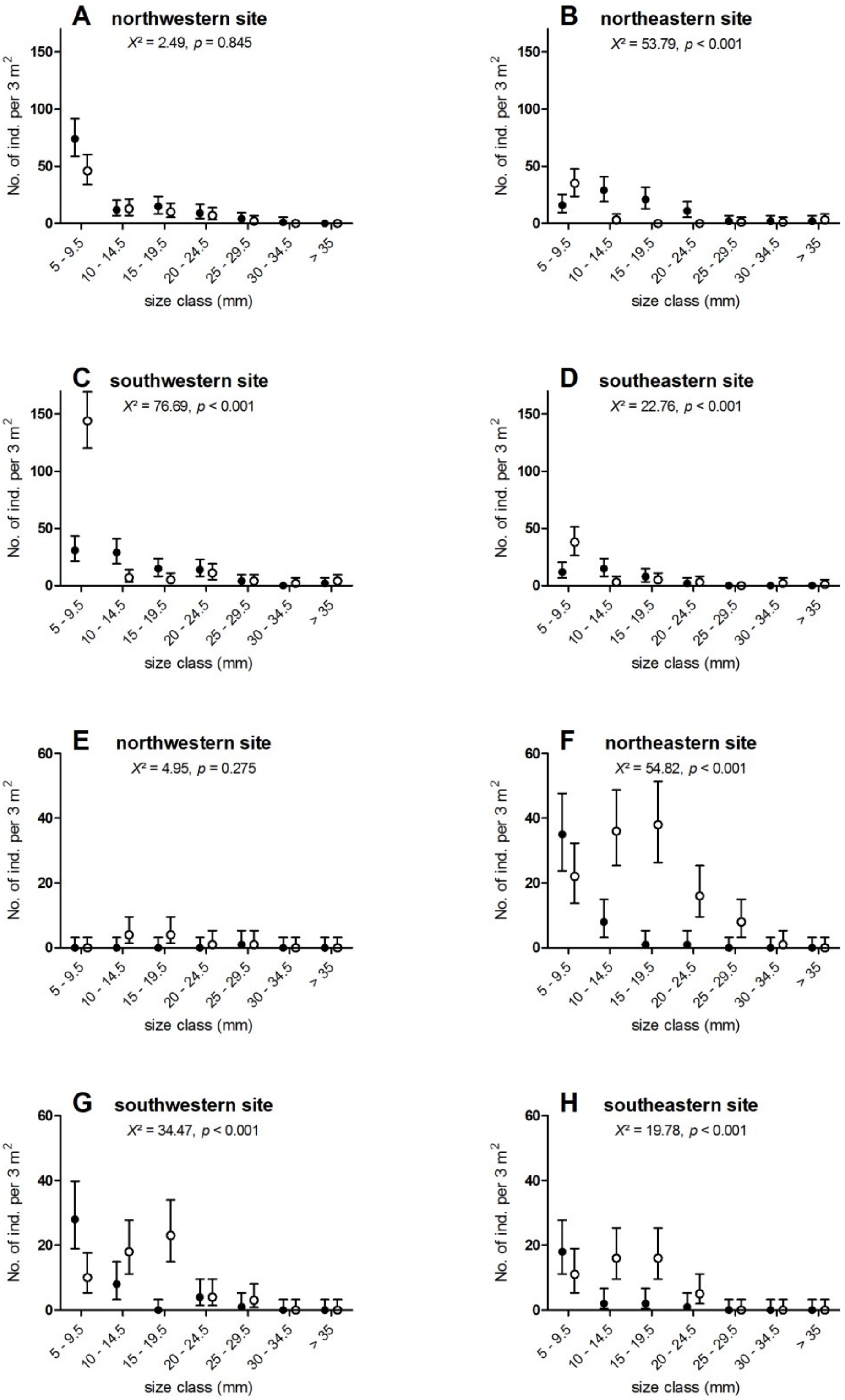

Figure 4.3: Size frequency distributions of *Carcinus maenas* (A to D) and *H. sanguineus* (E to H) at the different sampling sites around the island of Helgoland, German Bight (southern North Sea) in 2009 and 2014 (total number ± confidence intervals). At each site, all 12 samples were pooled, adding up to a total of 3 m^2. Inter-annual comparisons for each size class were significant, when confidence intervals did not overlap.

Similar to the numerical abundance data, the ‘second approach models’ revealed significant three-way interactions for biomasses at the southwestern and the northeastern sites (LRT: $F_{36,11} = 4.3918$, $p < 0.001$ and LRT: $F_{36,11} = 6.6993$, $p < 0.001$, respectively). At the southwestern site, the biomass of *C. maenas* strongly decreased at the high shore level from 15.1 in 2009 to 1.0 g/0.25 m^2 in 2014 (Figure 4.4C). In contrast, the biomass of *C. maenas* increased up to threefold at the other levels. The biomass of *H. sanguineus* increased at all three shore levels from 2009 to 2014. Increases between years were most pronounced at the mid and low-levels with increases from 0.7 to 9.4 and from 1.5 to 7.6 ind./0.25 m^2, respectively. At the northeastern site, biomasses of *C. maenas* decreased between years at the high and the mid-shore levels from 7.2 to 0.5 g/0.25 m^2 and from 13.4 to 1.4 g/0.25 m^2, respectively. The biomass of *H. sanguineus* increased from 0.2 to 6.2 and from 2.5 to 11.2 g/0.25 m^2, respectively (Figure 4.4D). At the lower shore level *C. maenas* biomass did not differ substantially between the years (20.3 and 15.6 g/0.25m^2), while *H. sanguineus* showed a dramatic increase from 0.1 to 49.1 g/0.25 m^2.

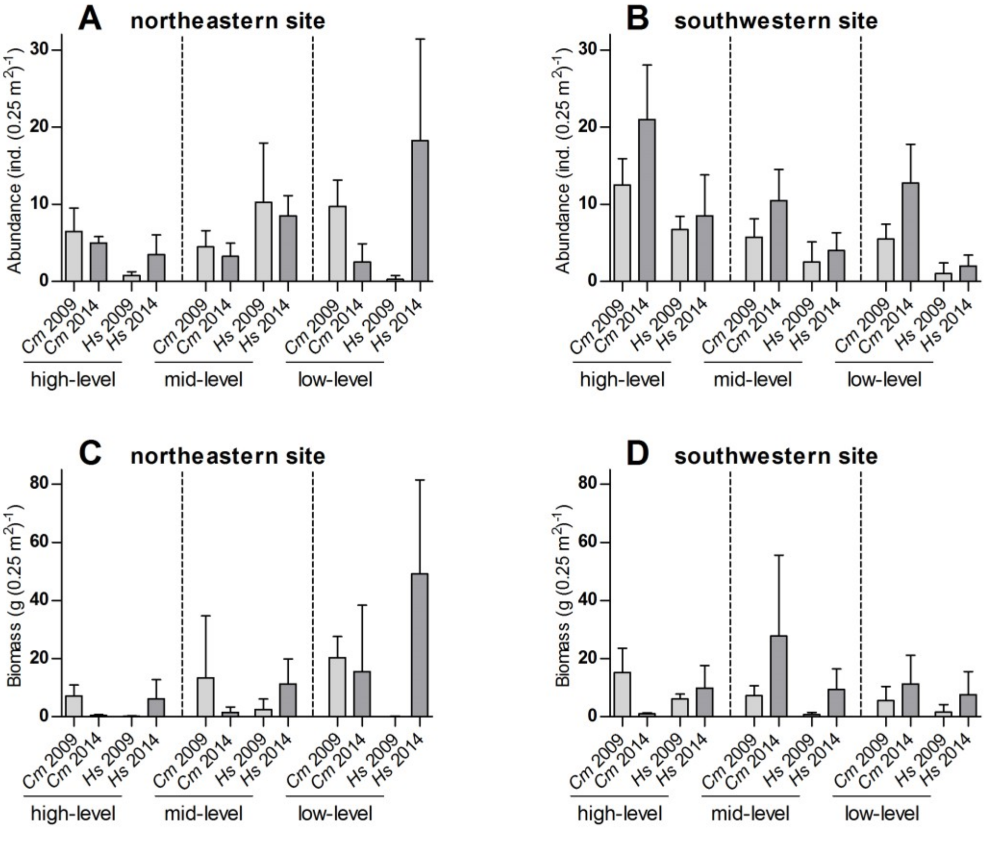

Figure 4.4: Abundances (A and B) and biomasses (C and D) of *Carcinus maenas* (*Cm*) and *Hemigrapsus sanguineus* (*Hs*) in 2009 (light grey bars) and 2014 (dark grey bars) at different intertidal shore levels at selected sites (see text) around the island of Helgoland, German Bight (southern North Sea) (means ± SD are presented).

4.5 Discussion

4.5.1 Invasion status of *Hemigrapsus sanguineus*

Numerical abundance and biomass of the invasive Asian shore crab *Hemigrapsus sanguineus* clearly increased around Helgoland from 2009 to 2014 and reached similar levels to those of the native green shore crab *Carcinus maenas*. During this period, *H. sanguineus* and *C. maenas* were the dominant decapod crustaceans in the intertidal of Helgoland. Population densities of *H. sanguineus* reached up to 144 ind./m^2 (36 ind./0.25 m^2) at the sheltered northeastern site, which represented a new maximum along European coasts. In North America, where *H. sanguineus* appeared in the late 1980s, population densities can be even higher with up to 305 ind./m^2 in Long Island Sound (Kraemer et al. 2007). Along the NW Atlantic coasts, increased abundance of *H. sanguineus* was accompanied by decreased numbers of *C. maenas,* as well as of several other species of panopeid brachyurans (Kraemer et al. 2007; O'Connor 2014). For the European coasts, this suggests that the abundance of *H. sanguineus* has not reached its maximum.

O'Connor (2014) separated the invasion process of *H. sanguineus* in Massachusetts into early, middle, and late phases. These phases were characterized by numerical dominance of local species, similar abundances, and numerical dominance of the invader species, respectively. Accordingly, the invasion at Helgoland is in the 'middle phase' because the populations of *H. sanguineus* the native *C. maenas* are of similar size. Alternatively, the possible expansion model for introduced species (Boudouresque et al. 2005) categorizes the phases after the new arrival of a species as 'naturalization', 'expansion phase' and 'persistence phase'. The presence of ovigerous *H. sanguineus* females in 2009 indicated that the species was naturalized around the island of Helgoland. The clear increase in abundance of *H. sanguineus* between 2009 and 2014 suggests the population has moved into the 'expansion phase'. Further monitoring of the population would be necessary to evaluate whether the species will attain a stable population size around Helgoland or if its population development will follow the 'boom and bust model' (Williamson 1996).

The invasion success of *H. sanguineus* in Europe may partly be due to its ability to dominate *C. maenas* in several ways. In the eastern USA, for example, *H. sanguineus* has been reported to prey on *C. maenas* to a much higher degree than the opposite, especially at the higher densities of *H. sanguineus* (Lohrer and Whitlatch 2002; Griffen and Byers 2006; Griffen and Williamson 2008). While intraspecific aggression was observed to be comparably low in *H. sanguineus* (Griffen 2006), feeding rates of adult *C. maenas* were already negatively affected by the presence of *H. sanguineus* (Griffen et al. 2008). The feeding behavior of *H. sanguineus*, however, was not affected by the presence of its presumed competitor. Nevertheless, in direct competition for food, *C. maenas* of similar mass can be superior over *H. sanguineus* (Jensen et al. 2002; MacDonald et al. 2007). In direct competition for shelter, *C. maenas* was displaced by *H. sanguineus* along the coast of New England, resulting in a higher risk of predation for the green crabs (Jensen et al. 2002). Intra-guild predation of *H. sanguineus* upon *C. maenas* as well as a higher predation risk of *C. maenas* may therefore also explain the rapid increase of the *H. sanguineus* population around Helgoland. In contrast to *H. sanguineus*, *C. maenas* also inhabits the subtidal areas around Helgoland and this refuge may supply the intertidal areas with green crab juveniles. Thus, it seems unlikely that the intertidal population of *C. maenas* will severely suffer from the *H. sanguineus* invasion.

Hemigrapsus sanguineus does not appear to be a common prey for seagulls in the Helgoland area. The piers and jetties around the island frequently show residues and carapaces of the edible crab *C. pagurus*, *C. maenas,* and the great spider crab *Hyas araneus* (Linnaeus, 1758). However, no body parts of *H. sanguineus* have been detected to date (S. Jungblut and J. Beermann pers. obs.). This is consistent with *H. sanguineus* being a cryptic species that is seldom observed outside of cover during daylight hours.

4.5.2 Size distributions

The size distributions of *H. sanguineus* and *C. maenas* differed significantly between 2009 and 2014 among three of the four locations around Helgoland. Individuals of *H. sanguineus* were on average larger (10 to 19.5 mm CW), whereas *C. maenas* were smaller. In fact, *C. maenas* showed a major recruitment event in 2014 resulting in high numbers of individuals in the smallest size class (5 to 9.5 mm CW). These very young specimens may have influenced the density measurements to a certain extent. The observed size patterns of *C. maenas* in August 2014 were similar to those reported for this species in the eastern USA during the 'middle phase' of the *H. sanguineus* invasion (Stephenson et al. 2009; O’Connor 2014). In addition to the observed shift in numerical abundance, this further supports the hypothesis that the *H. sanguineus* invasion at Helgoland has entered the ‘middle phase’ (*sensu* O’Connor 2014) or ‘expansion phase’ (*sensu* Boudouresque et al. 2005).

4.5.3 Influence of habitat and wave exposure

The densities of *H. sanguineus* at the northwestern site were comparatively low, which may be explained by the habitat structure. The northwestern site is mainly characterized by a rock plateau with boulders and cobbles. The habitats of the remaining three sites are based on coarse sands. This is consistent with previous reports that cobbles on coarse sands seem to be the preferred habitat of *H. sanguineus* in Europe (Landschoff et al. 2008; Dauvin 2009; Dauvin et al. 2009; Gittenberger et al. 2010; van den Brink et al. 2012). The generally low abundances of a non-native congener, *H. takanoi*, may be due to the lack of its preferred habitat type of muddy sediments around Helgoland (Dauvin et al. 2009, Gothland et al. 2014).

At the southeastern site, only a slight increase in biomass of *H. sanguineus* was detected from 2009 to 2014. Abundance and size distribution of *H. sanguineus* and *C. maenas* remained similar. Population growth in many decapod crustaceans is often limited by available shelter (see van den Brink et al. 2012 and references therein). The populations of both species may have reached a local carrying capacity situation at this relatively narrow and bordered site and that currently prevents *H. sanguineus* from increasing in abundance.

Although the southwestern and the northeastern sites showed a similar habitat structure and composition, they strongly differed with respect to wind exposure and, thus, wave action. The strongly exposed southwestern site seemed to be beneficial to *C. maenas*, whereas *H. sanguineus* was highly successful at the sheltered northeastern site. This pattern may partly be explained by the behavior of the megalops larvae of the two species. Unlike *C. maenas*, megalops of *H. sanguineus* respond positively to odors of adult and juvenile conspecifics (and even to odors of adult *C. maenas*) with a higher moulting and thus higher settlement rate (Zeng et al. 1997; Kopin et al. 2001; O'Connor 2007; Anderson and Epifanio 2009; Anderson et al. 2010; O'Connor and Judge 2010). The resulting 'gregarious settlement' of megalops and/or juveniles would facilitate the establishment of larger populations at more sheltered sites where odors likely persist longer in the water (Kopin et al. 2001; Steinberg et al. 2007; Anderson and Epifanio 2009; Anderson and Epifanio 2010).

Around the island of Helgoland, *H. sanguineus* was most abundant at the sheltered northeastern site. In contrast, in most other studies, *H. sanguineus* inhabited moderately to very exposed coasts and habitats with high hydrodynamic activity (Fukui 1988; Lohrer et al. 2000; Dauvin 2009; Dauvin et al. 2009; van den Brink et al. 2012). However, similar to the current findings at Helgoland, O'Connor (2014) detected higher densities of *H. sanguineus* at a more sheltered site in Narragansett Bay as opposed to more exposed sites along the outer coast of Massachusetts. The whole island of Helgoland may be regarded as highly hydrodynamic, due to its relatively remote location in the center of the German Bight. However, even in a generally high-hydrodynamic location, more sheltered sites may act as stepping-stones for *H. sanguineus* during its invasion process.

4.5.4 Effects at different tidal levels

At the highly exposed southwestern site, abundances of *H. sanguineus* decreased from higher to lower tidal levels, whereas at the sheltered northeastern location, this pattern was reversed. The intertidal vertical distribution of *H. sanguineus* has repeatedly been a matter of discussion. Previous studies have revealed no clear patterns, some of which have even produced contrasting results (Lohrer et al. 2000a, b; Lohrer and Whitlatch 2002). Lohrer et al. (2000a) therefore suggested that the vertical distribution of *H. sanguineus* may be predetermined by the structural complexity of the habitat (i.e. available shelter) rather than by tidal height. Although structural habitat complexity was not directly measured in the present study, overall habitat structures in the different tidal heights were similar between the two sites. The observed vertical distribution patterns may therefore result from the different degrees of wave exposure, although other factors, e.g. behavior, mortality or recruitment, may also be relevant (Crowe and Underwood 1998).

4.6 Conclusions

Differences in numerical abundance, biomass, and size distribution of the two crab species between the four sampling sites around Helgoland are likely caused by species-specific habitat preferences. The observed patterns may also partly result from differing intensities of wave exposure, promoting the recruitment of *H. sanguineus* megalopae in sheltered areas. The current findings resemble remarkably well the ‘mid-phase’ invasion status of *H. sanguineus* in North America, where *H. sanguineus* and *C. maenas* are both non-indigenous species. It seems the invasion process of *H. sanguineus* around Helgoland is still ongoing. As a consequence, reported interspecific competition for shelter and direct interference competition or even intra-guild predation between *H. sanguineus* and *C. maenas* will likely increase in the future, potentially revealing clearer competitive and predator-prey patterns. The temperature response to climate warming is up to four times higher in the North Sea than the global average (Belkin 2009). In addition, the increasing anthropogenic influences in coastal areas can cause severe habitat alterations (Bulleri and Chapman 2010). Both factors have the potential to change competitive relationships in favor of non-indigenous species

(Byers 2002). Invasion progression of *H. sanguineus* and possible effects on the native *C. maenas* population are thus still difficult to predict.

4.7 Acknowledgements

We thank M. Berggren (Sven Lovén Centre for Marine Sciences – Kristineberg, Fiskebäckskil, Sweden), H. Auel (Bremen Marine Ecology, University of Bremen, Bremen, Germany) and M. Molis (Alfred Wegener Institute, Bremerhaven, Germany) for communicating their personal observations on the occurrence of H. sanguineus. We also thank four reviewers for constructive comments, which greatly improved the earlier versions of the manuscript and Morgan McCarthy for proofreading the final version.

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5 Publication II

Native versus invasive brachyuran crabs in a European rocky intertidal: respiratory performance and energy demand with implications on their habitat

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under review in *Marine Biology*

Native *versus* invasive brachyuran crabs in a European rocky intertidal: respiratory performance and energy demand with implications on their habitat

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5.1 Abstract

The invasive Asian shore crab *Hemigrapsus sanguineus* is now the second most abundant intertidal crab in the North Sea behind the native European green crab *Carcinus maenas*. The respiratory performance and energy demand of individuals of both species from around the island of Helgoland, North Sea, Germany (54°11'N, 7°53E) were assessed in 2015. Respiration rates were measured in a flow-through setup between 5 and 20 °C. At lower temperatures, small and mid-sized *H. sanguineus* had similar respiration rates compared to corresponding *C. maenas* but significantly higher rates at higher temperatures. Larger crabs of either species showed no differences in respiration rates at all temperatures. Numerical models for the calculation of individual respiration rates were established and applied to the entire intertidal populations around Helgoland to compare the metabolic energy demands of both species. Abundance and biomass of *H. sanguineus* were already recorded around Helgoland in August 2014. They accounted for 21% and 59%, respectively, compared to the values of *C. maenas*. The extrapolated annual energy demand of the *H. sanguineus* population in 2014 reached, depending on the assumed diet of both species (complete herbivory *versus* complete carnivory), 76 to 119% of the value for *C. maenas*. As population densities of *H. sanguineus* are likely to increase in the North Sea, the energy demand of the invader and thus

its impact on the energy flux in the intertidal habitat will further increase. Whether this will lead to community impacts, depends on species-specific dietary preferences and remains to be investigated.

Keywords

Asian shore crab *Hemigrapsus sanguineus*, European green crab *Carcinus maenas*, southern North Sea, Helgoland, respiration, oxygen consumption

5.2 Introduction

Biological invaders are generally seen as a major threat to biodiversity in the marine realm (Grosholz 2002). As non-indigenous species, they can drastically change the native community structure and the function of an ecosystem. If the ecological impact is high, it may ultimately affect ecosystem services and thus human economic interest and health (Ruiz et al. 2000; Simberloff et al. 2013).

Brachyuran decapods represent one of the most prominent taxa, which frequently include species that invade coastal and marine ecosystems (Brockerhoff and McLay 2011). Due to their pronounced invasion potential, two of these crab species are relatively well studied: the European green crab *Carcinus maenas* and the Asian shore crab *Hemigrapsus sanguineus*. The first is native to European and African coasts of the North Atlantic. It crossed the Atlantic and invaded the coasts of North America by 1817. Subsequently, *C. maenas* appeared on coasts all around the globe, including the North American Pacific coast, South Africa, Australia, and Japan (Carlton and Cohen 2003). The latter, *H. sanguineus*, is native to Japanese, Korean, and Chinese coasts. It invaded the Atlantic coasts of North America in the late 1980´s and the European Atlantic coasts in the late 1990´s (Stephenson et al. 2009). On the Atlantic coasts of North America, *H. sanguineus* can be found from Cape Hatteras in North Carolina up to the Schoodic Peninsula in Maine (Williams and McDermott 1990; Delaney et al. 2008; Epifanio 2013). Its current European distribution ranges from the French Atlantic coast to the German Wadden Sea (Dauvin et al. 2009). A few specimens were also reported in the Adriatic Sea, the Black Sea and the Swedish Skagerrak (Schubart 2003; Micu et al. 2010; M. Berggren, Sven Lovén Centre for Marine Sciences – Kristineberg, Fiskebäckskil, Sweden, pers comm).

The importance of *C. maenas* in native as well as invaded areas stimulated investigations, which yielded a substantial amount of ecological and physiological data (e.g. Crothers 1967; Roman and Palumbi 2004; Breen and Metaxas 2012 and references therein).

Ecological information on *H. sanguineus* is growing, mostly from areas, where increasing numbers of *H. sanguineus* share habitat and possibly compete for resources with *C. maenas* (for native areas, e.g. Fukui 1988; Lohrer et al. 2000; for invaded areas, e.g. McDermott 1998; Jensen et al. 2002; Lohrer and Whitlatch 2002; Epifanio 2013 and references therein; Jungblut et al. 2017). However, compared to the available ecological information, physiological data of *H. sanguineus* are rather scarce (Breen and Metaxas 2012) and essentially all of these investigations stem from the invaded U.S. east coast. For *H. sanguineus*, changes in diet preferences correlated with season and crab size (Griffen et al. 2012). *Hemigrapsus sanguineus* was also identified as an income breeder, meaning it utilizes energy ingested during reproductive times directly for egg production (Griffen et al. 2012). *Carcinus maenas*, in contrast, is known as a capital breeder, who stores dietary energy first in the hepatopancreas, before allocating it to the ovaries for egg production (Griffen et al. 2011). Respiration rates and physiological condition of *H. sanguineus* were measured in relation to consumed food (Griffen et al. 2015). The metabolic rate of *H. sanguineus* increased with the amount of animal diet, due to increasing costs in digestion, but it was not influenced by algal diet. The two condition parameters, hepatosomatic index and total lipid content of the hepatopancreas were positively correlated. Both increased with increasing mussel consumption, but not with increasing algae consumption (Griffen et al. 2015).

Respiration, integrating all physiological processes of an organism, can serve as a proxy of the energetic impact that an organism has on its environment via food uptake (Brey 2010). Physiological processes include transformation of energy and matter. They require the uptake of O_2 (respiration) and release CO_2 (Ikeda et al. 2000). Hence, respiration rates can be used to calculate the carbon uptake, metabolic energy demand and, thus, the energetic impact of an organism on its habitat in terms of food uptake (Ikeda et al. 2000). Body mass of an organism and ambient temperature are the most important predictors for estimating aquatic invertebrate respiration rates (Brey 2010). Models for the calculation of respiration from mass and temperature are available for marine pelagic copepods, for calanoid copepods, euphausiids, and some decapod species (e.g. Crear and Forteath 2001; Ikeda et al. 2007; Bode et al. 2013; Ikeda 2013).

We measured respiration rates of *C. maenas* and *H. sanguineus* in a flow-through system using optical sensors. Crabs of both sexes and different biomass were incubated at four ambient temperatures between 5 °C and 20 °C. First, the generated data were used to compare the oxygen uptake of mass-standardized crabs. In a second approach, we established temperature and mass-dependent respiration models for both species. These equations were used to calculate the energy demands of the intertidal populations of both species around the island of Helgoland, Germany, as a measure of their energetic impact on their habitat and to assess the contribution of the two species to the energy flow.

5.3 Material and Methods

5.3.1 Origin of animals

Carcinus maenas and *Hemigrapsus sanguineus* (carapace widths 10 to 40 mm) were collected in April, June, and August 2015 during low tide at an intertidal site at the southwestern tip ('Kringel') on the island of Helgoland, southern North Sea, Germany (Fig. 5.1). This site is wave-exposed and the intertidal is composed of many small and large rocks, boulders, as well as coarse sand patches. Hard structures were covered by small to medium-sized macroalgae. Dominating species were: *Ulva* spp., *Enteromorpha* spp., *Fucus serratus*, *Chondrus crispus*, and *Mastocarpu*s *stellatus*. For details refer to Bartsch and Tittley (2004).

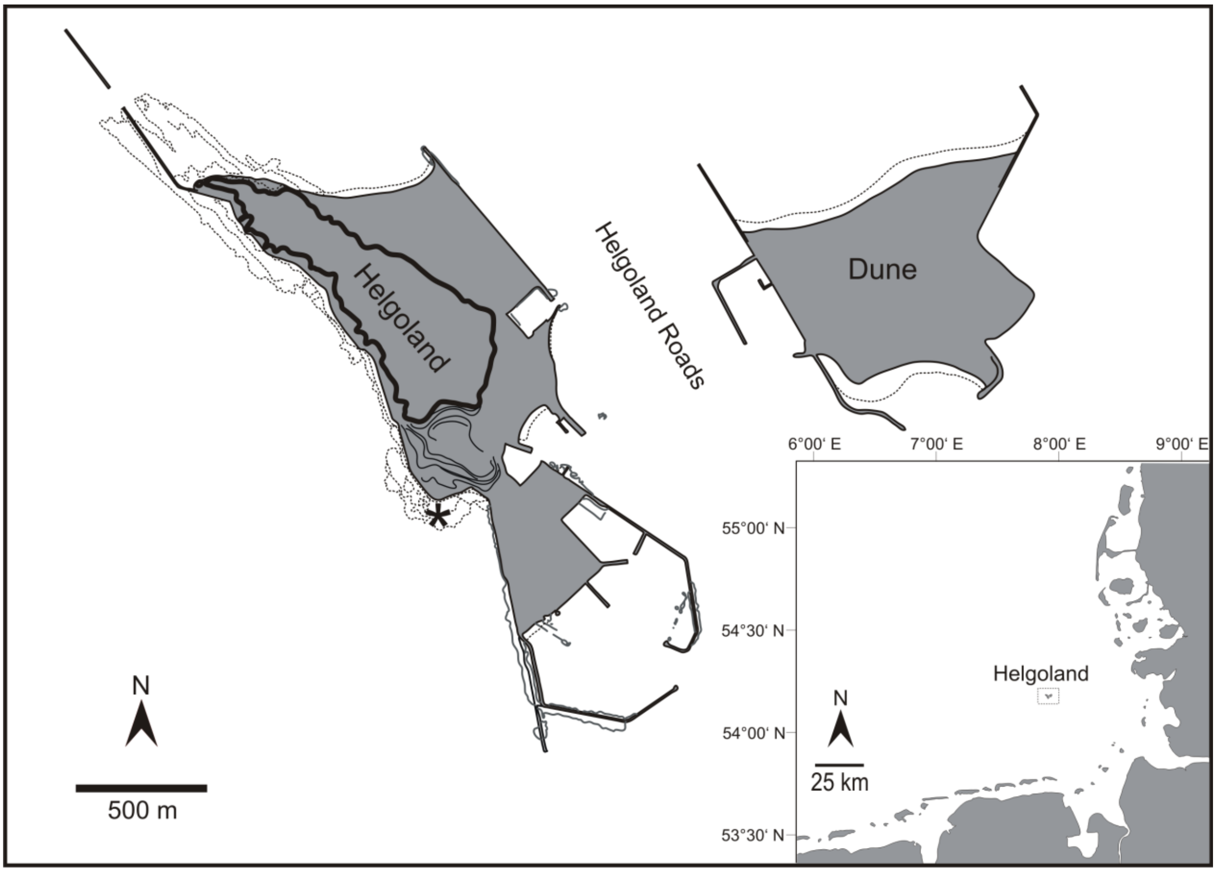

Fig. 5.1: The island of Helgoland and its location in the German Bight, southern North Sea (54°11'N, 7°53E). The sampling area at the southwestern tip ('Kringel') of the island is indicated by an asterisk (from Beermann 2014, adapted, with permission from Elsevier)

5.3.2 Respiration measurements

After sampling, the animals were immediately transferred to the laboratories of the Marine Station and kept for 24 h in plastic aquaria in the dark at 10 °C and a salinity of approx. 32.5 to standardize hunger levels and to let the crabs clear their gut. The carapace width of each crab was measured by Vernier calipers to the nearest 0.5 mm. The animals were carefully blotted dry with paper towels for 10 s and weighed to the nearest 0.001 g to obtain their fresh mass.

The oxygen consumption of crabs was measured with a 10-channel Fiber-Optic Oxygen Meter (OXY-10, PreSens, Precision Sensing GmbH, Regensburg, Germany) in a flow-through setup. Eight cylindrical acrylic glass respiration chambers (approx. 475 mL each) were used. In each series of measurements, one chamber was randomly selected to serve as a control chamber without an animal. For small crabs with less than a 15 mm carapace width, chamber volumes were reduced to approx. 190 mL by inserting a plastic plate. Each series of measurements (seven crabs and a control) was performed in the dark at consecutively increasing temperatures of 5, 10, 15, and 20 °C. The crabs remained in the chambers during the transition to the next highest temperature - usually taking about 5 min. The seawater (salinity approx. 32.5) used for the measurements was stored in 100-L tanks in each of four temperature-controlled rooms. The water was continuously aerated and the temperature never deviated more than ± 0.5 °C from the target temperature. The respiration chambers were placed on a laboratory trolley. To measure a set of crabs at any of the temperature levels, the laboratory trolley was rolled into the respective temperature-controlled room, aeration of the pre-tempered water was switched off, and the supply hose of the flow-through device was connected with the tank. Care was taken that no air bubbles entered the hose. The water flow through the entire experimental setup is presented in Fig. 5.2a. At the start of a measurement, the hose pump was switched on and the velocity was adjusted according to the approximate size of the crabs in the chambers. The flow rates were determined in preceding experiments. The tempered water flowed through the supply hose passing an oxygen-sensitive and fluorescent foil that was glued to the inner side of an acrylic holder (Fig. 5.2b, inflow measuring point). Directly behind this point, the tube branched out to supply the eight respiration chambers with water (Fig. 5.2c). Each chamber contained one crab (except for the control chamber). Behind each chamber, the water passed another oxygen-sensitive foil (outflow measuring point for each chamber), through the hose pump, and was directed to the sink. On the outside of each acrylic holder, a fiber optic cable was placed directly opposite of the oxygen-sensitive foil. The cable transmitted a light impulse from a 10-channel Fiber-Optic

Oxygen Meter to the foil and the emitting light, which is proportional to the oxygen concentration of the water, back to the device. The oxygen meter was connected to a laptop computer where the measurements of all nine channels (one entrance and eight exit measuring points) were recorded. The optical oxygen concentration measurements were performed once a minute for eight hours in total per temperature level. Prior to each set of measuring trials, the oxygen meter was calibrated according to the manufacturer's instructions. Oxygen concentrations below 70 % were avoided by increasing the velocity of the hose pump. Adjustments were always completed within the first two hours of each trial. In total, 63 *C. maenas* (31 males, 32 females) and 64 *H. sanguineus* (32 males, 32 females) were measured at the four different temperatures.

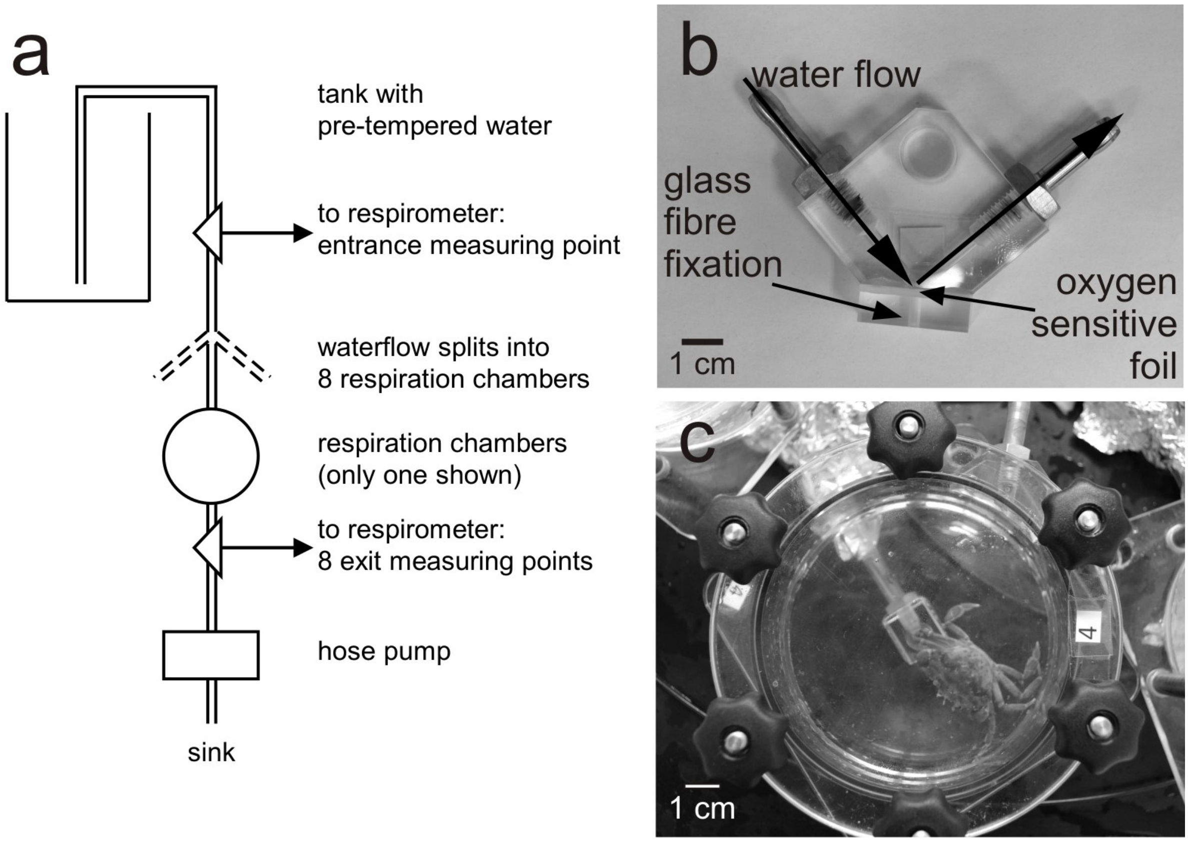

Fig. 5.2: Experimental setup; a) Water flow during respiration measurements. Tubes between the two measuring points were gas-tight, b) Acrylic holder for oxygen-sensitive foil (photo: S. Jungblut). c) Respiration chamber with *Carcinus maenas* inside (photo: S. Jungblut)

5.3.3 Data processing

The oxygen concentrations of the seawater were measured at the joint inflow and at the outflow of each respiration chamber and the differences were calculated. The recorded oxygen consumption rates of the crabs in the chambers were corrected for unspecific signal fluctuation by comparison with the signal from the control chamber. Preliminary measurements showed that within three hours, oxygen concentration readings reached a plateau. This indicates that the water in the respiration chambers and the metabolic rates of the crabs had acclimated to the new temperature. Thus, these first three hours of each measurement were omitted to account for handling stress and temperature acclimation. The remaining five hours of data collection provided about 300 measurements per crab and temperature. All values were standardized to the fresh mass of the respective animal.

Data were processed and figures were produced with the GraphPad Prism software (version 5.03). For each set of about 300 measurements per crab and temperature, a frequency distribution was established. Bin width of the frequency distributions was set to 1 and the lower limit to 0. Natural logarithms of the median values of these frequency distributions were used to obtain linear regressions between mass-specific respiration and crab mass for each incubation temperature. These linear regressions were used to calculate individual respiration rates of standardized crabs of 0.5, 5, and 10 g mass at each temperature. For these rates, Q_{10} temperature coefficients were calculated with van't Hoff's generalization:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)},$$

R_1 and R_2 are the metabolic rates, in this case respiration rates, and T_1 and T_2 the corresponding temperatures (Ikeda et al. 2000; Willmer et al. 2000).

5.3.4 Statistical analysis and respiration models

The O_2 consumption was modelled as a function of the following main effects: species (*C. maenas* and *H. sanguineus*), month (April, June, and August), sex (male and female), temperature (5, 10, 15, and 20 °C as a covariate) and individual fresh mass (as covariate). During the modelling process the factors sex and month were found to be not significant for the different crab groups (see section 'Results') and these factors were omitted from the model. Empirical regression models were described in detail for zooplankton organisms. In these models, R represents the respiration rate, m the individual´s biomass and T the temperature. Derived from the empirical relationships between respiration and biomass, $R = a \cdot m^b$ and $R = c \cdot d^T$ (with a, b, c, d as constants), the regression model is as follows:

$$\ln R = a^0 + a^1 \cdot \ln m + a^2 \cdot T$$

(e.g. Ikeda 1985; Ikeda et al. 2007; Bode et al. 2013; Ikeda 2013). In the main model, all factors were included. For the establishment of the respiration models, however, we constructed two species-specific models, which only included the factors mass and temperature. All statistical models were fitted in R, version 3.2.3 (R Development Core Team

2015) using the functions ‘lmer’ from the package ‘lme4’ (Bates et al. 2015) and ‘lm’ from the generic stats package for the reference models. In all models, we checked the following diagnostics for model stability: Cook’s distance and mdffits from the package ‘HMLdiag’ for lmerMod class objects in R (Loy and Hofmann 2014). In all models, the checks confirmed the absence of influential cases.

Main model

The main model included three interactions of first order: we combined species with either sex, temperature or ln body mass, in order to test, whether these three factors were affecting the respiration differently in the two species.

Each individual was measured consecutively at each of the four temperatures. Since these measurements represent longitudinal data, and consecutive data points are dependent data, we treated temperature as a within-subject’s factor and included temporal nesting into the model by including a random factor, where individual is nested in time. Although the factor month was non-significant, the individuals used in the present experiment were still collected in three seasonally distinct batches. Hence, it seems reasonable to include ‘month’ as a random factor in order to control for any grouping effects over season. Because the temporal nesting was individual-specific, the seasonal grouping effect was already accounted for by the random nested term. For the model defined above, the choice of a general linear mixed effects model (LMM) was most appropriate.

We fitted the model using the maximum-likelihood method. With the ln transformed response variable (see above) we met the assumptions of normal distribution and homogeneous variances of the residuals. In order to establish the significance of individual terms, we conducted likelihood ratio tests (LRT) using the R-function ‘anova’ with the argument ‘test’ set to ‘F’. Hereby, we compared the deviances of the respective full model with the corresponding reduced model not comprising the respective factor and/or term of interest. When testing for main factors, which were combined into a random factor, we created reference models, from which we omitted the respective random factors and subsequently applied the LRT for our term of interest (e.g. species and temperature). Non-significant interactions and terms were removed from the full model before (main factors sex and month, see ‘Results’).

Respiration models

The data were also used to establish mass and temperature specific respiration models for each species, *C. maenas* and *H. sanguineus*. Therefore, we separated the final model into two species-specific models. Both included only the main factors temperature and ln of mass (which revealed to be significant), as well as the random within-subjects-factor (individual nested in temperature). The coefficients of each model were then used to establish a respiration model for rates of the two individual species from habitats where information on temperature and biomasses were available. Thereby, however, we used only the coefficients of the fixed effects (i.e. intercept and slopes for temperature and mass) which could then be multiplied with empirical measurements of temperature and ln of mass. For the final predictions in the actual scale, the inverse natural exponential function was applied to the ln transformed predictions. We omitted the random term (subject nested in temperature) in the equation for the calculations of respiration rates, because they are only relevant for the present experimental design. In fact, future data might be collected in other months than those given in the present model. In addition, the estimates should not be specific for individual animals that were used in the present experiment, but should reflect individuals of a certain species in general.

5.3.5 Energy demands of the Helgoland crab populations

We assessed the energetic impact, which the *C. maenas* and *H. sanguineus* populations imply on their rocky intertidal habitat around the island of Helgoland via their food uptake, similar to a procedure used by Hu et al. (2012). Thereby, the respiration and energy demands of both populations were calculated. These calculations were based on the respiration models developed in this study, as well as the abundance and biomass data of both species from the four main intertidal sites in August 2014 (see Jungblut et al. 2017; Table 5.1). For the calculation of a crab´s energy demand, each individual was assigned its theoretical species-specific respiration via the respective respiration model. The average water temperature in August 2014 was 18.4 ± 0.8 °C (mean ± SD, water temperature data of the Helgoland Ferrybox downloaded from the COSYNA data web portal http://codm.hzg.de/codm/, 12 November 2016). Considering its mass, the total daily respiration of each crab was calculated. By multiplication with a given respiratory coefficient, the daily respiration was transformed into the daily CO_2 release (Gnaiger 1983). Respiratory coefficients depend on the ingested food. Herbivorous diets yield coefficients close to 1, while carnivorous diets yield a value close to 0.8 (Willmer et al. 2000). Since stomach content analyses were not yet performed for both species around Helgoland, we calculated the daily energy demand for both extremes. The daily CO_2 release was multiplied by the molar mass of carbon (12 $g \cdot mol^{-1}$) to obtain the daily carbon uptake. Because 1 g organic carbon equals 41.4 kJ stored energy (Salonen et al. 1976), the daily carbon uptake was converted into the daily energy demand of the respective crab. Thus, the daily metabolic energy demand E ($kJ \cdot d^{-1}$) for each crab can be calculated:

$$E = R \cdot m \cdot RQ \cdot 12 \cdot 10^{-6} \cdot 41.4,$$

with R ($\mu mol \cdot g^{-1} \cdot d^{-1}$) as the previously calculated respiration rate of the crab, the crabs body mass m (g) and the respiratory quotient RQ (ratio, no unit). For both species, the daily energy demand was calculated per square meter at each site, in total per site, and for the whole intertidal populations of both crab species around Helgoland. The intertidal areas (excluding large tide pools and concrete blocks) of the four main sites (*sensu* Jungblut et al. 2017) were calculated on the basis of the GIS map published by Bartsch and Tittley (2004) (H. Pehlke, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, pers comm).

Table 5.1: Intertidal sites around the island of Helgoland, Germany, abundance, and biomass of *Carcinus maenas* and *Hemigrapsus sanguineus* in August 2014

sites (*sensu* Jungblut et al. 2017)	**sites and habitats (*sensu* Bartsch and Tittley 2004)**	**total area (m²)**	**abundance**				**biomass**			
			C. maenas		***H. sanguineus***		***C. maenas***		***H. sanguineus***	
			($ind \cdot m^{-2}$)	**tot. (ind.)**	**($ind \cdot m^{-2}$)**	**tot. (ind.)**	**($g \cdot m^{-2}$)**	**tot. (kg)**	**($g \cdot m^{-2}$)**	**tot. (kg)**
northwestern	wave-cut platforms NE and W; red sandstone ridges, boulder fields	145,207	26.0	3,775,382	3.3	479,183	17.1	2,483.0	8.4	1,219.7
northeastern	boulder field east of "beach N"	763	14.3	10,911	40.3	30,749	23.3	17.8	88.8	67.8
southwestern	\`Kringel\`-area; red sandstone ridge with boulders	37,255	59.0	2,198,045	19.3	719,022	53.3	1,985.7	35.9	1,337.5
southeastern	boulder field south of "landing pier"	2,396	17.0	40,732	16.0	38336	11.6	27.8	24.2	2,682.9
total		185,621		6,025,070		1,267,290		4,514.3		2,682.9

In the same way as described above, we calculated the metabolic energy demand for the other months and the cumulative value of the year 2014 for both species. Most recent crab abundance and biomass data were those from August 2014 (Jungblut et al. 2017). Since no data are available for the other months of 2014 or some few years before, we assumed stable populations as described for August 2014 and used monthly means of seawater temperature for the calculations (seawater temperature data of the lightship "German Bight" downloaded from the COSYNA data web portal http://codm.hzg.de/codm/, 18 February 2017). For the months January, February, November, and December we excluded *C. maenas* from the calculations, as this species migrates to the subtidal in winter and, thus, does not contribute to the energy flux in the intertidal (Janke 1986; Aagaard et al. 1995; S. Jungblut, personal observation). In contrast, *H. sanguineus* is present in the intertidal all around the year (S. Jungblut, personal observation).

5.4 Results

5.4.1 Oxygen consumption

Respiration rates were measured in April, June, and August 2015. No seasonality was apparent, when *Carcinus maenas* or *Hemigrapsus sanguineus* data were treated separately or both species combined were tested, (LRT_{Month}: df = 2, $\chi^2 = 4.3978$, p = 0.1109 for *C. maenas*, LRT_{Month}: df = 2, $\chi^2 = 5.8441$, p = 0.0538 for *H. sanguineus*, LRT_{Month}: df = 2, $\chi^2 = 4.3606$, p = 0.1130 for both species combined). Likewise, differences between sexes and interactions between sexes and species were not significant (LRT_{Sex}: df = 1, $\chi^2 = 0.686$, p = 0.4075 and $LRT_{Sex*Species}$: df = 1, $\chi^2 = 0.3654$, p = 0.5455).

Overall, the native *C. maenas* showed slightly higher respiration rates than did the non-native *H. sanguineus* ($LRT_{Species}$: df = 1, $\chi^2 = 8.6594$, p = 0.0033), and both species showed an increase in respiration with increasing temperatures ($LRT_{Temperature}$: df = 1, $\chi^2 = 151.21$, $p < 0.0001$) (Fig. 5.3). The interaction between species and temperature was determined as the only relevant interaction. It showed that respiration rates increased with temperature at a higher rate in *H. sanguineus* than in *C. maenas* ($LRT_{Species*Temperature}$: df = 1, $\chi^2 = 4.5865$, p = 0.0322). Furthermore, respiration rates showed a negative relationship with body mass (LRT_{Mass}: df = 1, $\chi^2 = 62.591$, $p < 0.0001$). Respiration rates were mostly dependent on temperature and mass and less on the two species or the interaction between species and temperature.

Table 5.2: Regression parameters of mass-dependent respiration measurements of *Carcinus maenas* and *Hemigrapsus sanguineus* at different temperatures: *m* represents the crabs fresh mass (g) and *R* the corresponding respiration rate ($nmol \cdot d^{-1} \cdot g^{-1}$)

Temperature (°C)	***C. maenas***	***H. sanguineus***
5	$R = -0.095 \cdot m + 10.71$	$R = -0.044 \cdot m + 10.59$
10	$R = -0.084 \cdot m + 10.98$	$R = -0.097 \cdot m + 11.15$
15	$R = -0.065 \cdot m + 11.21$	$R = -0.092 \cdot m + 11.72$
20	$R = -0.039 \cdot m + 11.44$	$R = -0.079 \cdot m + 11.94$

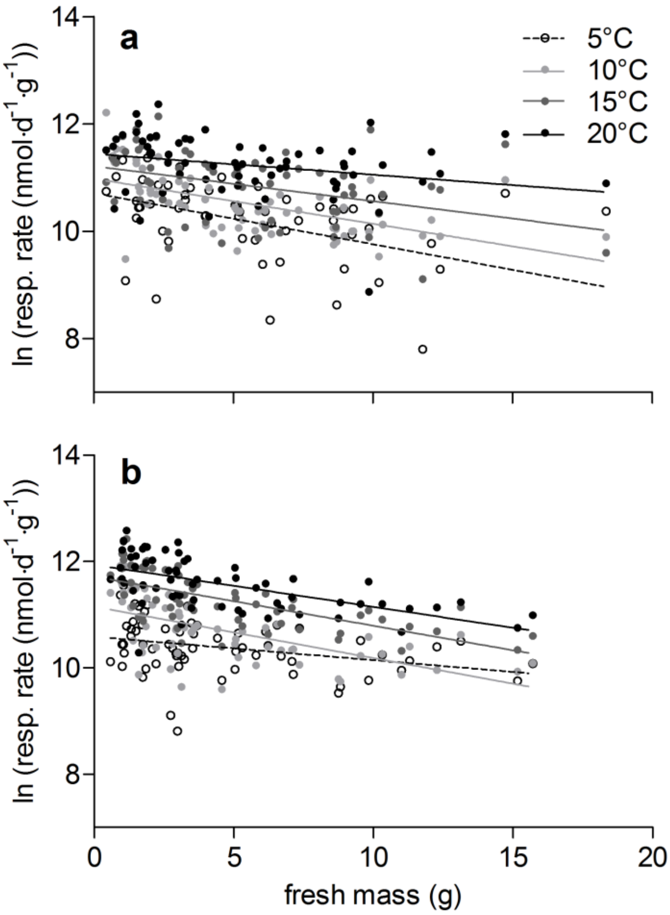

Fig. 5.3: Median respiration rates for a) *Carcinus maenas* (n = 63 ind.) and b) *Hemigrapsus sanguineus* (n = 64 ind.) at 5, 10, 15, and 20 °C. The equations of the linear regressions are presented in Table 5.2. Note that one data point measured at 5 °C is not shown for *C. maenas*: fresh mass = 8.67 g, resp. rate = 4.47 nmol·d^{-1}·g^{-1}

Linear regression lines between the crab´s wet mass and the natural logarithm of the corresponding respiration rates were almost parallel and increased with increasing temperatures. The regression slope of *H. sanguineus* measured at 5 °C was more even and crossed the 10 °C regression at approximately 11 g body mass (Table 5.2, Fig. 5.3).

The specific respiration rates of the standardized crabs increased with temperature and decreased with body mass (Fig. 5.4). At 5 °C and 10 °C, respiration rates were similar in both species and all sizes. At 15 °C and 20 °C, however, the specific respiration rates of small and medium-sized *H. sanguineus* were significantly higher than those of *C. maenas* (Fig. 5.4a and b). For the smallest size class of *H. sanguineus*, the increase in respiration between 15 °C and 20 °C was noticeably smaller than the increase from 10 °C to 15 °C. The differences between both species and between the temperatures are also mirrored in the Q_{10} values (Table 5.3).

5.4.2 Respiration models

The coefficients of the two species-specific statistical models were used to construct two models for the calculation of crab respiration rates. Here, R represents the individual respiration rate (nmol·g^{-1}·d^{-1}), m the crab´s body mass (wet mass, g) and T the environmental temperature (°C):

a) $\ln R = 10.39 + (-0.34 \cdot \ln m) + (0.06 \cdot T)$ for *C. maenas*

b) $\ln R = 10.42 + (-0.39 \cdot \ln m) + (0.08 \cdot T)$ for *H. sanguineus*.

The resulting Q_{10} values of the models were 1.82 for *C. maenas* and 2.23 for *H. sanguineus*.

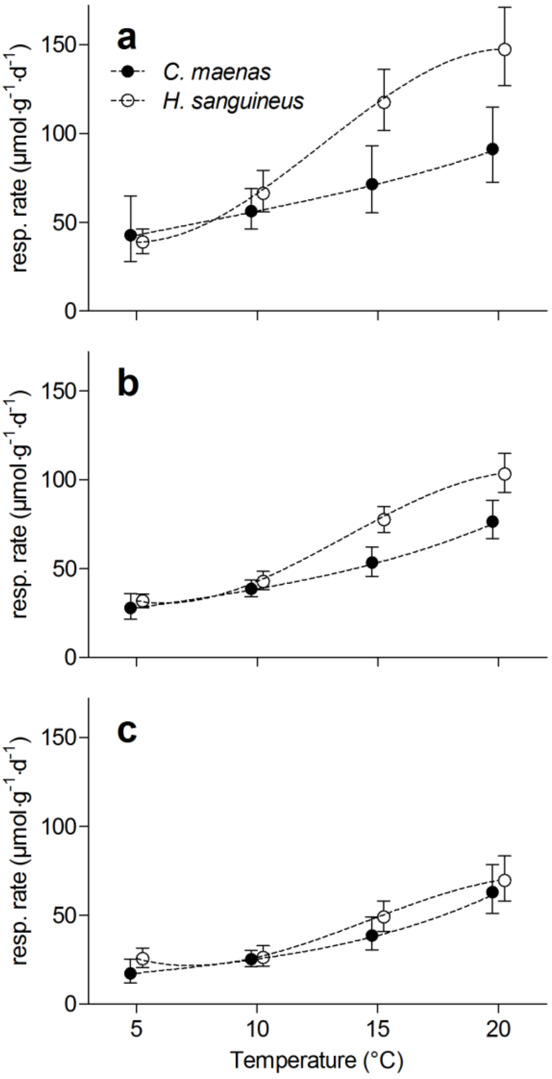

Fig. 5.4: Specific respiration rates of standardized *Carcinus maenas* and *Hemigrapsus sanguineus* of a) 0.5 g, b) 5 g, and c) 10 g wet mass. The rates were calculated from the regressions of respiration measurements at 5, 10, 15, and 20 °C (Table 5.2). Error bars represent 95 % confidence intervals

Table 5.3: Q_{10} values for standardized *Carcinus maenas* and *Hemigrapsus sanguineus* of 0.5, 5, and 10 g biomass, calculated from respiration measurements at 5, 10, 15, and 20 °C

Mass (g)	Temperature (°C)	Q_{10} *C. maenas*	Q_{10} *H. sanguineus*
0.5	5 to 10	1.73	2.91
	10 to 15	1.61	3.14
	15 to 20	1.63	1.57
5	5 to 10	1.92	1.80
	10 to 15	1.92	3.29
	15 to 20	2.05	1.77
10	5 to 10	2.14	1.06
	10 to 15	2.32	3.46
	15 to 20	2.66	2.01

5.4.3 Energy demands of the Helgoland crab populations

The respiration models were used to calculate respiration and energy demands of the intertidal populations of *C. maenas* and *H. sanguineus* around the island of Helgoland, Germany. Detailed information about abundance and biomass of the four main intertidal areas are available for August 2014 (see Jungblut et al. 2017, Table 5.1). Total abundance and biomass of *H. sanguineus* around Helgoland comprised about 21 % and 59 % of the values for *C. maenas*, respectively.

Depending on the animal density, respiration rates of *C. maenas* varied from 0.9 to 3.2 mmol $O_2 \cdot m^{-2} \cdot d^{-1}$ and those of *H. sanguineus* ranged from 0.8 to 8.3 mmol $O_2 \cdot m^{-2} \cdot d^{-1}$ (Table 5.4). Total respiration of the entire intertidal crab populations around Helgoland comprised about 343 mol $O_2 \cdot d^{-1}$ for *C. maenas* and about 267 mol $O_2 \cdot d^{-1}$ for *H. sanguineus*. Assuming a more carnivorous diet (RQ = 0.8), the carbon uptake rates of *C. maenas* varied from 8.4 to 30.8 mg $C \cdot m^{-2} \cdot d^{-1}$. For *H. sanguineus* the values ranged between 7.7 and 80.1 mg $C \cdot m^{-2} \cdot d^{-1}$. On the basis of an herbivorous diet (RQ = 1.0), the carbon uptake rates varied from 10.4 to 38.5 mg $C \cdot m^{-2} \cdot d^{-1}$ and 9.7 to 100.1 mg $C \cdot m^{-2} \cdot d^{-1}$ for *C. maenas* and *H. sanguineus*, respectively. For the entire Helgoland intertidal, carnivorous *C. maenas* would consume about 3293 g $C \cdot d^{-1}$, whereas herbivorous *C. maenas* would require about 4116 g $C \cdot d^{-1}$. The daily carbon uptake of *H. sanguineus* would amount to about 2561 g $C \cdot d^{-1}$ in case of a carnivorous diet and to 3201 g $C \cdot d^{-1}$ in case of a primarily herbivorous diet (Table 5.4). Correspondingly, the energy demands varied from 0.4 to 1.3 $kJ \cdot m^{-2} \cdot d^{-1}$ for carnivorous *C. maenas* and 0.4 to 1.6 $kJ \cdot m^{-2} \cdot d^{-1}$ for herbivorous *C. maenas*. *H. sanguineus* requires between 0.3 and 3.3 $kJ \cdot m^{-2} \cdot d^{-1}$ and 0.4 and 4.2 $kJ \cdot m^{-2} \cdot d^{-1}$, being carnivorous or herbivorous, respectively (Table 5.4). For the whole intertidal area of Helgoland, the total energy demands amounted to 136,314 and 106,017 kJ·d^{-1} for *C. maenas* and *H. sanguineus*, respectively, assuming a mostly carnivorous diet (respiratory quotient 0.8). On the basis of a mostly herbivorous diet (respiratory quotient 1.0), the corresponding values are higher: 170,393 and 132,521 kJ·d^{-1} for *C. maenas* and *H. sanguineus*, respectively (Table 5.4). For both feeding modes, the total energy demand of *H. sanguineus* in August 2014 was thus about 62 to 97% of those calculated for *C. maenas*.

The trends of the energy demands of the two crab populations were almost parallel over the year, with *H. sanguineus* on a lower level than *C. maenas*, and well resembling the temperature trend during the course of the year (Fig. 5.5a). The energy demand of both populations was also calculated cumulatively over the year, i.e. the monthly values were subsequently added. The cumulative energy demand of the *C. maenas* population increased faster over the year than that of the *H. sanguineus* population (Fig. 5.5b). While *C. maenas* is usually present in the intertidal only from March until October, *H. sanguineus* is present year-round. The cumulative energy demands of both species were similar at the end of the year. Depending on the respiratory quotient, the *C. maenas* population required between 26,370 and 32,970 MJ per year, whereas the *H. sanguineus* population consumed between 25,120 and 31,400 MJ per year. Thus, the cumulative energy demand of the whole *H. sanguineus* population reaches about 76 to 119% of the value for the whole *C. maenas* population.

Table 5.4: Respiration, carbon uptake and energy demand of the intertidal populations of *Carcinus maenas* and *Hemigrapsus sanguineus* at four sites around the island of Helgoland in August 2014. Carbon uptake and energy demand were calculated for respiratory quotients of $RQ = 0.8$ (complete carnivory) and $RQ = 1.0$ (complete herbivory)

sites (*sensu* Jungblut et al. 2017)	**population respiration**		**population carbon uptake (for $RQ = 0.8$)**		**population carbon uptake (for $RQ = 1.0$)**		**population energy demand (for $RQ = 0.8$)**		**population energy demand (for $RQ = 1.0$)**	
	per m^2 (mmol O$_2$·m^{-2} ·d^{-1})		**per m^2 (mg C·m^{-2}·d^{-1})**		**per m^2 (mg C·m^{-2}·d^{-1})**		**per m^2 (kJ·m^{-2}·d^{-1})**		**per m^2 (kJ·m^{-2}·d^{-1})**	
	C. maenas	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***
northwestern	1.5	0.8	14.6	7.7	18.2	9.7	0.6	0.3	0.8	0.4
northeastern	1.1	8.3	10.7	80.1	13.4	100.1	0.4	3.3	0.6	4.2
southwestern	3.2	3.7	30.8	35.3	38.5	44.1	1.3	1.5	1.6	1.8
southeastern	0.9	2.7	8.4	25.4	10.4	31.8	0.4	1.1	0.4	1.3
	total (mol O$_2$·d^{-1})		**total (g C·d^{-1})**		**total (g C·d^{-1})**		**total (kJ·d^{-1})**		**total (kJ·d^{-1})**	
	C. maenas	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***	***C. maenas***	***H. sanguineus***
northwestern	220.7	117.1	2,118.6	1,124.4	2,648.2	1,405.5	87,708	46,551	109,636	58,189
northeastern	0.9	6.4	8.2	61.1	10.2	76.4	339	2,530	424	3,163
southwestern	119.4	136.9	1,145.9	1,314.3	1,432.3	1,642.9	47,438	54,414	59,298	68,017
southeastern	2.1	6.3	20.0	60.9	25.0	76.1	828	2,521	1,036	3,151
total	343.1	266.7	3,292.6	2,560.8	4,115.8	3,201.0	136,314	106,017	170,393	132,521

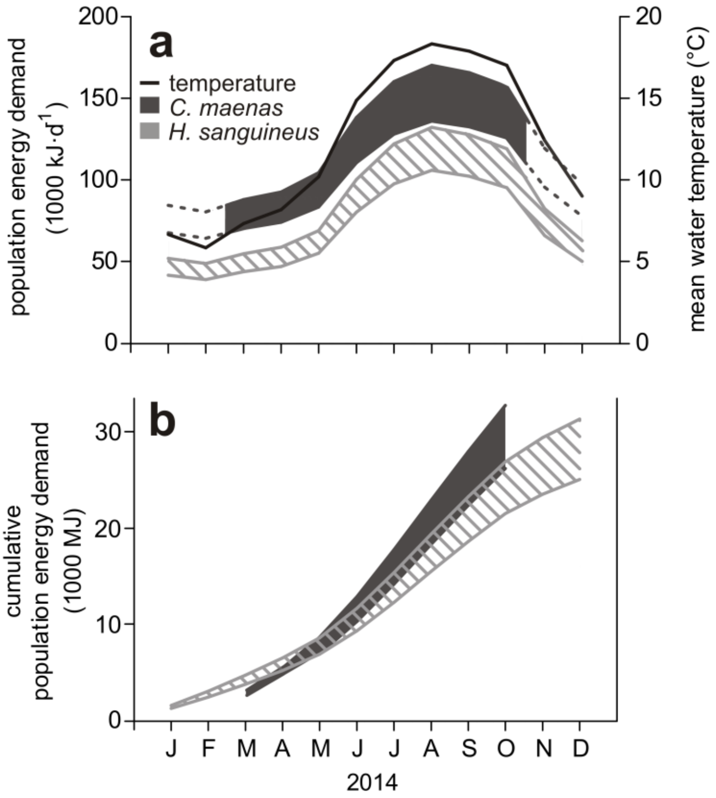

Fig. 5.5: Population energy demand of *Carcinus maenas* and *Hemigrapsus sanguineus* and mean water temperatures around Helgoland in 2014. Energy demands were calculated for respiratory quotients of 0.8 (carnivory, lower margin of each band) and 1.0 (herbivory, upper margin of each band); a) Energy demand per month. *C. maenas* in winter months is shown in dashed lines to illustrate their absence from the intertidal area, b) Cumulative energy demand

5.5 Discussion

Respiration rates of *Carcinus maenas* and *Hemigrapsus sanguineus* differed significantly in their relations between animal size and temperature. A positive exponential correlation between respiration and temperature was present in all size classes of *C. maenas*, however, this was not the case in *H. sanguineus*. On the other hand, both species showed an inverse exponential correlation between mass-specific respiration and body mass, as reported for other crustaceans (Du Preez 1983; Cockcroft and Wooldridge 1985; Booth and McMahon 1992; Robertson et al. 2002; Brey 2010).

In both species, no significant difference in respiration was evident between sexes. Similarly, no significant differences were observed between seasons, as for example noted for Northern krill *Meganyctiphanes norvegica* from the Ligurian Sea, Mediterranean (Saborowski et al. 2002). Overall respiration rates resembled those of other brachyurans, e.g. *Ovalipes punctatus*, *Callinectes sapidus*, *C. maenas*, *Hemigrapsus takanoi* (Du Preez 1983; Houlihan et al. 1990; Booth and McMahon 1992; Robertson et al. 2002; Shinji et al. 2009).

5.5.1 Respiration-temperature dependence

The measurements for this study were performed in temperature-controlled rooms in darkness without external stimuli and will thus mainly reflect standard respiration rates. However, the physical activity of the crabs was not controlled and, therefore, the obtained respiration curves could reflect a combination of standard and routine metabolism, which would lead to higher respirations values than pure standard respiration rates (e.g. Du Preez 1983). The classical thermodynamic relationship between standard respiration and temperature describes an exponential increase of oxygen consumption towards a maximum and a subsequent drop beyond the critical temperature due to biochemical and biophysical limitations (e.g. Pörtner 2002; Frederich and Pörtner 2000; Verberk et al. 2016). *C. maenas* of all size classes closely followed this relationship and showed quite uniform Q_{10} values around 2 (Fig. 5.4, Table 5.3), which are slightly higher than those reported for *C. maenas* by Wallace (1972). In contrast, *H. sanguineus* exhibited a sigmoidal curve, which was most distinct in the smallest animals (0.5 g), but leveled out to an almost exponential curve in the largest size group (10 g). Moreover, respiration rates of small and medium-sized *H. sanguineus* significantly exceeded those of similar-sized *C. maenas* at 15 and 20 °C. At first glance, such a sigmoidal curve seems to indicate that the small animals already approach their critical temperature, which then would be much lower than that of the larger animals (Pörtner 2002). This explanation, however, appears unlikely, because the temperature range of (native) *H. sanguineus* in Asia ranges from about 2 to 28 °C (Stephenson et al. 2009) and the intermediate temperature of 15 to 20 °C should comprise the thermal optimum of this species. Moreover, the species showed no differences in the respiration-temperature dependences between summer and winter and is apparently not able to adjust their metabolism to environmental variations, but is probably also not required to do so.

We propose two alternative explanations for the respiration curves, which are based on different growth dynamics and different behavioral patterns of the two species. A similar result as described here for small *H. sanguineus* was found for juvenile Dungeness crabs, *Metacarcinus magister* (Gutermuth and Armstrong 1989). Juvenile 1+ crabs (>1 year old) showed a "classical" exponential increase with temperature from about 250 µl $O_2 \cdot g^{-1} \cdot h^{-1}$ at 6 °C to 950 µl $O_2 \cdot g^{-1} \cdot h^{-1}$ at 18 °C. In contrast, oxygen consumption of 0+ crabs (<1 year old) at 6 °C was more than twice as high as that of the 1+ crabs, increased almost linearly towards 950 µl $O_2 \cdot g^{-1} \cdot h^{-1}$ at 14 °C, and remained at this level at 18 °C. The authors explained the elevated resting metabolic rate and the low response between 14 and 18 °C by acclimation to warmer temperatures, which allow the juveniles to grow faster (see also Klein-Breteler 1975a, b). It should be noted that Gutermuth and Armstrong (1989) used different methods to measure the respiration of their two crab cohorts, probably limiting the comparability between the two groups and to our study. A higher growth rate may also explain the respiratory differences between small *C. maenas* and *H. sanguineus* found in the present study. Both species have similar carapace widths at the end of their first season after recruitment (~10 mm; Erikson and Edlund 1977; Dauvin 2009). However, with the same carapace width, *H. sanguineus* is remarkably heavier than *C. maenas* of the same size due to its squarish carapace shape: 0.42 g versus 0.29 g at 10 mm carapace width, respectively (Jungblut et al. 2017). Thus, in their early development, the body mass of *H. sanguineus* is growing faster than that of *C. maenas*.

We did not record the activity of crabs during the on-going respiration measurements. Therefore, it cannot be excluded that the generally higher respiration rates of smaller and mid-sized *H. sanguineus* may also be due to enhanced activity compared to *C. maenas* and not necessarily only due to a higher resting metabolism. Evidence for a generally higher activity of *H. sanguineus* compared to *C. maenas* might be found in several studies comparing direct interactions as well as the population developments of both species. Along the U.S. east coast, the invasion of *H. sanguineus* was generally accompanied by a massive decrease of *C. maenas* (Lohrer and Whitlatch 2002; Kraemer et al. 2007; O´Connor 2014). Studies on an individual and community level revealed competitive dominance of *H. sanguineus* over *C. maenas*. The invader dominated *C. maenas* in competition for food and shelter (Jensen et al. 2002). Furthermore, *H. sanguineus* was found to be highly mobile (Brousseau et al. 2002). These examples suggest an overall higher agility and activity of *H. sanguineus*. This behavior may partially explain the higher respiration and thus metabolic rates of this species compared to *C. maenas*.

5.5.2 Metabolic energy demand

The two species-specific respiration models are rather similar. However, the higher Q_{10} value of the *H. sanguineus* compared to the *C. maenas* respiration model suggests a higher dependency of respiration on temperature and body mass. This is also apparent in the generally higher rates of mass-standardized *H. sanguineus* specimens. The total energy demand per site and species is obviously dependent on the density and biomass of the crabs. Considering the biomass values of August 2014, the energy demands for *C. maenas* and *H. sanguineus* are in the same order of magnitude for each site. Consequently, the total August values for the entire Helgoland intertidal zone are similar, even if the biomass of *H.*

sanguineus is generally lower than that of *C. maenas* (Jungblut et al. 2017). The calculated cumulative energy demand of the two species for the whole year 2014 was found to be very similar. The energy demand for *C. maenas* generally exceeds that of *H. sanguineus*, but *C. maenas* is virtually absent from the intertidal area for about four months in winter and thus has no impact on this habitat (Janke 1986; Aagaard et al. 1995; S. Jungblut, personal observation). In contrast, the invasive species is present in the intertidal year-round (S. Jungblut, personal observation) and although less abundant, it has to cover its energy demand by foraging in winter as well. Generally, the energy demands calculated for both species only comprise first assessments, as these laboratory measurements did not consider field conditions like locomotor activity, photoperiod or exposure to air during low tide that might have an influence on metabolic rates (e.g. Dehnel 1958; Taylor et al. 1977).

Crabs are one of several important components of intertidal communities and their impact is density-dependent. Generally, their energetic demand is relatively small compared to the total primary production in rocky intertidal areas. The annual carbon demand of *C. maenas* and *H. sanguineus* in the Helgoland intertidal ranges between 3.5 to 4.3 and 3.3 to 4.1 $g\,C\cdot m^{-2}$, respectively (derived from the cumulative energy demand for 2014). Unfortunately, no primary production data are available for the Helgoland intertidal area. For kelp forests, as for example present in the subtidal area around Helgoland, a value of about $2\,kg\,C\cdot m^{-2}\cdot yr^{-1}$ is established (Thomas 2002). Primary production of a similar intertidal region, the rocky intertidal area of the Ria of Vigo (northwestern Spain), for instance, was estimated to be about 416.1 $g\,C\cdot m^{-2}$ (Niell 1977). At the Wadden Sea island of Sylt, a food web network analysis revealed a primary production of 81.2 $mg\,C\cdot m^{-2}\cdot d^{-1}$ for the intertidal sand flat area, while *C. maenas* was found to consume about 0.84 $mg\,C\cdot m^{-2}\cdot d^{-1}$ (Baird et al. 2004). Both comparisons indicate that primary production is not a limiting resource in intertidal areas.

The Helgoland population of *C. maenas* is not or not yet suffering from the invasion of *H. sanguineus* (Jungblut et al. 2017), since the abundance of both crab species together in the Helgoland intertidal roughly doubled from 2007 to 2014. However, the current invasion status and the increasing abundances of *H. sanguineus* around Helgoland suggest that the population will continue to grow (Jungblut et al. 2017). A considerably higher *H. sanguineus* density may then have stronger effects on the community patterns in the rocky intertidal compared to today. The ecological impact of *C. maenas* on this area was previously described as rather limited (Janke 1990). The feeding ecology of *H. sanguineus* at Helgoland is not yet understood, but if *H. sanguineus* is as omnivorous as *C. maenas*, this may result in strong competition for food between both species. If *H. sanguineus* prefers a more herbivorous diet, trophic competition would likely be less pronounced. In this case, however, total population energy demand of this species would be even higher and with the current invasion status of *H. sanguineus* around Helgoland, its energy demand could exceed that of *C. maenas*. Macroalgae, at least, appear not to be a limiting resource in the Helgoland intertidal.

In the nearby Wadden Sea, another invader, the Asian brush-clawed shore crab, *Hemigrapsus takanoi*, is also present in high abundances in addition to *H. sanguineus* and *C. maenas* (Landschoff et al. 2013). Competition for food could be stronger and more complex as macroalgae are not very abundant in the intertidal zone of the Wadden Sea. Energy flow models, as for instance proposed for the Sylt-Rømø Bight, should be updated to account for the impact of those successful invasive crab species (Baird et al. 2004, 2007, 2012).

5.6 Conclusions

Hemigrapsus sanguineus successfully invaded the rocky intertidal around Helgoland and established a prospering population. Abundance and biomass of the invader have reached values as high as 21% and 59%, respectively, of those recorded for the native *Carcinus maenas*. However, depending on the diet the energy demands of the invader reached 62 to 97% (calculated for August 2014) and 76 to 119% (whole year 2014) of the values calculated for the native crab. Energetic requirements of *H. sanguineus* in their new habitat are thus in the same range as those of *C. maenas*.

Present warming trends in Helgoland waters attributed to climate change (Wiltshire and Manly 2004) and the fourfold higher North Sea warming projection compared to the global average (Belkin 2009) should be favorable for *H. sanguineus*, as increasing ambient temperatures may accelerate growth and facilitate reproduction. Furthermore, increasing human influence and subsequent alterations in coastal habitats such as the creation of artificial hard substrate are also known to promote non-indigenous species in competitive situations (Byers 2002; Bulleri and Chapman 2010). Consequently, we predict that the overall energetic impact of *H. sanguineus* will increase, while asserting competitive dominance over its native ecological equivalent *C. maenas*. To assess the ecological influence of the invader more comprehensively, subsequent studies on e.g. preferred diet and competitive interactions are needed. Prevalence of the invader will eventually have implications for the original community patterns in rocky intertidal areas. Studies of the community impact of *H. sanguineus* in invaded habitats are, however, still pending.

5.7 Acknowledgements

We thank the staff of the Biologische Anstalt Helgoland and especially Dr. Jan Beermann for laboratory and temperature-controlled room space and for support, whenever needed. Dr. Beermann also provided and prepared the map of Helgoland. Hendrik Pehlke calculated the areas of the intertidal sites around Helgoland. Water temperature data were provided by the COSYNA system operated by Helmholtz-Zentrum Geesthacht, Zentrum für Material- und Küstenforschung GmbH. This study was partly supported by a RISE internship grant from the German Academic Exchange Service (DAAD) to Morgan McCarthy.

5.8 Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of the marine invertebrate animals were followed in this study. This article does not contain any studies with human participants performed by any of the authors.

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6 Publication III

Seasonal lipid storage and dietary preferences of native European vs. invasive Asian shore crabs

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submitted to *Marine Ecology Progress Series*

Seasonal lipid storage and dietary preferences of native European *vs.* invasive Asian shore crabs

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6.1 Abstract

The invasive Asian shore crab *Hemigrapsus sanguineus* and the native European green crab *Carcinus maenas* share intertidal habitats along the European North Atlantic shores and may compete for food. We evaluated the energy-storing capacities of the two species and determined their dietary preferences by means of lipid analysis and fatty acid trophic biomarker indices. Specimens of both sexes and various sizes were sampled in the rocky intertidal of the island of Helgoland, North Sea, in April, June, August and October 2015. Total lipid contents of the midgut glands of both sexes of *H. sanguineus* were significantly higher than in *C. maenas* and followed a distinct seasonal cycle. In contrast, the low lipid contents of males and females of *C. maenas* remained at a similar level throughout the seasons. For the females of *H. sanguineus*, the seasonal differences may be due to higher reproductive output and, thus, lipid turnover. The distinct seasonality of total lipids in males, however, remains unexplained. The biomarker indices for Bacillariophyceae, Chlorophyta and especially for Phaeophyceae were higher in *H. sanguineus* than in *C. maenas*, suggesting a higher degree of herbivory of the invader. In contrast, the Rhodophyta index and the relative carnivory index were higher in *C. maenas*. Thus, competition for food between the two species will probably be low in habitats rich in macroalgae. The ability of *H. sanguineus* to utilize mainly energy-poor algae but accumulate high-energy reserves may be a key adaptive advantage/feature for the successful establishment of persistent populations in new habitats.

Keywords

Asian shore crab *Hemigrapsus sanguineus*, European green crab *Carcinus maenas*, feeding preferences, competition, lipids, fatty acids, North Sea, Helgoland

6.2 Introduction

Biological invasions can entail severe ecological changes (Vitousek et al. 1997, Grosholz 2002, Didham et al. 2005). Non-indigenous species may alter structures of native communities and negatively influence biodiversity and ecosystem functioning. Ultimately, native species may become extinct and ecosystem services, economic interests, and human health might be at risk (Ruiz et al. 2000, Gurevitch & Padilla 2004, Simberloff et al. 2013).

Brachyuran crabs are common invaders of marine and coastal ecosystems (Brockerhoff and McLay 2011). Among this group, the European green crab *Carcinus maenas* (Linnaeus 1758) and the Asian shore crab *Hemigrapsus sanguineus* (De Haan 1835) show a very pronounced invasion potential. *C. maenas* is native to the European and northern African Atlantic coasts. After invading the North American east coast in the early 19th century, it appeared along other coasts worldwide, including the North American west coast, South Africa, Japan, and Australia (Carlton & Cohen 2003). *H. sanguineus*, in contrast, originally inhabited the coastlines of Japan, Korea, and China (Fukui 1988, Stephenson et al. 2009). It was first detected in the late 1980's along the U.S. east coast in Delaware Bay and is now abundant from North Carolina to Maine (Williams & McDermott 1990, Delaney et al. 2008, Epifanio 2013). In the late 1990´s *H. sanguineus* appeared in northern France and the Netherlands (Breton et al. 2002). It spread northwards along the European coasts and reached the German North Sea in 2007 (Obert et al. 2007, Landschoff et al. 2013, Jungblut et al. 2017). Hence, *C. maenas* and *H. sanguineus* share intertidal habitats in their native and invaded habitats in Europe, the U.S. east coast, and Asia, providing an interesting basis for species invasion studies.

The determining factors, which contribute to the success of an invader in a new habitat, are pivotal topics of ecological research (e.g. Elton 1958, Ruiz et al. 2000, Kolar & Lodge 2001, Sakai et al. 2001). Most studies are based on population structure and dynamics (e.g. Sakai et al. 2001, Russel et al. 2008, O´Connor 2014). Several ecological models and hypotheses were established or used as a framework to explain invasive processes, such as the ‘enemy release hypothesis’, ‘biotic resistance theory’ or the ‘community ecology theory’ (e.g. Lodge 1993, Shea & Chesson 2002, Colautti et al. 2004). Few studies, however, focus on the underlying physiological mechanisms and properties, which form the fundamental drivers of the invader´s success (e.g. Kelley 2014).

Feeding and nutrition are cardinal processes in heterotrophic organisms (Saborowski 2015). The ability to store energy reserves provides an advantage to overcome periods of starvation such as food scarcity or during ecdysis. In crabs, the midgut gland, or hepatopancreas, is the main storage organ of dietary components such as proteins, carbohydrates and, particularly, high-energy lipids (Jimenez & Kinsey 2015). The size and lipid content of the midgut gland provides a suitable indicator for the overall condition of the animal, its storage capacity and the seasonal dynamics of energy deposition (Kyomo 1988, Griffen et al. 2015, Jimenez & Kinsey 2015). Moreover, the nutritive state is closely related to reproductive processes and the preferred diet (Griffen et al. 2011, 2012, Zeng et al. 2014).

Gut content analysis of *C. maenas* and *H. sanguineus* classified both species as opportunistic omnivores (e.g. Ropes 1968, Tyrell & Harris 1999, Lohrer et al. 2000, Griffen et al. 2012), but with a higher preference of *H. sanguineus* for macroalgae. Gut content analysis tend to overestimate recently ingested particles and to underestimate easily digestible food; two biases, which can be eliminated by using fatty acids (FAs) as trophic markers (Graeve et al. 2001, Dalsgaard et al. 2003, Latyshev et al. 2004).

One aim of the study was thus to examine the lipid storage properties of *C. maenas* and *H. sanguineus*. Secondly, we aimed to determine longer-term feeding preferences for both species by applying the fatty acid trophic marker (FATM) concept to evaluate the potential for food competition. Samples of both sexes and different sizes of either species were taken in April, June, August and October 2015 at the island of Helgoland (North Sea). In order to meet these aims, we tested the following hypotheses:

1) *H. sanguineus* stores larger amounts of lipids in the hepatopancreas than *C. maenas*.
2) The seasonal variation in total lipid levels is stronger in *C. maenas* than in *H. sanguineus*.
3) The fatty acid compositions differ i) between species and within each species with ii) sex, and iii) seasons.
4) Trophic biomarker concentrations differ i) between *C. maenas* and *H. sanguineus* and differ for both species with ii) crab size, iii) sex, and iv) seasons.

6.3 Material and methods

6.3.1 Origin of samples

The sampling site was located at the southwestern tip of the island of Helgoland, southern North Sea, German Bight (Fig. 6.1). This wave-exposed site is composed of coarse sand with small to large rocks and boulders, which are covered with macroalgae (Jungblut et al. 2017). The prevailing species are listed in Table 6.3. For further details on habitats around the island of Helgoland, see Bartsch & Tittley (2004).

Males and females of European green crabs *Carcinus maenas* and Asian shore crabs *Hemigrapsus sanguineus* were sampled during low tides in April, June, August, and October 2015. Animals with carapace widths between 10 and 40 mm were collected. Macroalgae were sampled in August 2015.

After sampling, the crabs and algae were transferred into the laboratories of the Helgoland Marine Station, Germany. The crabs were maintained in aquaria with aeration until further processing on the same day. The carapace width of each crab was measured to the nearest 0.5 mm with Vernier calipers. After blotting dry with paper towels for 10 s, the fresh mass of the crabs was determined to the nearest 0.001 g. The midgut glands of the crabs were dissected and transferred into pre-weighed glass vials. The masses of the midgut glands were determined and the vials were immediately frozen at -80°C. The hepatosomatic index (HSI) was calculated as the mass of the fresh midgut gland in relation to the rest of the crab's fresh mass (Griffen et al. 2011, 2012, Kennish 1997, Table 6.1). The algae were briefly rinsed in distilled water, blotted dry, and frozen at -80°C.

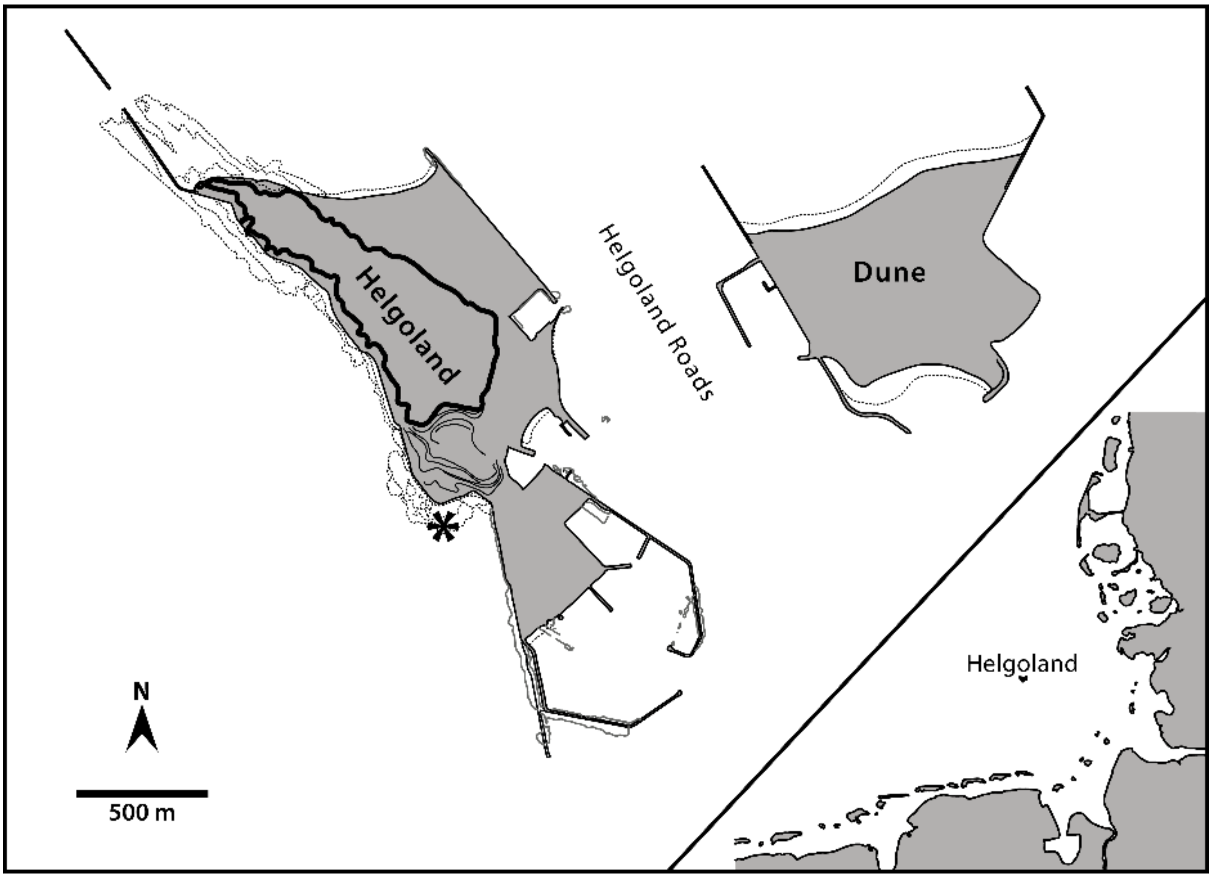

Fig. 6.1: The rocky intertidal sampling area (asterisk) at the southwestern tip of the island of Helgoland, southern North Sea, Germany. Lower right corner: The location of Helgoland in the German Bight (from Beermann 2014, adapted, with permission from Elsevier).

6.3.2 Total lipid extraction and fatty acid analysis

The frozen samples were transported on dry ice to the laboratories of the University of Bremen, Germany. The samples were lyophilized for 48 h and the dry masses were determined to the nearest 0.001 g. Lipids were extracted with dichloromethane:methanol (2:1 per volume) and an aqueous solution of KCl (0.88%) after Folch et al. (1957) and Hagen (2000). The total lipid content (TL) was determined gravimetrically to the nearest 0.001 g and calculated as the percentage of lipids in relation to the dry mass of the sample (%DM, Table 6.1). For the analysis of fatty acids, subsamples of the lipid extracts were transmethylated with methanol containing 3% concentrated sulphuric acid. This converted the fatty acids to their methyl ester derivatives (FAMEs), which could then be detected and quantified by gas chromatography (Kattner & Fricke 1986). The device (Agilent Technologies 7890A) was equipped with a DB-FFAP column (30 m length, 0.25 mm diameter) and run with helium as carrier gas. It was operated with a programmable temperature vaporizer injector. The fatty acids of the samples were identified by their retention times. Menhaden fish oil and lipids of the copepod *Calanus hyperboreus* Krøyer, 1838 were used as standards (Schukat et al. 2014, Bode et al. 2015).

Free fatty alcohols and unidentified components accounted for only about 1% each averaged over all samples and were not considered for further evaluation. The FA dataset was evaluated in view of the fatty acid trophic biomarker (FATM) concept. This concept is well established for marine zooplankton (reviewed by Dalsgaard et al. 2003, Lee et al. 2006) and has also been applied to benthic systems (reviewed by Kelly & Scheibling 2012). Benthic animals may consume other animals and macroalgae but may also ingest benthic microalgae. Since FAs for the three macroalgae groups, the microalgae, and animals are partly identical, we developed new dietary indices to distinguish between these prey items as best as possible (Table 6.1).

The FAs 18:1(n-7), 18:2(n-6), 18:3(n-3), and 18:4(n-3) are major components in Chlorophyta (green macroalgae). Additionally, 16:4(n-3) is a dominating FA in the order Ulvales (Kelley & Scheibling 2012 and references therein). Because 18:4(n-3) is the only FA that could be used as an indicator for Phaeophyceae (brown macroalgae) (Kelly & Scheibling 2012), it was omitted in the green algae indicator. Thus, I_{Ch} = 16:4(n-3) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) was used as dietary index for Chlorophyta and I_P = 18:4(n-3) as indicator for Phaeophyceae. Rhodophyta (red macroalgae) are particularly rich in 20:5(n-3), which is also an important biomembrane FA in marine animals (Kelley & Scheibling 2012). The red algae diet index I_R = 20:5(n-3) / [16:0 + 18:0 + 22:6(n-3)] sets 20:5(n-3) in relation to the three other FAs generally known as membrane FAs (Boissonnot et al. 2016, Lee et al. 2006). A high index might thus indicate consumption of red algae. The sum of I_B = 16:1(n-7) + 16:4(n-1) was used as an index for diatom ingestion. Also 18:1(n-1) is a Bacillariophyceae marker FA (Dalsgaard et al. 2003). However, the latter is also abundant in green algae and was, thus, omitted in the diatom index (Kelley & Scheibling 2012). The FA 18:4(n-3) is commonly used as dinoflagellate marker (Dalsgaard et al. 2003). It is also prevalent in green and brown algae (Kelley & Scheibling 2012) and, thus, cannot contribute to a dinoflagellate index. However, crabs might not distinguish between diatoms and dinoflagellates and a high diatom index might thus generally indicate ingestion of benthic microalgae. To assess carnivory of the crabs, we developed a carnivory index similar to that of zooplankton (Schukat et al. 2014, Bode et al. 2015). The carnivory marker FA 18:1(n-9) was set into relationship with all FAs which are abundant or are marker FAs for algae: I_{Ca} = 18:1(n-9) / [16:1(n-7) + 16:4(n-3) + 16:4(n-1) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) + 18:4(n-3) + 20:4(n-6) + 20:5(n-3)].

Table 6.1: Equations for the calculation of condition parameters and trophic marker indices.

Parameter	Equation
Hepatosomatic Index	HSI = wet $\text{mass}_{\text{hepatopancreas}}$ / (fresh $\text{mass}_{\text{crab}}$ - wet $\text{mass}_{\text{hepatopancreas}}$)
Total lipid content (%$_{\text{DM}}$)	TL = $\text{mass}_{\text{extracted lipids}}$ / dry $\text{mass}_{\text{sample}}$ * 100
Carnivory Index	I_{Ca} = 18:1(n-9) / [16:1(n-7) + 16:4(n-3) + 16:4(n-1) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3) + 18:4(n-3) + 20:4(n-6) + 20:5(n-3)]
Chlorophyta Index	I_{Ch} = 16:4(n-3) + 18:1(n-7) + 18:2(n-6) + 18:3(n-3)
Phaeophyceae Index	I_P = 18:4(n-3)
Rhodophyta Index	I_R = 20:5(n-3) / [16:0 + 18:0 + 22:6(n-3)]
Bacillariophyceae Index	I_B = 16:1(n-7) + 16:4(n-1)

6.3.3 Statistical analysis

We tested for the effects of 'species' (*C. maenas*, *H. sanguineus*), 'sex' (male, female), 'month' (April, June, August, October) and 'fresh mass' (covariable) on seven different response variables: two physiological parameters (HSI and TL) and five trophic marker indices (I_{Ca}, I_{Ch}, I_P, I_R, and I_B) (see Table 6.1). In total, seven individual linear models were developed - one per response variable. Each model was fully crossed with all above mentioned factors, including highest-order interactions of all factors and subsequent interactions of lower order. Prior to the analyses the data were log(+1)-transformed, if needed, to meet the assumptions of normal distribution and homogeneous variances of the residuals. The following diagnostics of model stability were checked for each model: Cook's distance, leverage (Quinn & Keough 2002) and dffits (Cohen & Cohen 2008). For the latter, data points were excluded one by one from the data sets and the derived fitted values were compared with those obtained from the models based on all data points. All models were fitted in R, version 3.2.3 (R Development Core Team 2015), using the generic function 'lm'. Graphs were produced with the software GraphPad Prism (version 5.03).

For all models, significances of individual terms (interaction terms and main factors) were established with likelihood ratio tests (LRT) using the R function 'anova' with the argument 'test' set to 'F'. Hereby, we compared the respective full models with the corresponding reduced models not comprising the respective factor and/or term of interest. Where possible, we excluded non-significant terms in order to create models that are more parsimonious and to avoid the risk of over-parametrization with four main factors. Due to the number of models and the complexity of each model, we refrain from showing the results of post-hoc tests and report the most distinct results descriptively (i.e. significant interactions of highest order or lower orders if adequate). Values presented in the results section are means ± standard deviations for all crabs per species and month, i.e. pooled over the different fresh masses.

The model stability checks (test for leverage) revealed difficulties with potentially influential cases in all seven linear models. In the HSI model, one value was detected to be influential, however, omitting it did not change the results. For the TL model, a slight indication for systematic variation in the variances of the residuals was found besides some influential cases. Potentially influential data were detected for the five models of trophic marker indices. In those models, however, the influential cases could not clearly be identified as being extraordinary high or low in their values. Additionally, the residuals of the brown algae indices showed a slight systematic pattern in a few data points. Omitting the largest values and recalculating the brown, red, and diatom index models did not change the results of the respective models. Other model checks suggested no influential data to exist and thus the models were accepted, but must be interpreted with caution. Only very clear, i.e. highly significant results were considered to actually be meaningful.

Principal component analyses (PCA) were conducted on the complete set of FA components (see list in Table 6.3) to reveal differences in the entire FA compositions between species (*C. maenas* and *H. sanguineus*), sexes (male and female) and months (April, June, August, October). Firstly, differences between sexes within one of the two crab species and each of the four months were tested. No sex-specific clustering of data was observed in either of the eight single PCAs (see Results). Therefore, sexes were pooled for each species and month. Secondly, differences between months within each of the crab species were tested. Additionally, the data were pooled for each species and a final PCA was conducted on all data to reveal differences between the crab species in relation to the sampled macroalgae species. Only those FAs were processed in the PCAs which revealed a mean value of >1% in any of the considered species. Prior to each PCA, FA percentages were transformed into proportions and arcsine-square-root transformed to achieve normality and homogeneity of variances. PCAs and generation of the corresponding graphs were conducted with the Primer v6 software (Clarke & Warwick 1994).

6.4 Results

The results of the statistical analysis of the hepatosomatic index (HSI), total lipid content (TL) and the indices for carnivory, Chlorophyta, Phaeophyceae, Rhodophyta, and Bacillariopyceae (I_{Ca}, I_{Ch}, I_{P}, I_{R}, I_{B}, resp.) are summarized in Table 6.2. Here, we focus on the results of interactions including the factor 'species'. Interactions excluding this factor (e.g. sex*month*fresh mass) were not meaningful in view of the aims and questions of our study.

Table 6.2: Results of Likelihood Ratio Tests (LRT) of seven individual linear models comparing midgut gland samples of *Carcinus maenas* and *Hemigrapsus sanguineus* with regard to hepatosomatic index (HSI), total lipid content (TL) and the indices for carnivory, Chlorophyta, Phaeophyceae, Rhodophyta, and Bacillariopyceae (I_{Ca}, I_{Ch}, I_{P}, I_{R}, I_{B}. resp.). Samples were collected in April, June, August, and October 2015 from the island of Helgoland, Germany. Results of LRTs are listed, which include the factor 'species'. Statistically significant p-values are bold.

		$\mathrm{LRT}_{\mathrm{species*sex*month*fresh\ mass}}$	$\mathrm{LRT}_{\mathrm{species*sex*month}}$	$\mathrm{LRT}_{\mathrm{species*sex*fresh\ mass}}$	$\mathrm{LRT}_{\mathrm{species*month*fresh\ mass}}$	$\mathrm{LRT}_{\mathrm{species*month}}$	$\mathrm{LRT}_{\mathrm{species*fresh\ mass}}$	$\mathrm{LRT}_{\mathrm{species*sex}}$	$\mathrm{LRT}_{\mathrm{species}}$
HSI	DF	376,3	379,3	379,1	379,3	394,3	389,1	389,1	401,1
	F	0.006	1.689	1.448	4.625	20.295	3.136	0.484	3.251
	p	0.999	0.169	0.230	**0.003**	**< 0.001**	0.077	0.487	0.072
TL	DF	376,3	379,3	379,1	383,3	389,3	389,1	389,1	401,1
	F	0.953	0.266	0.084	3.308	31.579	4.452	11.348	327.28
	p	0.415	0.850	0.772	**0.020**	**< 0.001**	**0.036**	**< 0.001**	**< 0.001**
I_{Ca}	DF	377,3	387,3	380,1	380,3	390,3	390,1	390,1	402,1
	F	0.859	3.673	1.195	1.153	15.679	0.008	1.660	77.228
	p	0.463	**0.012**	0.275	0.328	**< 0.001**	0.931	0.198	**< 0.001**
I_{Ch}	DF	377,3	380,6	380,4	380,6	390,3	390,1	390,1	402,1
	F	0.797	1.874	3.768	2.156	0.432	6.573	5.805	265.11
	p	0.496	0.084	**0.005**	**0.046**	0.730	**0.011**	**0.016**	**< 0.001**
I_{P}	DF	377,3	389,3	389,1	380,3	380,3	390,1	390,1	402,1
	F	0.816	3.574	7.395	1.695	9.182	3.274	0.068	1047.5
	p	0.486	**0.014**	**0.007**	0.167	**< 0.001**	0.071	0.794	**< 0.001**
I_{R}	DF	377,3	380,3	392,1	380,3	390,3	390,1	390,1	402,1
	F	0.694	0.601	10.320	2.568	7.611	4.411	2.414	729.24
	p	0.556	0.615	**0.001**	0.054	**< 0.001**	**0.036**	0.121	**< 0.001**
I_{B}	DF	377,3	380,3	380,1	380,3	390,3	390,1	390,1	402,1
	F	0.582	2.367	0.969	0.273	6.806	4.492	4.998	186.63
	p	0.627	0.071	0.326	0.845	**< 0.001**	**0.035**	**0.026**	**< 0.001**

6.4.1 Hepatosomatic Index

Carcinus maenas and *Hemigrapsus sanguineus* showed diverging relationships between the hepatosomatic indices (HSI) and fresh mass (significant species*month*fresh mass interaction, pooled over sex, $p = 0.003$). While differently sized *C. maenas* showed the same HSI, a negative relationship between HSI and fresh mass was evident in *H. sanguineus* from June and August (Fig. 6.2).

C. maenas and *H. sanguineus* showed different seasonal patterns of HSI (significant species*month interaction, pooled over sex and fresh mass, $p < 0.001$). In *C. maenas*, the highest average HSIs were present in April (0.097 ± 0.024) and June (0.096 ± 0.019). The values became lower in August (0.078 ± 0 033) and October (0.065 ± 0.020) (Fig. 6.2A and B). In contrast, *H. sanguineus* showed no significant differences in the average HSI between seasons (min. 0.077 ± 0 016 in August and max. 0.094 ± 0.016 in April, Fig. 6.2C and D).

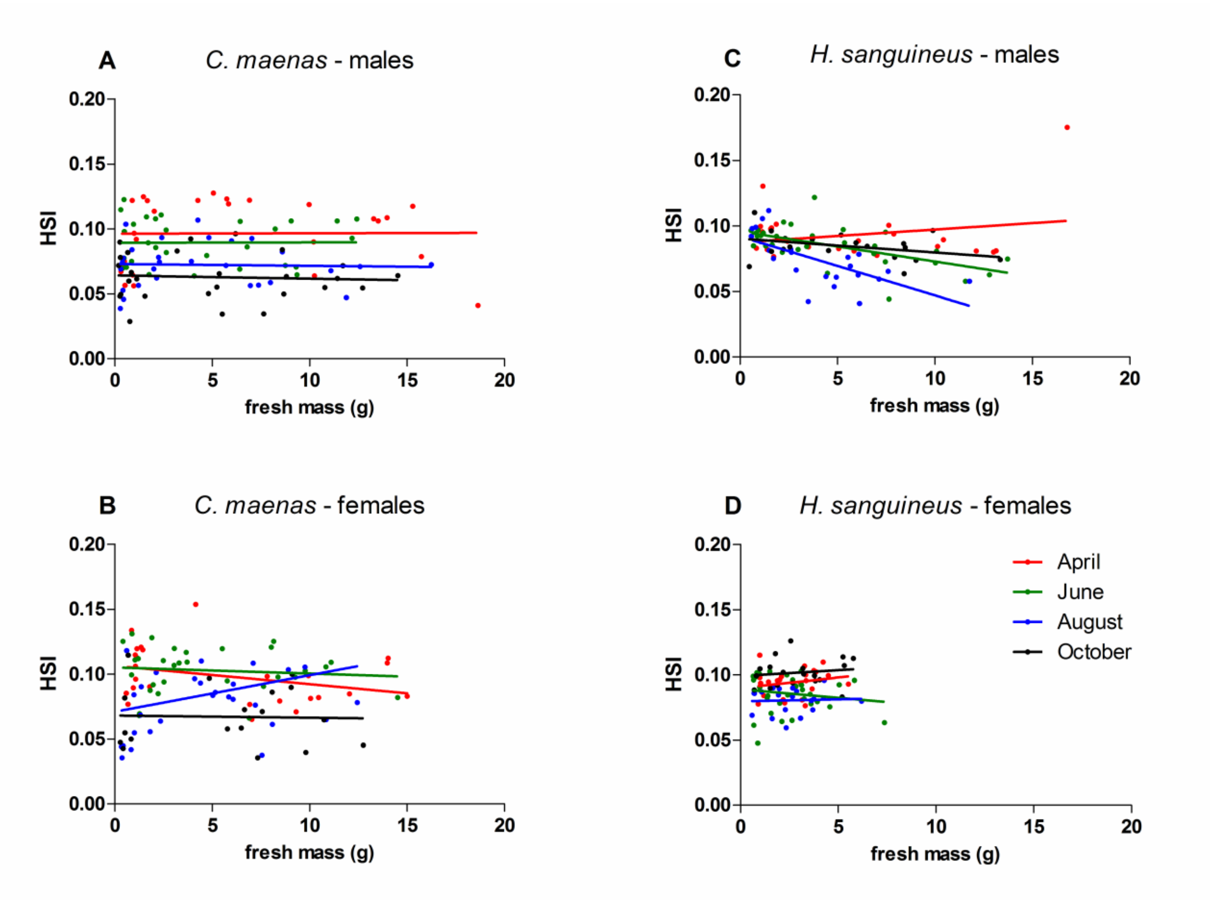

Fig. 6.2: Hepatosomatic indices (HSI) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of HSI see Table 6.1.

6.4.2 Total lipid content

The midgut glands of *H. sanguineus* had higher total lipid (TL) contents than those of *C. maenas* (significant main factor species, $p < 0.001$, Fig. 6.3). When sexes were pooled, the species showed different seasonal relationships between TL and fresh mass (significant species*month*fresh mass interaction, $p = 0.020$). TL levels did not change with fresh mass in *C. maenas* but showed a significant negative correlation in August and October for *H. sanguineus*.

TL values per species and month revealed differences between *C. maenas* and *H. sanguineus* (significant species*month interaction, pooled over sex and fresh mass, $p < 0.001$). *C. maenas* showed quite similar average values between 17.6 ± 5.5 $\%_{DM}$ in June and 21.0 ± 5.8 $\%_{DM}$ in August (Fig. 6.3A and B). In *H. sanguineus,* the average TL values differed remarkably between seasons: 38.2 ± 9.0 $\%_{DM}$ in April, 47.4 ± 9.2 $\%_{DM}$ in June, 27.8 ± 12.3 $\%_{DM}$ and August, and 34.4 ± 12.9 $\%_{DM}$ in October (Fig. 6.3C and D).

The significance in the species*sex interaction (pooled over fresh mass and month, $p < 0.001$) was due to diverging patterns in the sexes: In *C. maenas*, males showed lower TL values than females (18.8 ± 6.6 vs. 20.0 ± 7.4 $\%_{DM}$, resp.) (Fig. 6.3A and B) whereas in *H. sanguineus*, the males had higher TL values than the females (39.3 ± 13.3 $\%_{DM}$ vs. 36.7 ± 12.5 $\%_{DM}$, resp.) (Fig. 6.3 C and D).

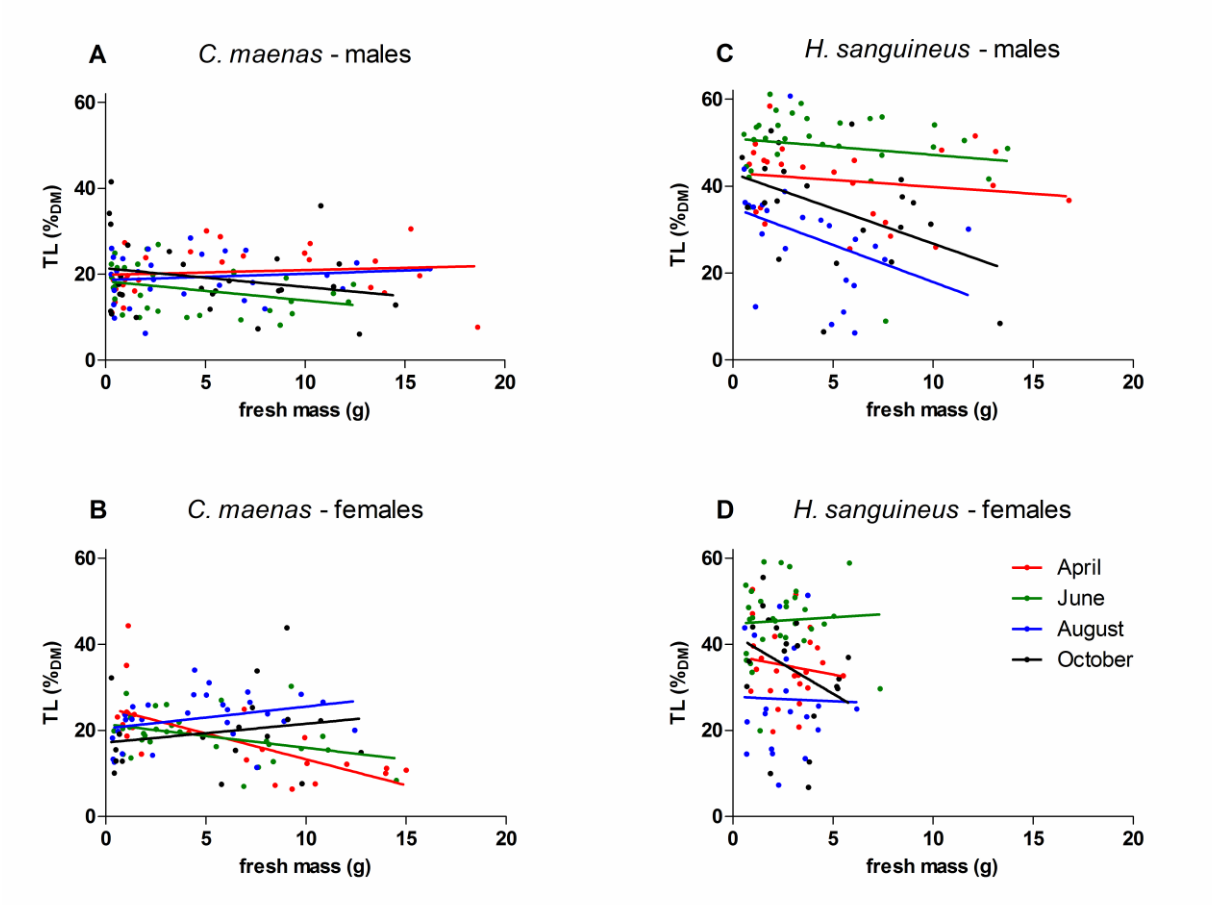

Fig. 6.3: Total lipid content (TL, in $\%_{DM}$) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of TL see Table 6.1.

6.4.3 Fatty acid compositions

Fatty acid (FA) compositions of the midgut glands of both crab species and the macroalgae are presented in Table 6.3 and in Table S1 (Supplementary Material).

The main FAs in the midgut glands of *C. maenas* and *H. sanguineus* were 16:0 (18.5 and 22.1%, resp.), 20:5(n-3) (14.4 and 7.5%, resp.), 18:1(n-9) (11.0 and 14.2%, resp.), and 16:1(n-7) (6.9 and 10.8%, resp.) (Table 6.3). Summarized over all months and fresh masses, *C. maenas* showed lower values of 16:0, 16:4(n-3), 18:1(n-9), and 18:3(n-3) than *H.*

sanguineus (Table 6.3). In contrast, the FAs 18:0, 20:1(n-11), 20:1(n-7), and especially 20:5(n-3) were higher in *C. maenas*.

The macroalgae showed the group-specific patterns as described by Kelley and Scheibling (2012) (Table 6.3). The Chlorophyta *Ulva* sp. was richest in 16:0 (21.7%). Other prevalent FAs were 18:3(n-3) (17.3%), 16:4(n-3) (14.2%) and 18:1(n-7) (11.0%). The three species of Phaeophyceae were richest in 16:0 (18.2 - 25.8%), 18:1(n-9) (8.4 - 33.5%), 20:4(n-6) (9.7 - 13.9%), 18:2(n-6) (6.1 - 12.0%), 14:0 (4.5 - 11.6%), and 20:5(n-3) (5.6 - 10.5%). In *F. serratus*, the FA 18:1(n-9) dominated with about 33.5%, whereas this FA comprised only about 8 and 20% in *S. muticum* and *D. aculeate*, respectively. The four species of Rhodophyta were rich in 20:5(n-3) (17.2 - 33.1%), 16:0 (24.9 - 30.3%), 20:4(n-6) (7.3 - 17.9%), and 18:1(n-9) (2.7 - 9.8%). Within this group, *C. virgatum* and *C. officinalis* showed comparably low values of 20:4(n-6) but high values of 20:5(n-3).

Principal Component Analyses (PCAs) of the FA compositions were conducted separately for males and females of either species and in each of the four months. The PCAs did not show sex-specific differences within species (Fig. S1a-h of the Supplementary Material).

PCA of FAs of *C. maenas* did not reveal seasonal clustering (Fig. 6.4A). The three most important principal components together explained about 64% of the variance in the data and PC1 and 2 together, about 52%. PC1 is mainly represented by positive eigenvector values for 20:5(n-3), 20:4(n-6), 18:0, and 22:6(n-3) (in decreasing order) as well as negative values for 16:1(n-7), 14:0, and 18:1(n-9) (in decreasing order) (Fig. 6.4A). Positive eigenvector values for 20:1(n-7), 20:1(n-11), and 22:5(n-3) as well as negative values of 18:1(n-9) and 20:4(n-6) (in decreasing order) are contributing most to PC2.

Table 6.3 (next two pages): Number of replicates (n), fresh mass, hepatosomatic index (HSI), total lipid content (TL, in $\%_{DM}$), fatty acid composition (in %total fatty acids, %TFA) and the trophic indices for carnivory, Chlorophyta, Phaeophyceae, Rhodophyta and Bacillariopyceae (I_{Ca}, I_{Ch}, I_P, I_R, I_B. respectively) of *Carcinus maenas*, *Hemigrapsus sanguineus*, and several macroalgae species from Helgoland, Germany. Values of *C. maenas* and *H. sanguineus* are averages from April, June, August, and October. Macroalgae were sampled in August 2015. All values are means ± standard deviations; "-": not measured/not detected; "+": traces (<0.1%). For the calculation of HSI, TL and the dietary indices, see Table 6.1.

	Brachyura		**Chlorophyta**	**Phaeophyceae**		
	Carcinus maenas (Linnaeus 1758)	*Hemigrapsus sanguineus* (De Haan 1835)	*Ulva* sp. Linnaeus 1753	*Fucus serratus* Linnaeus 1753	*Sargassum muticum* (Yendo) Fensholt 1955	*Desmarestia aculeata* (Linnaeus) J.V.Lamouroux 1813
n	214	195	5	5	5	5
fresh mass (g)	5.0 ± 4.4	3.8 ± 3.1	-	-	-	-
HSI	0.08 ± 0.03	0.09 ± 0.02	-	-	-	-
TL (%DM)	19.4 ± 7.0	38.7 ± 15.8	0.6 ± 0.2	2.3 ± 0.8	2.2 ± 0.2	3.5 ± 1.1
Fatty acids (%TFA)						
14:0	2.7 ± 1.1	3.1 ± 0.8	1.1 ± 0.3	11.6 ± 0.6	4.5 ± 0.3	8.4 ± 0.3
15:0	1.0 ± 0.3	0.9 ± 0.3	0.4 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
16:0	18.5 ± 2.4	22.1 ± 3.0	21.7 ± 0.6	18.2 ± 0.9	25.8 ± 0.7	21.3 ± 0.2
16:1(n-7)	6.9 ± 3.3	10.8 ± 3.2	1.9 ± 0.3	1.1 ± 0.6	7.7 ± 0.7	0.2 ± 0.3
16:2(n-4)	0.6 ± 0.4	3.3 ± 1.8	-	-	0.7 ± 0.1	-
16:4(n-3)	1.7 ± 1.0	3.2 ± 1.3	14.2 ± 0.9	0.5 ± 0.1	0.6 ± 0.2	0.5 ± 0.1
16:4(n-1)	+	0.2 ± 0.4	-	-	0.6 ± 0.1	-
iso 17:0	1.4 ± 0.6	1.0 ± 0.2	1.9 ± 0.1	0.2 ± 0.2	0.7 ± 0.1	0.4 ± 0.0
18:0	5.5 ± 1.8	3.0 ± 0.8	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.3	3.1 ± 0.2
18:1(n-9)	11.0 ± 3.5	14.2 ± 2.9	1.6 ± 0.4	33.5 ± 5.2	8.4 ± 0.2	19.7 ± 1.3
18:1(n-7)	7.5 ± 1.2	6.2 ± 1.0	11.0 ± 0.9	-	0.6 ± 0.2	+
18:2(n-6)	2.1 ± 0.6	3.2 ± 0.5	7.6 ± 0.7	7.1 ± 0.6	6.1 ± 0.4	12.0 ± 0.3
18:3(n-6)	+	0.3 ± 0.1	1.8 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	1.4 ± 0.1
18:3(n-3)	2.0 ± 1.1	4.2 ± 1.2	17.3 ± 1.6	3.9 ± 0.7	8.6 ± 0.9	4.6 ± 0.1
18:4(n-3)	0.8 ± 0.6	3.4 ± 1.5	11.4 ± 0.5	3.6 ± 1.0	5.8 ± 0.5	7.9 ± 0.4
20:1(n-11)	3.2 ± 1.1	1.0 ± 0.3	-	0.3 ± 0.0	1.1 ± 0.1	-
20:1(n-9)	1.1 ± 0.3	0.9 ± 0.2	-	-	-	-
20:1(n-7)	2.9 ± 1.3	1.0 ± 0.3	-	-	-	-
20:2(n-6)	1.6 ± 0.4	0.7 ± 0.2	-	+	-	-
20:4(n-6)	4.9 ± 2.4	3.5 ± 1.3	2.2 ± 0.2	9.7 ± 1.4	13.9 ± 0.2	10.0 ± 0.5
20:5(n-3)	14.4 ± 3.6	7.5 ± 2.2	1.8 ± 0.2	5.6 ± 1.3	10.5 ± 0.8	7.0 ± 0.3
22:5(n-3)	2.8 ± 1.0	1.3 ± 0.5	1.6 ± 0.1	-	-	-
22:6(n-3)	2.4 ± 1.4	1.9 ± 1.1	-	-	-	-
$\sum_{\text{Saturated}}$	29.2 ± 3.4	30.1 ± 3.9	25.7 ± 0.6	31.2 ± 1.0	32.0 ± 0.9	33.8 ± 0.6
$\sum_{\text{Monosaturated}}$	32.6 ± 6.1	34.0 ± 5.3	14.6 ± 1.5	34.9 ± 5.5	17.8 ± 0.8	20.0 ± 1.5
$\sum_{\text{Polysaturated}}$	33.2 ± 6.4	32.6 ± 5.7	57.9 ± 2.4	30.9 ± 4.7	47.5 ± 1.6	43.5 ± 1.3
$\sum_{\text{Others/Undetermined}}$	4.9 ± 1.1	3.3 ± 0.6	1.8 ± 0.7	3.0 ± 0.8	2.7 ± 0.2	2.8 ± 0.3
Dietary indices						
I_{Ca}	0.3 ± 0.1	0.3 ± 0.1	+	1.1 ± 0.3	0.2 ± 0.0	0.5 ± 0.0
I_{Ch}	13.3 ± 1.9	16.7 ± 2.4	50.2 ± 1.2	11.5 ± 1.1	15.9 ± 1.0	17.1 ± 0.4
I_P	0.8 ± 0.6	3.4 ± 1.5	11.4 ± 0.5	3.6 ± 1.0	5.8 ± 0.5	7.9 ± 0.4
I_R	0.6 ± 0.2	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
I_B	6.9 ± 3.4	11.0 ± 3.3	1.9 ± 0.3	1.1 ± 0.6	8.3 ± 0.8	0.2 ± 0.3

	Rhodophyta			
	Mastocarpus stellatus	*Chondrus crispus*	*Ceramium virgatum*	*Corallina officinalis*
	(Stackhouse) Guiry 1984	Stackhouse 1797	Roth 1797	Linnaeus 1758
n	5	4	5	5
fresh mass (g)	-	-	-	-
HSI	-	-	-	-
TL (%DM)	0.2 ± 0.1	0.1 ± 0.0	2.6 ± 0.3	0.2 ± 0.1
Fatty acids (%TFA)				
14:0	3.8 ± 0.3	4.3 ± 0.3	6.3 ± 0.2	3.4 ± 1.3
15:0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
16:0	29.2 ± 1.6	28.4 ± 2.2	30.3 ± 0.6	24.9 ± 0.5
16:1(n-7)	4.3 ± 1.3	5.4 ± 1.7	6.0 ± 0.7	6.9 ± 3.2
16:2(n-4)	0.6 ± 1.0	0.8 ± 0.4	0.4 ± 0.1	0.9 ± 0.4
16:4(n-3)	0.8 ± 0.2	0.6 ± 0.4	0.7 ± 0.2	1.2 ± 0.1
16:4(n-1)	0.1 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.9 ± 0.7
iso 17:0	0.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.1
18:0	1.9 ± 0.5	1.9 ± 0.5	1.0 ± 0.1	0.9 ± 0.1
18:1(n-9)	9.8 ± 1.3	8.3 ± 0.7	5.6 ± 0.4	2.7 ± 0.2
18:1(n-7)	4.6 ± 1.6	4.4 ± 1.0	2.7 ± 0.3	3.4 ± 0.5
18:2(n-6)	1.6 ± 0.3	2.3 ± 0.7	0.9 ± 0.1	2.8 ± 0.4
18:3(n-6)	0.9 ± 0.1	0.7 ± 0.0	0.7 ± 0.2	0.5 ± 0.2
18:3(n-3)	0.8 ± 0.4	2.0 ± 1.0	0.1 ± 0.2	1.4 ± 0.3
18:4(n-3)	0.5 ± 0.3	1.4 ± 0.7	0.5 ± 0.1	1.3 ± 0.2
20:1(n-11)	0.5 ± 0.9	0.2 ± 0.3	-	-
20:1(n-9)	0.8 ± 0.1	0.1 ± 0.1	-	0.5 ± 0.1
20:1(n-7)	0.1 ± 0.3	0.1 ± 0.2	0.2 ± 0.2	+
20:2(n-6)	0.1 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.6 ± 0.1
20:4(n-6)	17.9 ± 2.3	16.4 ± 5.0	7.3 ± 1.3	13.2 ± 2.9
20:5(n-3)	17.2 ± 1.5	18.0 ± 1.2	33.1 ± 1.6	29.6 ± 2.4
22:5(n-3)	-	-	-	-
22:6(n-3)	1.0 ± 0.9	0.7 ± 0.6	-	0.7 ± 0.4
$\sum_{\text{Saturated}}$	35.7 ± 1.1	35.5 ± 1.6	38.6 ± 0.7	30.2 ± 1.2
$\sum_{\text{Monosaturated}}$	20.1 ± 2.2	18.6 ± 3.7	14.5 ± 0.8	13.5 ± 3.0
$\sum_{\text{Polysaturated}}$	41.4 ± 2.1	43.5 ± 3.3	44.3 ± 1.0	53.1 ± 3.9
$\sum_{\text{Others/Undetermined}}$	2.7 ± 0.8	2.4 ± 0.7	2.6 ± 0.3	3.2 ± 0.4
Dietary indices				
I_{Ca}	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	+
I_{Ch}	7.8 ± 1.8	9.3 ± 2.5	4.4 ± 0.3	8.7 ± 1.1
I_P	0.5 ± 0.3	1.4 ± 0.7	0.5 ± 0.1	1.3 ± 0.2
I_R	0.5 ± 0.1	0.6 ± 0.0	1.1 ± 0.1	1.1 ± 0.1
I_B	4.3 ± 1.5	5.9 ± 1.9	6.4 ± 0.8	7.8 ± 3.9

In *H. sanguineus* as well, the PCA did not show any distinct seasonal clustering. However, an arrangement of samples according to the first two months (April and June) vs. the last two months (August and October) became apparent (Fig. 6.4B). The first three PCs account for about 68% of the variance and the first two PCs together explained about 54%. In decreasing order, PC1 is mainly represented by positive eigenvectors of 16:2(n-4), 18:4(n-3), and 16:4(n-3) as well as negative values for 20:4(n-6), 16:0, 18:1(n-7), and 18:1(n-9) (in decreasing order). For PC2, the positive eigenvectors of 16:1(n-7) and 18:1(n-9), and 18:4(n-3) as well as the negative values for 16:2(n-4), 20:5(n-3), 20:4(n-6), and 22:6(n-3) are contributing most (in decreasing order).

The PCA, which included all FA compositions of crab and macroalgae tissues, revealed distinct clusters for *C. maenas* and *H. sanguineus*. Likewise, the macroalgae formed distinct groups, clearly separated from the crab species and from each other (Fig. 6.5). The first three PCs account for about 67% of the variance, while the first two together explained about 56%. The main contributing eigenvectors of PC1 were, in decreasing order, 18:4(n-3), 18:3(n-3), 16:2(n-4), and 16:1(n-7) with positive values, as well as 20:5(n-3), 18:0, 20:1(n-11), and 20:1(n-7) with negative values. PC2 was mostly characterized by positive values of 20:4(n-6) and 20:5(n-3), as well as by negative values of 16:1(n-7), 16:4(n-4), and 20:1(n-7), again in decreasing order.

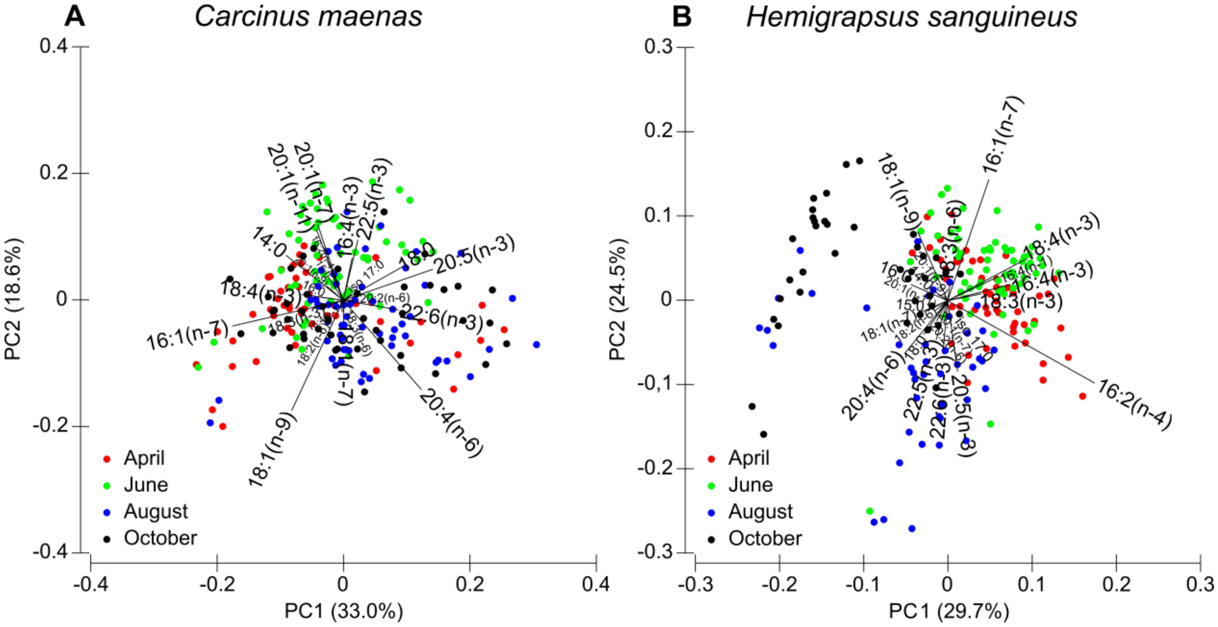

Fig. 6.4: Principal component analyses (PCAs) of hepatopancreas fatty acid compositions of (A) *Carcinus maenas* and (B) *Hemigrapsus sanguineus* sampled at Helgoland, Germany in 2015.

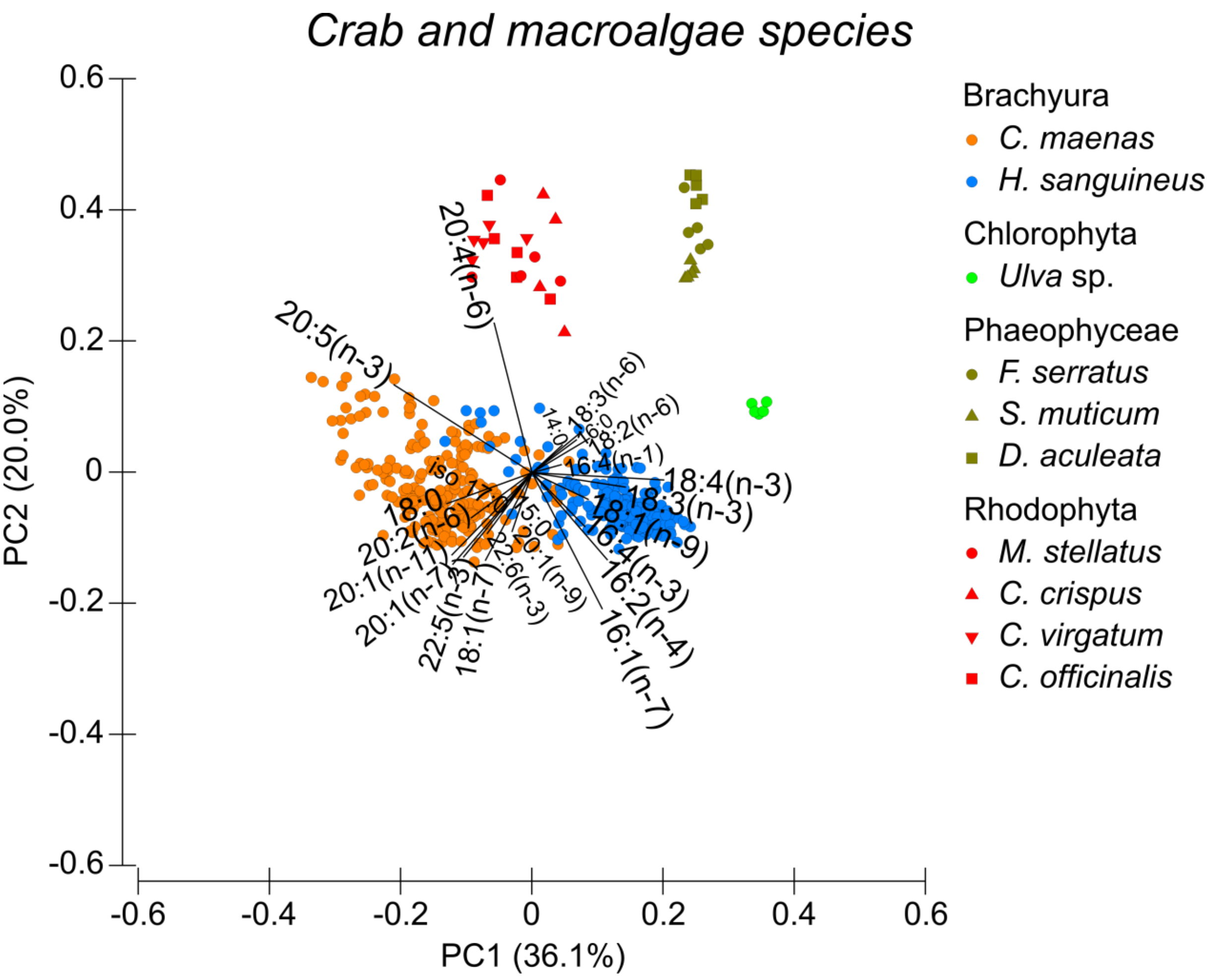

Fig. 6.5: Principal component analysis (PCA) of fatty acid compositions of hepatopancreas samples of *Carcinus maenas*, *Hemigrapsus sanguineus*, and of different macroalgae species sampled at Helgoland, Germany, in 2015.

6.4.4 Carnivory index

Levels of the carnivory index (I_{Ca}) were higher in *H. sanguineus* compared to *C. maenas* (significant main factor species, pooled over fresh mass, sex and months, $p < 0.001$) and showed significantly differing seasonal patterns between the two species with regard to the species*sex*month interaction (pooled over fresh mass, $p = 0.012$) and the species*month interaction (pooled over fresh mass and sex, $p < 0.001$). Males and females of *C. maenas* showed similar seasonal patterns (Fig. 6.6A and B). In June, the average I_{Ca} was lower (0.20 ± 0.06 and 0.21 ± 0.07, resp.) than in the other months (I_{Ca} between 0.29 ± 0.07 and 0.34 ± 0.12). In *H. sanguineus*, males showed similar average I_{Ca} values in April, June, and August (0.32 ± 0.08, 0.35 ± 0.08, 0.30 ± 0.06, resp.) but an increase in October (0.39 ± 0.05) (Fig. 6.6C). In female *H. sanguineus* I_{Ca} levels were similar in April and June (0.30 ± 0.09 and 0.31 ± 0.06, resp.), increased in August (0.39 ± 0.08), and remained at this level in October (0.40 ± 0.07).

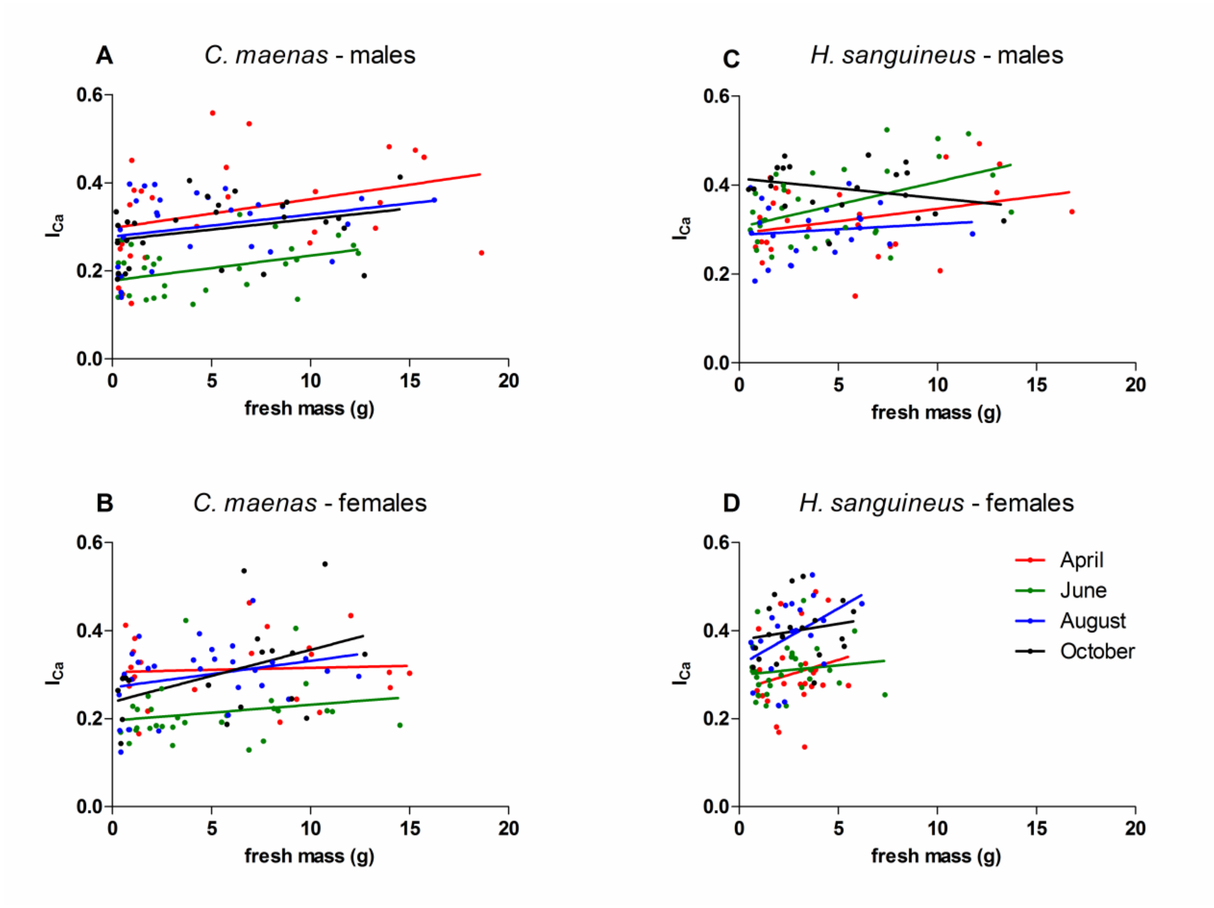

Fig. 6.6: Carnivory index (I_{Ca}) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of I_{Ca} see Table 6.1.

6.4.5 Chlorophyta index

H. sanguineus showed higher Chlorophyta index (I_{Ch}) levels than *C. maenas* (significant main factor species, pooled over fresh mass, sex and months, $p < 0.001$). The average values of *C. maenas* ranged between 12.8 ± 1.7 for males in August and 13.8 ± 1.8 for males in June (Fig. 6.7A and B). For *H. sanguineus*, in contrast, the average I_{Ch} values were between 15.5 ± 2.8 for males in October and 17.8 ± 2.0 for females in females in June (Fig. 6.7C and D).

I_{Ch} differed between males and females of *C. maenas* and *H. sanguineus* with fresh mass (significant species*sex*fresh mass interaction, pooled over months, $p = 0.005$). Only *H. sanguineus* females showed a positive correlation between I_{Ch} and fresh mass. When pooled over sexes, the two species also differed in their seasonal relationship of I_{Ch} with fresh mass (significant species*month*fresh mass interaction, $p = 0.046$). Then, only *H. sanguineus* individuals from October showed a positive I_{Ch} correlation to fresh mass whereas all other *H. sanguineus* and all *C. maenas* individuals did not. Pooling the different months and sexes revealed that I_{Ch} levels of *H. sanguineus* specimen were correlated to fresh mass, whereas this is not the case in *C. maenas* (significant species*fresh mass interaction, p = 0.011).

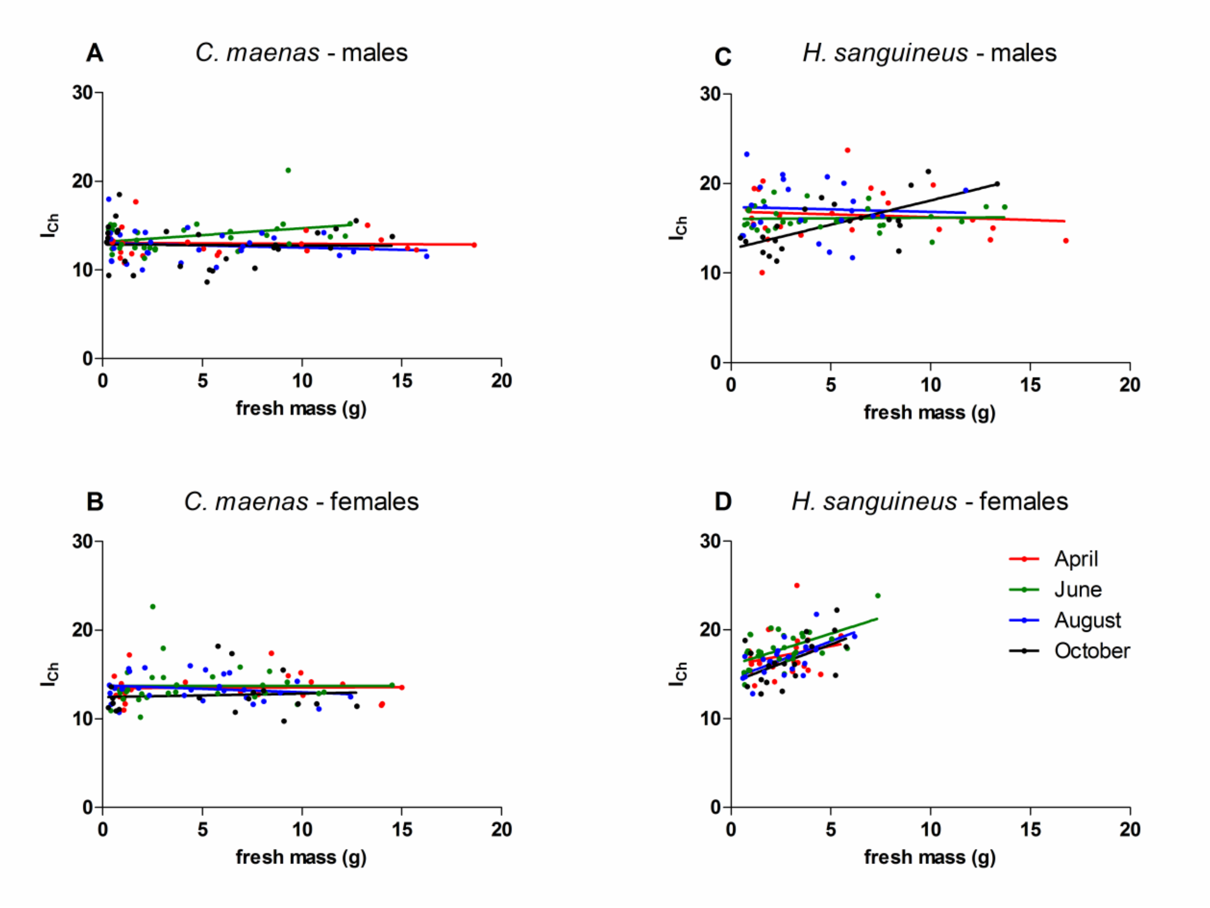

Fig. 6.7: Chlorophyta index (I_{Ch}) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of I_{Ch} see Table 6.1.

6.4.6 Phaeophyceae index

Levels of the Phaeophyceae index (I_P) were higher in *H. sanguineus* than in *C. maenas* (significant main factor species, p < 0.001). The seasonal patterns of I_P differed according to sex and month (significant species*sex*month interaction, pooled over fresh mass, p = 0.014). In males, a similar pattern was detected in both species: similar levels in April and June, a decrease towards August followed by a similar level in October (*C. maenas*: 0.9 ± 0.7, 1.1 ± 0.6, 0.5 ± 0.3, 0.4 ± 0.3, resp., Fig. 6.8A; *H. sanguineus*: 4.3 ± 1.3, 3.8 ± 1.1, 2.2 ± 0.9, 2.4 ± 0.8, resp., Fig. 6.8C). The seasonal patterns in female *H. sanguineus* were similar to that of males (4.5 ± 1.5, 4.5 ± 1.1, 1.7 ± 0.6, 2.3 ± 0.7 from April to October Fig. 6.8D). Females of *C. maenas* showed a decrease from August to October (1.2 ± 1.0, 1.0 ± 0.5, 0.6 ± 0.3, 0.4 ± 0.3 from April to October, resp., Fig. 6.8B). Additionally, when both sexes and fresh masses were pooled, the two species differed in their seasonal IP patterns (significant species*month interaction, $p < 0.001$).

Only females of *H. sanguineus* showed a positive correlation between I_P and fresh mass. No correlation occurred in *H. sanguineus* males and in both sexes of *C. maenas* (significant species*sex*fresh mass interaction, pooled over months, $p = 0.007$).

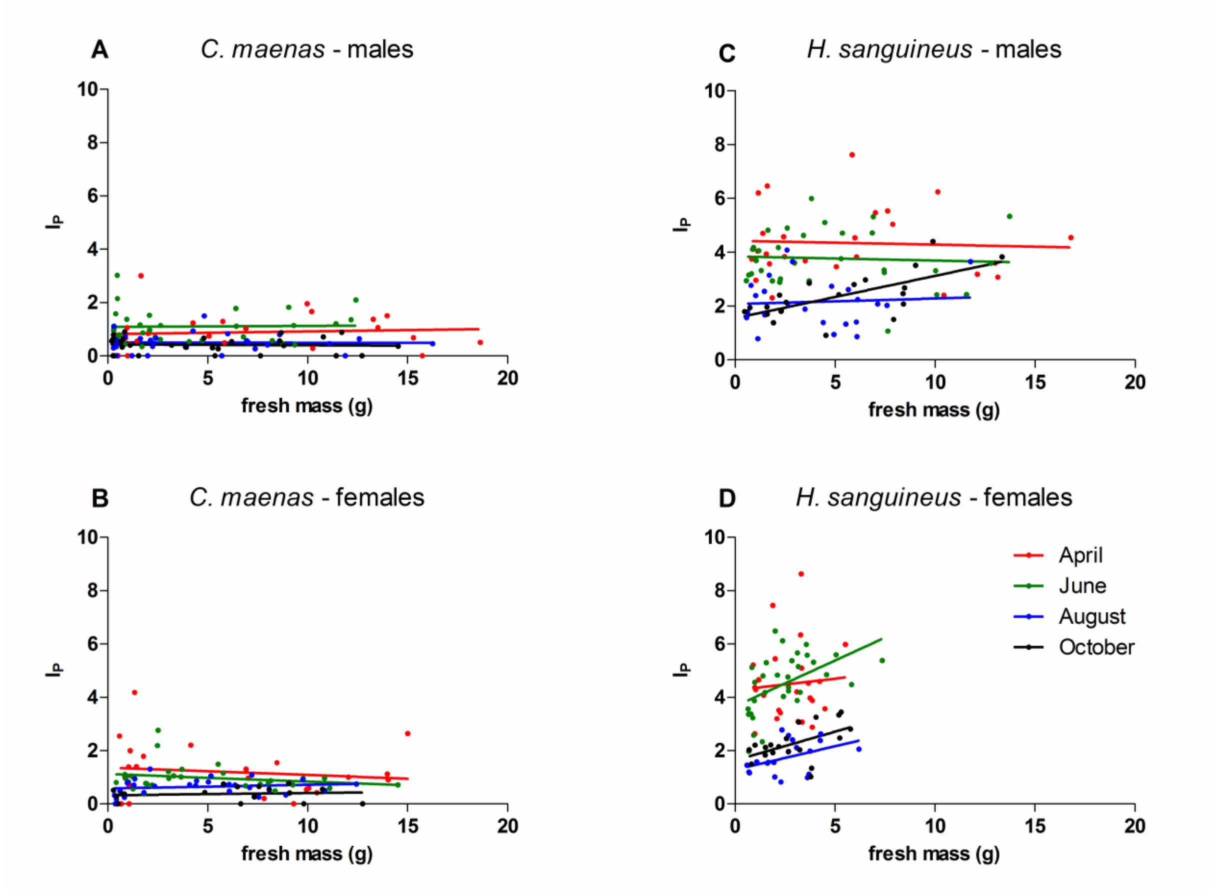

Fig. 6.8: Phaeophyceae index (I_P) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of I_P see Table 6.1.

6.4.7 Rhodophyta index

Overall, values of the Rhodophyta index (I_R) were lower in *H. sanguineus* than in *C. maenas* (significant main factor species, $p < 0.001$). Only the I_R values of *H. sanguineus* females (Fig. 6.9D) showed a negative correlation with fresh mass (significant species*sex*fresh mass interaction, pooled over months). When the data were pooled over months and sexes, a negative relation of I_R and fresh mass was found for *H. sanguineus* but not for *C. maenas* (significant species*fresh mass interaction, $p = 0.036$).

The seasonal I_R patterns differed for the two species (significant species*month interaction, pooled over sex and fresh mass, $p < 0.001$). The I_R-levels of *C. maenas* were similar in April and June (0.6 ± 0.2 and 0.6 ± 0.1), decreased in August (0.5 ± 0.1), and remained at the same level in October (0.5 ± 0.2), (Fig. 6.9A and B).

In *H. sanguineus*, the I_R-levels decreased from April to June and August and again to October (0.4 ± 0.1, 0.3 ± 0.1, 0.3 ± 0.1, 0.2 ± 0.1, resp., Fig. 6.9C and D).

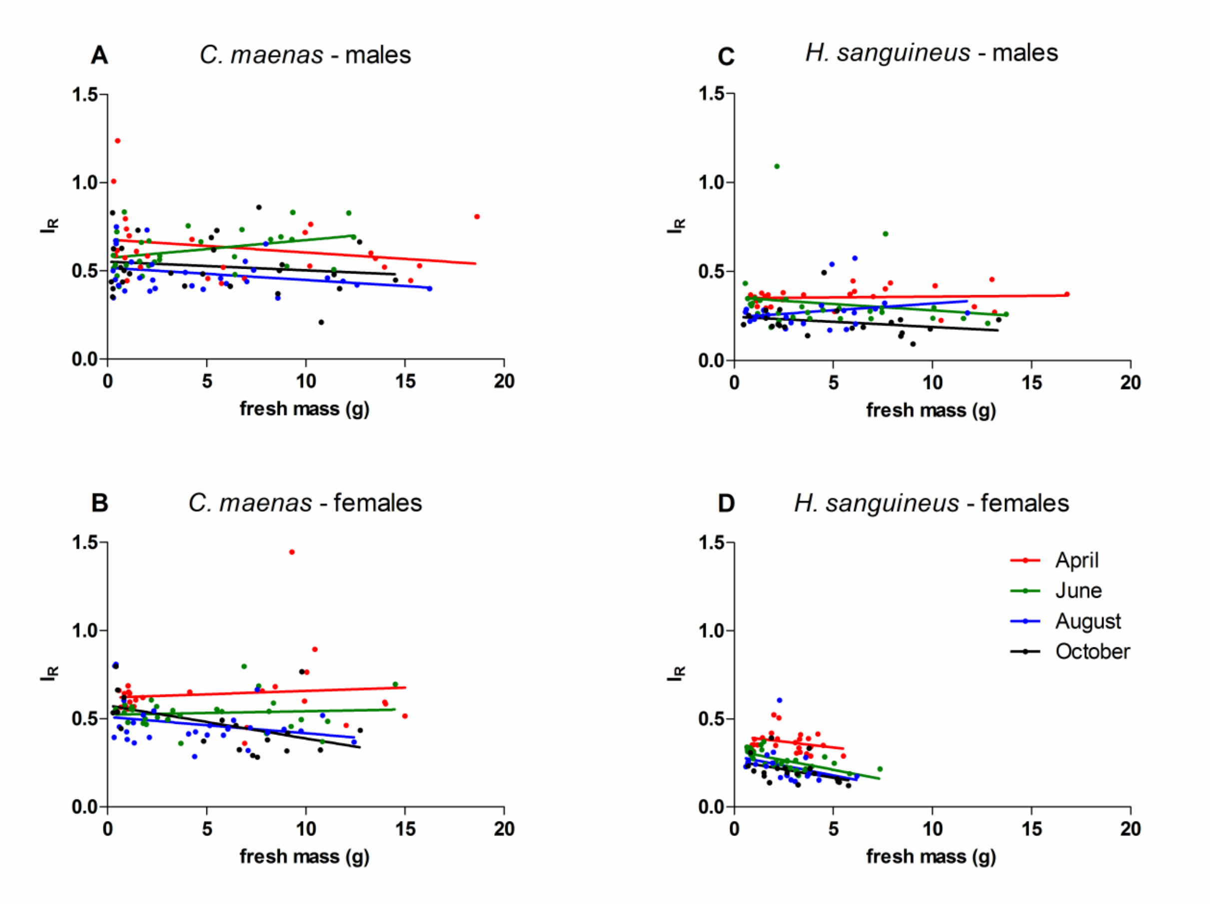

Fig. 6.9: Rhodophyta index (I_R) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of I_R see Table 6.1.

6.4.8 Bacillariophyceae index

Values of the Bacillariophyceae index (I_B) were higher in *H. sanguineus* than in *C. maenas* (significant main factor species, $p < 0.001$). Seasonal patterns, however, differed between species (significant species*month interaction, pooled over sex and fresh mass, $p < 0.001$). *C. maenas* showed decreasing values from April to August and an increase in October (8.9 ± 3.5, 7.4 ± 3.2, 5.0 ± 2.7, 6.5 ± 2.9, resp., Fig. 6.10A and B). In contrast, *H. sanguineus* had similar

I_B-values in April and June, decreasing values in August followed by an increase to October (12.3 ± 2.7, 13.0 ± 2.4, 7.3 ± 1.9, 10.4 ± 3.0, resp., Fig. 6.10C and D).

When pooled over month and fresh mass (significant species*sex interaction, $p = 0.026$) *C. maenas* males had lower I_B-values than females (6.6 ± 3.1 and 7.3 ± 3.6, resp.). The opposite appeared in *H. sanguineus* males and females (11.2 ± 3.7 and 10.8 ± 2.9, resp.).

Positive correlations between I_B-values and fresh masses were present in both sexes of *C. maenas*, but no significant correlation was apparent in *H. sanguineus* (significant species*fresh mass interaction, pooled over sex and month, $p = 0.035$).

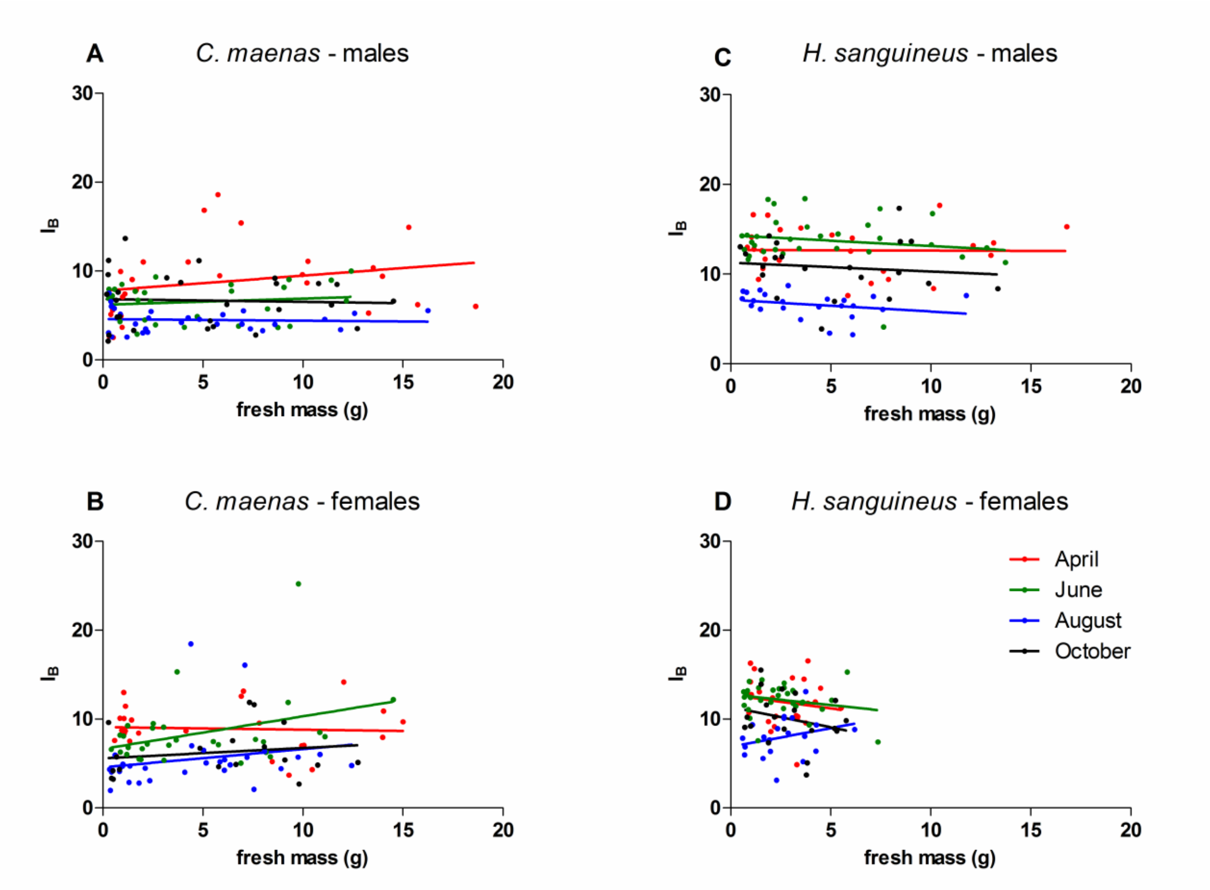

Fig. 6.10: Bacillariophyceae index (I_B) of *Carcinus maenas* males (A) and females (B) as well as *Hemigrapsus sanguineus* males (C) and females (D) sampled at the island of Helgoland, Germany, in 2015. For the calculation of I_B see Table 6.1.

6.5 Discussion

6.5.1 Energy storage

We used the hepatosomatic index (HSI) and the total lipid content (TL) as measures for the energy storage capacities of *Carcinus maenas* and *Hemigrapsus sanguineus.* HSI values were in the same range as reported previously for these two species from the east coast of North America (0.02 - 0.13 for both species, Griffen et al. 2011, 2012). In the present study, the HSI did not differ significantly between species and the only seasonal variation was a decrease of the HSI in *C. maenas* from June to August. This result differs from observations from Scotland, where no HSI seasonality was detected in *C. maenas* (Heath & Barnes 1970).

The amount of total lipids in the hepatopancreas is a good assessment of the energy stores in crabs (Jimenez & Kinsey 2015). TL values, 20$\%_{DM}$ for *C. maenas* and 20 to 50$\%_{DM}$ for *H. sanguineus*, were in a range reported previously for *C. maenas* (Aagaard 1996, Styrishave & Andersen 2000) and *H. sanguineus* (Griffen et al. 2015). The TL levels for *H. sanguineus* showed a strong seasonal pattern, whereas TL values of *C. maenas*, did not differ between seasons around Helgoland. This observation is in contrast to distinctly seasonal TL values reported for *C. maenas* from Denmark (Styrishave & Andersen 2000).

Energy stores are required, when animals need to overcome periods of food scarcity, e.g. low productive seasons, or when energy stores are used to support reproduction (Alava et al. 2007, Barrento et al. 2009, Kennish 1997, Kyomo 1988, Kucharski & Da Silva 1991b, Yamaguchi 2004). The different annual lipid patterns of the two species can hardly be explained by the differing reproductive strategies described for *C. maenas* and *H. sanguineus*. On the basis of HSI and gonadosomatic indices (GSI), North American *C. maenas* were identified as 'capital breeders', i.e. they store energy in the hepatopancreas, before they use it for oogenesis (Griffen et al. 2011). However, our data did not show lipid accumulation prior to and lipid depletion during the reproductive season of *C. maenas*. In contrast to *C. maenas*, *H. sanguineus* was described as an 'income breeder', i.e. energy taken up by feeding immediately fuels oogenesis (Griffen et al. 2012). This strategy should result in similar TL levels in the hepatopancreas throughout the reproductive season. Instead, we detected a strong seasonality in the lipid depots of *H. sanguineus*.

The differences in *H. sanguineus* and *C. maenas* in TL levels and seasonality may rather be explained by deviations in the duration of their reproductive periods and in their egg-production capacities. Around Helgoland, the reproductive period of *H. sanguineus* covers at least five months (June to October), whereas that of *C. maenas* lasts only for about 3 months (April to June) (S. Jungblut, pers. obs.). Per year, *H. sanguineus* females produce about five egg clutches with up to 56,000 eggs each (Fukui 1988), *C. maenas* females only one clutch with max. 185,000 eggs (Klassen & Locke 2007). Around Helgoland, single egg clutches of female *C. maenas* comprised on average 15.1% of their body fresh mass, whereas *H. sanguineus* females produced a corresponding mean clutch mass of 8.4%. Thus, annual egg production of *C. maenas* accounts for about 15% of the body mass, the five clutches of *H. sanguineus* together comprise 42% of their body mass (M. McCarthy, unpubl. data). Additionally, freshly spat *H. sanguineus* eggs contained lipid levels of about $28\%_{DM}$, those of *C. maenas* only about $23\%_{DM}$. Hence, *H. sanguineus* produces more egg mass and transfers more lipids into their eggs than *C. maenas*. Thus, *H. sanguineus* females invest in higher egg quantity and quality, they accumulate more lipid reserves and must have a higher lipid turnover than *C. maenas*.

The same pronounced seasonal pattern of lipid deposition as in females was also found in the males of *H. sanguineus*. Styrishave & Andersen (2000) suggested that energy-demanding mating behavior causes the seasonal lipid depletion in *C. maenas* males from Denmark. The energy demand of the mating behavior of male *H. sanguineus* is unknown. Furthermore, there is no evidence that *H. sanguineus* males cease feeding during the mating season and would thus not be able to maintain their energy depots.

Energy storage levels may also be influenced by the amount and quality of the ingested diet. Higher HSI and TL levels occurred in both species, when they were fed in the laboratory with an animal diet instead of algae (Griffen et al. 2011, 2012, Griffen 2017). In order to store larger amounts of lipids, e.g. for reproductive efforts, *H. sanguineus* might ingest more food or increase carnivory to compensate for the low energy content of the usually preferred algae material.

6.5.2 Fatty acid compositions of midgut glands

The FA compositions of the midgut glands of *C. maenas* and *H. sanguineus* formed two well separated clusters in the PCA. No distinct differences in FA compositions in relation to sex or seasons were detected in each of the two species. Other studies, however, reported variable FA compositions, which were attributed to ovarian maturation, sex, and seasons (Alava et al. 2007, Barrento et al. 2009). In *C. maenas* from the Kattegat, diverging FA compositions were found in males and females as well as between males of different color morphs (Styrishave & Andersen 2000). In the same study, the levels of dominating FAs were reported to differ between seasons. Moreover, acclimation of *C. maenas* in the laboratory to lower temperatures resulted in higher portions of unsaturated FAs, which were incorporated to maintain membrane fluidity and permeability (Chapelle 1978).

The formation of two separate clusters in the PCA may partly be due to the large differences in TL levels. The cluster of *C. maenas* is oriented towards the direction of three membrane FAs, 18:0, 20:5(n-3), and 22:6(n-3). These FAs gained more overall importance in *C. maenas* samples, because the TL levels were rather low and hence biomembrane FAs prevail. However, other components seemed to contribute to this separation as well. For instance, the vector of the FA 18:1(n-9) is directed towards the samples of *H. sanguineus*. On a first glance, this could indicate a higher degree of carnivory. Indeed, 18:1(n-9)-values were influenced by consumed animal tissue, but were most likely elevated after consumption of 18:1(n-9)-rich Phaeophyceae, especially *Fucus serratus* and *Desmarestia aculeata*.

6.5.3 Dietary preferences

The example of the FA 18:1(n-9) illustrates the need for a closer examination of the dietary preferences of benthic animals and the significance of fatty acid trophic markers. The five taxa-specific FA trophic marker indices are suitable tools to reveal more detailed information about the dietary preferences of *C. maenas* and *H. sanguineus*. Each of the five indices clearly showed differences between the two species. For some indices, differences were also found between seasons and between sexes and among different sizes of crabs. These differences, however, did not follow similar trends, e.g. for smaller versus larger crabs during different seasons.

Similar to our study on fatty acids, the few studies on the stomach contents of differently sized *C. maenas* showed inconsistent results (Ropes 1968, Elner 1981, Ropes 1988, Baeta et al. 2006). Therefore, we suggest to encourage FATM analysis as the intermediate way between biased short-term gut content studies and rather unspecific long-term SI analysis, in order to evaluate complex dietary preferences and to explore competition for food in co-occurring crabs (Graeve et al. 2001, Latyshev et al. 2004, Griffen 2014).

Three of the four algae indices were higher in *H. sanguineus* than in *C. maenas*, which indicates a higher level of herbivory for *H. sanguineus*. These results corroborate findings from the U.S. east coast, where *C. maenas* relied to 24% on herbivorous feeding and *H. sanguineus* to 65% (Griffen & Mosblack 2011).

The Bacillariophyceae index was higher in *H. sanguineus* than in *C. maenas*, indicating higher ingestion of benthic microalgae, e.g. from the sediment or by feeding on a food source (prey or macroalgae) covered or surrounded by a biofilm. For instance, juvenile *C. maenas* feed on detritus from the seafloor and extract meiofauna from the sediment (Eriksson & Edlund 1977, Pihl 1985) and *H. sanguineus* consumes settling cyprid larvae (Brousseau & Goldberg 2007). However, the effect of the primary food source may dominate the effect of the biofilm and the importance of sediment ingestion in foraging of *H. sanguineus* is unknown.

The Chlorophyta index and especially the Phaeophyceae index were higher in *H. sanguineus* than in *C. maenas*. High consumption of Chlorophyta by *H. sanguineus* is in accordance with a preference for *Enteromorpha intestinalis* (now *Ulva intestinalis*, L. 1753) observed in a multi-species choice experiment (Tyrell & Harris 1999) and with reported food preferences of the congener *Hemigrapsus nudus* (Dana 1851) (Birch 1979). A higher Phaeophyceae index in *H. sanguineus* indicates a higher consumption of brown algae compared to *C. maenas*. It may partially be elevated due to the higher consumption of Chlorophyta and benthic microalgae, as Chlorophyta and dinoflagellates also show relatively high levels of the FA 18:4(n-3), which is the basis of the Phaeophyceae index (Kelley & Scheibling 2012). However, the species-specific difference in the Phaeophyceae index was higher than the differences for the Chlorophyta and the Bacillariophyceae indices. Therefore, it is likely that *H. sanguineus* indeed consumed Phaeophyceae at a much higher rate than *C. maenas*. In the multi-species choice experiment mentioned above, the Phaeophyceae *Fucus vesiculosus* was the second-most preferred algae after *E. intestinalis* (Tyrell & Harris 1999).

The higher Rhodophyta level in *C. maenas* as compared to *H. sanguineus* corroborates studies from North America. *Chondrus crispus* was the major algae species in the guts of female *C. maenas* (Griffen et al. 2011). Interestingly, *C. maenas* also significantly shifted consumption towards red algae, when *H. sanguineus* was present (Griffen et al. 2008). *H. sanguineus* was also observed to consume red algae in its native and its U.S.-invaded habitat (Lohrer et al. 2000, Griffen & Delaney 2007).

Both, *C. maenas* and *H. sanguineus*, were described as omnivorous predators feeding on a variety of food organisms (for. *C. maenas*: e.g. Ropes 1968, Elner 1981, Scherer & Reise 1981, Ropes 1988, Baeta et al. 2006; for *H. sanguineus*: e.g. Gerard et al. 1999, Tyrell & Harris 1999, Lohrer et al. 2000, Brousseau & Goldberg 2007, Brousseau et al. 2014). The carnivory index levels were higher for *H. sanguineus* than for *C. maenas*. This result, however, is distorted in *H. sanguineus* by the high consumption of 18:1(n-9)-rich Phaeophyceae, which is also the numerator FA of the carnivory index ratio. Thus, in fact, *H. sanguineus* is less carnivorous than *C. maenas.*

We demonstrated that *H. sanguineus* prefers a herbivorous diet and thus competition for food between *C. maenas* and *H. sanguineus* may be low in macroalgae-rich habitats. So far, the trophic impact of *H. sanguineus* was mostly evaluated for potential animal prey organisms like barnacles, mytilid mussels or littorinid snails (e.g. Lohrer et al. 2000, Lohrer & Whitlatch 2002, Brousseau & Baglivo 2005, Tyrell et al. 2006, Brousseau & Goldberg 2007, Brousseau et al. 2014). Only few studies consider the more likely case: the impact of *H. sanguineus* on the algal community (Tyrell & Harris 1999). In contrast to *C. maenas*, *H. sanguineus* does not retreat to subtidal areas or bury in the sediment in winter (Janke 1986, Aagaard et al. 1995). Thus, *H. sanguineus* is present and forages in the intertidal area during the whole year and may reduce the winter recruitment success of e.g. Phaeophyceae in invaded habitats. Given the macroalgae richness, this effect might be low around Helgoland. For macroalgae-poor habitats like the Wadden Sea, however, the competition for food between *C. maenas* and *H. sanguineus* and the effect of *H. sanguineus* on Phaeophyceae might be significant.

6.6 Conclusions

We demonstrated that *Hemigrapsus sanguineus* stored much higher amounts of lipids as energy reserve than *Carcinus maenas* (hypothesis 1 accepted). In contrast to *C. maenas*, *H. sanguineus* showed a pronounced seasonality in lipid deposition and depletion (hypothesis 2 rejected). This difference may be due to deviating reproductive capacities of the two species. The fatty acid compositions of *C. maenas* and *H. sanguineus* differed distinctly from one another (hypothesis 3i accepted), but within each species, no differences with respect to sex or seasons became apparent (hypotheses 3ii and 3iii rejected). *H. sanguineus* was able to accumulate high lipid quantities in relatively short periods, apparently by mainly utilizing macroalgae. The dietary indices for Chlorophyta, Bacillariophyceae and especially for Phaeophyceae were higher for *H. sanguineus* than for *C. maenas* (hypothesis 4i accepted). Only the Rhodophyta index suggested a higher consumption of red algae by *C. maenas*. The index for relative carnivory was higher for *H. sanguineus* than for *C. maenas*. However, it was clearly distorted for *H. sanguineus* and must be interpreted with caution, as the fatty acid 18:1(n-9), the nominator in the carnivory index, is also prevalent in Phaeophyceae, making this index ambiguous. There was no clear pattern in the dietary preferences of *C. maenas* and *H. sanguineus* with regard to crab size, sex, and season (hypothesis 4ii, iii, and iv without decision). The competition for food between *H. sanguineus* and *C. maenas* may be low in macroalgae-rich habitats. *H. sanguineus* also naturally consumes energy-poor macroalgae to a much higher degree than *C. maenas*, apparently occupying a quite different trophic niche.

However, *H. sanguineus* is able to maintain large lipid reserves, which can buffer periods of food paucity, facilitate its reproductive output and thus contributes to the invasion success of this invasive crab.

6.7 Acknowledgements

We thank the staff of the Biologische Anstalt Helgoland and especially Dr Jan Beermann for laboratory space and for support, whenever needed. Dr Beermann also provided and prepared the map of Helgoland. This study was partly supported by a RISE internship grant from the DAAD (German Academic Exchange Service) to Morgan L McCarthy. Dominik A Nachtsheim proofread the final version of the manuscript.

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7 Synoptic discussion

7.1 Baseline information

This thesis project compared ecological and ecophysiological properties of the invasive Asian shore crab *Hemigrapsus sanguineus* and the native European green crab *Carcinus maenas* around the island of Helgoland. The most important results of the thesis and some additional information for the direct comparison of the two species are compiled in Table 7.1. These data provide the basis of the following synoptic discussion.

Table 7.1: Compilation of relevant results to compare the invasive Asian shore crab *Hemigrapsus sanguineus* and the native European green crab *Carcinus maenas*. FATM = fatty acid trophic marker.

Parameter	**Region**	***H. sanguineus***	***C. maenas***	**Source**
Abundance (2009 - 2014)	Helgoland	increasing	stagnating	Publication I
Site of max. abundance (2014)	Helgoland	sheltered site	exposed site	Publication I
Site fidelity	US east coast	low	unknown	Brousseau et al. 2002
Parasite infection	The Netherlands	low	high	Goedknegt et al. 2017
Annual population energy demand (GJ yr^{-1})	Helgoland	26-33	25 -31	Publication II
Reproductive duration (months)	Helgoland	5	3	Jungblut, pers. obs.
Breeding strategy	US east coast	income breeder	mixed breeder	Griffen et al. 2012 / Griffen 2017
Annual no. of egg clutches per female	Asia / US east coast	5	1	Fukui 1988 / Klassen and Locke 2007
Annual egg mass per female (% fresh mass)	Helgoland	40	15	McCarthy, unpubl. data
Egg no. per clutch	Asia / US east coast	56,000	185,000	Fukui 1988 / Klassen and Locke 2007
Annual no. of eggs per female	Asia / US east coast	280,000	185,000	Fukui 1988 / Klassen and Locke 2007
Lipid content eggs (% dry mass)	Helgoland	28	23	McCarthy, unpubl. data
Lipid content midgut gland (% dry mass)	Helgoland	30-50	20	Publication III
Chlorophyta consumption (rel. FATM index units)	Helgoland	16.7	13.3	Publication III
Phaeophyceae consumption (rel. FATM index units)	Helgoland	3.4	0.8	Publication III
Rhodophyta consumption (rel. FATM index units)	Helgoland	0.3	0.6	Publication III
Bacillariophyceae consumption (rel. FATM index units)	Helgoland	11	6.9	Publication III
Herbivory (%), based on gut morphology	US east coast	65	24	Griffen and Mosblack 2011
Algae material (% gut contents)	Helgoland	38	23	Saborowski et al. in prep.

7.2 Invasion dynamics of *H. sanguineus* in Europe

The progress of invasions of *H. sanguineus* at the North American east coast and at the European Atlantic shores were characterized by initial rapid population growth at the colonized spots and subsequent spreading into adjacent areas (see Introduction and references therein). The first step of this process was evident at Helgoland as well: the population of *H. sanguineus* showed a strong increase from 2009 to 2014 (publication I). The second step, the expansion of the population, is limited because the island of Helgoland represents a special case: it offers the only and, thus, isolated rocky intertidal in the German Bight.

This special situation prevented the establishment of another invasive crab, the brush-clawed shore crab *Hemigrapsus takanoi*. It can only be found in extremely low numbers around Helgoland, where the intertidal areas are dominated by coarse sand with cobbles and boulders on top (publication I). *H. takanoi*, however, prefers muddy sand with shelter opportunities (Dauvin et al. 2009, Gothland et al. 2014). Both habitat conditions, coarse and muddy sands with shelter opportunities can be found in the German Wadden Sea, where both species are abundant (Landschoff et al. 2013). Therefore, two invading *Hemigrapsus* species need to be taken into account, when considering invasive intertidal crabs in Europe and their competitive interactions with the native species, the European green crab *Carcinus maenas*.

Facing the new competitors in its native range, the local German *C. maenas* population seems to perform relatively well for the moment (Landschoff et al. 2013, publication I). Likewise, in the Dutch part of the Wadden Sea, the invasion of *Hemigrapsus* spp. was only discussed as a facilitating factor for an already on-going decrease of *C. maenas* abundances but not as the determining factor (van den Brink et al. 2012).

In one of the four intertidal sites, which were sampled around Helgoland, *C. maenas* still outnumbered *H. sanguineus*. This was explained by the extremely high wave exposition at this site and it was suggested that the native *C. maenas* might be better adapted to such conditions. Furthermore, *C. maenas* has established a relatively large, more sheltered subtidal population, which may supply the intertidal areas with recruits (publication I). Three other sites around Helgoland, which are dominated by *H. sanguineus*, are also highly hydrodynamic but less wave-exposed. Here, *H. sanguineus* probably benefits from the gregarious settlement behaviour of its megalopa larvae when detecting waterborne cues of adult conspecifics (e.g. Anderson and Epifanio 2009, Anderson et al. 2010).

An important factor for the success of *H. sanguineus* in Europe is probably the absence of influential predators. The only predators of adult *H. sanguineus* in European waters could be large *C. maenas*. Seabirds do not seem to feed on *H. sanguineus*. On the piers around the island Helgoland we found no remains of *H. sanguineus* while remains and shell fragments of other crab species were frequent (publication I). However, a more detailed study is needed to confirm this observation.

Laboratory experiments from North America revealed a preference of crab-eating fishes for *H. sanguineus* over native crab species (Kim and O'Connor 2007, Heinonen and Auster 2012). However, stomach content analyses of wild-caught fishes did not confirm this finding and it was concluded that the predation pressure of fish on *H. sanguineus* seems to be rather low in the wild (Kim and O'Connor 2007, Brousseau et al. 2008). Similarly, the predation pressure of fish in the Helgoland intertidal can be expected to be low, as very few crab-eating fishes (mostly the shanny *Lipophrys pholis*) occur in this habitat (S. Jungblut, J. Beermann, pers. obs.).

In addition to a low number of predators, *H. sanguineus* is less infected by parasites in Europe compared to the invaded North American range and the native Asian range (Kroft and Blakeslee 2016, Goedknegt et al. 2017 and references therein). In contrast, the native *C. maenas* was found to be heavily infected. Whether or not *H. sanguineus* benefits from lower infection levels is still unclear (Goedknegt et al. 2017).

Currently, the southern North Sea represents the northernmost area, which is highly invaded by *H. sanguineus*. Further north, so far, only some dozens of individuals were detected at the Swedish west coast (Jungblut et al. in prep.) and, thus, close to the entrance of the Baltic Sea. Given the availability of suitable habitats along the Scandinavian coast and in the Baltic Sea, the question arises about the abiotic limits of *H. sanguineus*, i.e. how far can they progress north (limited by temperature) and into the Baltic Sea (limited by lower salinities). The congener of *H. sanguineus*, *H. takanoi*, has already been found in the Baltic Sea, which indicates that *H. sanguineus* might also be able to establish there. *H. takanoi* was found in the Kiel fjord, most likely introduced as fouling on ship hulls through the Kiel Channel (Geburzi et al. 2015). Likewise, also the first German record of *H. takanoi* (at that time still considered as *H. penicillatus*) was found as hull fouling on a car-carrier in the port of Bremerhaven (Gollasch 1999). Thus, the introduction of *H. sanguineus* into the Baltic might be possible vial larval transport into the Skagerrak region, but also as ship hull fouling species.

The potential of adult *H. sanguineus* to tolerate quick transfer to colder and less saline waters, as for instance via ships, was examined in a laboratory experiment. Survival and activity of *H. sanguineus* was unaffected by temperatures as low as 10°C and by salinities as low as 10 (S. Jungblut, unpubl. data). Thus, a transport of adults into colder and/or less saline habitats, as for instance with hull fouling of ships, might be successful.

An on-going study combines field larvae data with survival and developmental rates of *H. sanguineus* larvae reared under different temperature and salinity regimes in the laboratory. A modelling approach (Giménez et al. in prep.) will establish the temperature and salinity limits and show in which areas of Europe larvae of *H. sanguineus* will be able to develop and to establish self-sustaining (reproductive) populations. Beyond those limits, the spread of *H. sanguineus* might reach even further given that 'sink populations' may establish. Sink populations are supplied by recruits from other areas, where *H. sanguineus* is able to reproduce. A similar case can already be found in the Baltic Sea, where in certain localities the invasive Chinese mitten crab *Eriocheir sinensis* is only able to maintain a population, as larvae are supplied externally to this region (Ojaveer et al. 2007, Wójcik and Normant 2014).

Depending on the ability of *H. sanguineus* to tolerate elevated temperatures and salinities, it may also be able to colonize the Mediterranean or Black Sea. So far, however, only a few scattered specimens were found in either area (Schubart 2003, Micu et al. 2010).

There is no apparent reason, why *H. sanguineus* should not be able to further spread along the Swedish Skagerrak coast, southern Norway or the German Baltic coast. Given the currently increasing numbers of *H. sanguineus* specimens reported for the Swedish Skagerrak coast (M. Berggren, pers. comm.), a continuous monitoring should take place to evaluate the population development in this and in adjacent areas. Changes of water level due to tides are very small or absent in the Skagerrak and the Baltic Sea. An intertidal area to investigate and to sample by turning rocks in defined areas (e.g. publication I) is absent. Thus, monitoring can only be managed with traps to be deployed in a habitat of interest for a certain duration. Abundances of North American *H. sanguineus* were clearly positively correlated to the amount of shelter opportunities (Lohrer et al. 2000a). Thus, a monitoring should probably be carried out using 'artificial habitat collectors' or 'shelter traps', e.g. rack cubes filled with oyster shells or other natural material that provides plenty of shelter opportunities and which are deployed for several weeks (Fowler et al. 2013, Hewitt and McDonald 2013). Such traps are expected to be more efficient compared to fish-baited traps, which were less successful in catching *H. sanguineus* in an actually highly invaded site at Helgoland (Jungblut et al. in prep.).

H. sanguineus provides a good example to follow the invasion history of an invasive brachyuran crab in several locations around the world. For instance, the temperature-related distribution limits in North America and Europe could be examined first by model predictions and then verified by actual monitoring data. However, a distribution limit of *H. sanguineus* in Europe is not yet reached as abundances are still increasing and new habitats are still being invaded.

7.3 Energy demand, food preferences and habitat impact

Small and medium-sized *H. sanguineus* consume significantly more oxygen than *C. maenas* of similar size (publication II). Two alternative explanations were provided for this observation, which do not exclude each other. The first explanation is based on a different growth pattern (e.g. Gutermuth and Armstrong 1989). At a similar carapace width, *H. sanguineus* individuals have a higher mass than *C. maenas*, due to their quadratic carapace shape (see Figs. 1.1 and 1.3). Both species measure about 10 mm in carapace widths at the end of their first season after recruitment (Erikson and Edlund 1977, Dauvin 2009). Applying the carapace width-biomass regressions established for Helgoland, *H. sanguineus* is remarkably heavier than *C. maenas* at this stage: 0.42 versus 0.29 g, respectively (publication I). Therefore, the body mass of early life stages of *H. sanguineus* is growing faster compared to *C. maenas*, which may lead to a higher oxygen consumption. The second explanation is based on the activity and mobility of *H. sanguineus*. Unfortunately, it was not possible to record activity patterns of the crabs during the respiration measurements. Higher respiration might be due to higher activity in *H. sanguineus*. This seems likely, as in the field *H. sanguineus* generally appears to be more agile and mobile than *C. maenas* (e.g. Brousseau et al. 2002, Jensen et al. 2002).

Higher respiration rates of *H. sanguineus* result in a higher energy demand than for *C. maenas*, which the invasive crabs have to compensate by appropriate food uptake (publication II). Based on abundance and biomass data from August 2014 (publication I), the energy demand of the populations of both species were extrapolated for the whole Helgoland intertidal. Although abundance and biomass of *H. sanguineus* reached only 21% and 59%, respectively, of the values of *C. maenas*, the annual energy demand of *H. sanguineus* was calculated to be as high as 76-119% of that of the *C. maenas* population (publication II). The energy demand depends on the composition of the diet (publication II). The lower number was calculated on the assumption of complete carnivory of *H. sanguineus* and complete herbivory of *C. maenas*. In the opposite case, complete herbivory of *H. sanguineus* and complete carnivory of *C. maenas*, the result of the calculation is 119%. The second assumption reflects the true conditions in the field much better, as several studies showed *H. sanguineus* to be more herbivorous than *C. maenas*. Based on gut functional morphology, Griffen and Mosblack (2011) calculated that North American *C. maenas* were about 24% herbivorous, whereas *H. sanguineus* showed a value of about 65%. Thus, the herbivory value of *H. sanguineus* was about three times higher than that of *C. maenas* (Table 7.2). This finding was corroborated by data of fatty acid trophic marker analyses (publication III). Four fatty acid trophic marker (FATM) indices were developed to assess the ingestion of different algae groups. Three of these indices were higher in *H. sanguineus*, only one was higher in *C. maenas* (Table 7.2). On average, herbivory of *H. sanguineus* was about twice as high as in *C. maenas*. This result is also supported by stomach content analysis of crabs sampled at Helgoland. The amount of algae material in *H. sanguineus* stomachs was about 65% higher than in *C. maenas* (Saborowski et al. in prep., Table 7.2). Thus, these three different approaches yielded similar results. Furthermore, comparisons of digestive enzyme activities and stomach morphology analysis suggest that *H. sanguineus* is better adapted to utilize algae material than *C. maenas* (Bartolin 2016, Saborowski et al. in prep.).

Table 7.2: Relative herbivory of *Carcinus maenas* (*C.m.*) and *Hemigrapsus sanguineus* (*H.s.*) revealed by gut functional morphology, fatty acid trophic marker (FATM) and gut content analysis. Data of publication III and Saborowski et al. (in prep.) averaged for males and females of both species sampled over a period of 6 months.

Source	**Method (unit)**	***Carcinus maenas***	***Hemigrapsus sanguineus***	**Percentage (*H.s./C.m.**100)**
Griffen and Mosblack 2011 (North America, east coast)	gut morphology (%herbivory)	24	65	271
Publication III, Table 6.3 (Europe, Helgoland)	FATM: Chlorophyta Index	13.3	16.7	126
	FATM: Phaeophyceae Index	0.8	3.4	425
	FATM: Rhodophyta Index	0.6	0.3	50
	FATM: Bacillariophyceae Index	6.9	11	159
			average	190
Saborowski et al. in prep. (Europe, Helgoland)	gut contents (%algae material)	23	38	165

With the invasion of *H. sanguineus* another crab species with a different life history occurs in European intertidal areas, which before was mainly dominated by *C. maenas*. During winter, *C. maenas* migrates to the subtidal areas or buries in the sand (Janke 1986, Aagaard et al. 1995). In contrast, *H. sanguineus* is present in the intertidal throughout the year. Although exhibiting a relatively low metabolism due to low temperatures, *H. sanguineus* also needs to cover its energy demand during winter (publication II). Thus, the appearance of *H. sanguineus* represents an additional new foraging pressure on the intertidal areas. During the whole year, but especially in spring, *H. sanguineus* forages on Phaeophyceae at a much higher degree than *C. maenas* (publication III, Table 7.1). The intertidal *Fucus* species have a relatively long reproductive phase around Helgoland and young *Fucus* specimens (likely most palatable for crabs) are present year-round (Kornmann and Sahling 1977). Because *H. sanguineus* may also utilize shallow subtidal areas (Gilman and Grace 2009), the winter and spring recruits of subtidally occurring Laminariales (*Laminaria digitata* (Hudson) J.V.Lamouroux 1813, *Laminaria hyperborea* (Gunnerus) Foslie 1884 and *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders, 2006) may also be eaten (Kornmann and Sahling 1977).

In habitats rich in macroalgae like the rocky intertidal around Helgoland, the invasion of *H. sanguineus* may not be a major problem, even if they increased the foraging pressure on Phaeophyceae. Around Helgoland, *C. maenas* and *H. sanguineus* populations together have a carbon demand of max. 8.5 g C m^{-2} y^{-1} (publication II). As similar rocky intertidal areas have a primary production of about 416 g C m^{-2} y^{-1} (Niell 1977), a detrimental impact of *H. sanguineus*, at least in the near future, may be unlikely around Helgoland. The situation may be fundamentally different in the Wadden Sea, where macroalgae are not abundant. Hence, *H. sanguineus* is probably more omnivorous in the Wadden Sea and competition between *H. sanguineus* and *C. maenas* may be stronger. If the second crab invader, *H. takanoi*, shows similar ecophysiological traits as *H. sanguineus*, competitive interactions may be even stronger in the Wadden Sea. It is probable that the abundances of both invaders will increase in the future (Landschoff et al. 2013, publication I). Thus, detrimental effects on the native *C. maenas* population may be more likely in the Wadden Sea than around Helgoland.

7.4 Energy storage

Hemigrapsus sanguineus showed a higher degree of herbivory than *C. maenas* (publication III, Saborowski et al. in prep.). Although it mainly ingested energy-poor macroalgae, *H. sanguineus* was able to build up and maintain large, lipid-rich energy reserves (publication III). These lipid depots exhibit a pronounced seasonality with maxima of about $50\%_{DM}$ in June and minima of about $30\%_{DM}$ in August. *C. maenas*, in contrast, showed lower but rather stable lipid levels of about $20\%_{DM}$ year-round.

Larger lipid reserves may enable *H. sanguineus* to better overcome food paucity in winter or periods of reduced feeding, e.g. during moulting. This would result in higher survival rates compared to *C. maenas* and may partially explain the success of *H. sanguineus* as an invasive species. In *H. sanguineus*, high lipid reserves could fuel gonad formation, e.g.

oogenesis, independent of food supply. This species may thus start their mating season immediately with rising temperatures in spring.

On the bases of hepatosomatic (HSI) and gonadosomatic indices, two North American studies suggested that *H. sanguineus* and *C. maenas* probably have different energy storage and reproductive strategies (Griffen et al. 2011, 2012). In *H. sanguineus*, higher GSI values were not correlated with lower HSI levels, i.e. a conversion of lipids stored in the hepatopancreas to storage lipids in newly produced eggs in the ovaries was not apparent. Instead, *H. sanguineus* utilizes energy from the diet it is consuming during the reproductive period directly for the production of eggs (following the definition of 'income breeding'), i.e. it does not reduce the level of already stored lipids in the hepatopancreas (Griffen et al. 2012). In *C. maenas*, an inverse correlation between GSI and HSI values was found. Therefore, this species was described as a 'capital breeder', which uses previously stored energy reserves to fuel reproduction (Griffen et al. 2011). However, Griffen (2017) recently suggested that *C. maenas* is able to partially utilize the income breeding strategy as well.

The lipid levels of *H. sanguineus* showed a strong seasonal pattern (publication III). This is in conflict with the strategy of an income breeder, which would not utilize lipids stored in the hepatopancreas for egg production and would show a rather constant lipid level over the seasons. On the other hand, lipid levels of *C. maenas* remained constant during seasons. This is in contrast to the expectation of a strong seasonality for a capital breeder, which utilize their stored lipids for egg production.

High storage levels and strong seasonality of lipids may result from a high reproductive output of *H. sanguineus* compared to *C. maenas*. Around Helgoland, *C. maenas* was reproductively active for about three months (April to June) (S. Jungblut, pers. obs.). During this time, the females usually produce a single clutch of eggs comprising max. 185,000 eggs (Klassen and Locke 2007). These eggs contain about 23% of lipids (% dry mass) and account for about 15% of the body fresh mass of the crab (M. McCarthy, unpubl. data). *H. sanguineus*, in contrast, has a longer reproductive period of at least five months (June to October) (S. Jungblut, pers. obs.). This species is able to produce about five clutches per year of up to 56,000 eggs each (Fukui 1988). These eggs contain 28% lipids (% dry mass) and account for 8% of a female's body mass per clutch, which sums up to 40% per year (M. McCarthy, unpubl. data). Hence, compared to *C. maenas, H. sanguineus* produces more eggs per year, with more lipid for the development of the embryos. The high energy demand for reproduction may be an explanation for the high lipid levels in *H. sanguineus.* The production of several clutches of eggs per season is most likely responsible for the observed seasonality.

The strong reproductive efforts (high quantity and quality of eggs, long reproductive period) are obvious advantages and may explain the success of *H. sanguineus* as an invader of new habitats.

7.5 The invasibility of Helgoland and the invasiveness of *H. sanguineus*

Most research on species invasions can be allocated to either the concept of 'invasibility' or the concept of 'invasiveness'. The concept of invasibility focuses on the habitat that is being invaded and how easy this invasion is. On the other hand, the concept of invasiveness concentrates on the properties of species that are beneficial to invade new habitats.

The concept of 'invasibility' focuses on environmental and biological properties of the recipient habitat. For instance, the diversity of the native community was assumed as an important biological determinant, how easy non-native species are able to establish. Communities of higher diversity are, in general, more resistant against invasions than communities of low diversity (e.g. Lonsdale 1999, Kennedy et al. 2002 and references therein). In marine habitats, the availability of open space, e.g. for settlement of benthic organisms, was demonstrated to be a controlling factor of the establishment of invasive species: the more open space available, the higher were the recruitment rates of the invaders (Stachowicz et al. 2002).

The most basic environmental properties, which fall under the concept of invasibility are abiotic factors. Temperature and salinity may be the most striking factors determining survival and abundance of marine invaders (e.g. Nicholson 2002, Braby and Somero 2006). However, also other factors as for instance wave exposure may control species abundance in marine, especially intertidal habitats (e.g. Hampton and Griffiths 2007, Russel et al. 2008). Around the island of Helgoland, *H. sanguineus* was outnumbering *C. maenas* at a more sheltered site, whereas *C. maenas* was dominant at a site of very high wave exposition (publication I). This observation may be extrapolated and used for the predictions of future invasion scenarios. If, for instance, invading the Irish coasts, *H. sanguineus* will most likely show lower abundances at the exposed west coast than at the comparably sheltered east coast.

Also biotic factors can contribute to the invasibility of habitats. For the intertidal area of Helgoland, the enormous amounts of macroalgae provide a valuable food source. Especially primarily herbivorous species like *H. sanguineus* benefit from this unlimited food supply (publication III). This enables the invader to cover its high carbon demand (publication II) and to accumulate large amounts of lipid reserves (publication III).

The concept of ‘invasiveness’ focuses on the characteristics of species, which favour their potential to invade new habitats (Elton 1958, Richardson and Pyšek 2006). To explore these characteristics, numerous studies compared the properties of invasive and native species (e.g. Jensen et al. 2002, Breen and Metaxas 2013, publications II, III). Such studies identified high propagule pressure (e.g. number of offspring), high growth rates, and short generation times as important factors facilitating a successful invasion (reviewed e.g. in Sakai et al. 2001, Parker et al. 2013). Such life history traits, in turn, depend on the ability of the invader to utilize food resources. High plasticity in food preference is thus an advantage of invasive species (Blasi and O’Connor 2016). Food preferences may also be adjusted according to competitive interactions between native and invasive species (e.g. Griffen et al. 2011). Thus, an assessment of the diets of co-occurring species may always be a mix between the actual food preference of the species and shifts due to competitive interactions between the co-occurring species (e.g. publication III).

Physiological aspects of life history traits have only partly been considered. In the context of invasive species, temperature is the most intensely studied abiotic factor (reviewed in Kelley 2014). For instance, invasive ectotherms show a wider thermal window, which is correlated to a higher upper thermal tolerance threshold - a measure of the upper thermal limit, at which a species is able to survive (Kelley 2014).

Other physiological characteristics have been largely neglected. For example, the ability to accumulate and maintain large energy reserves, utilizing energy-poor but abundant food, could directly be connected to the reproductive success of marine invasive species. This was the case for *H. sanguineus* (publication III). Also seasonal patterns in physiological variables, which are related to reproduction, as for instance energy deposition, are important to investigate. For *H. sanguineus*, for instance, a pronounced seasonality in the high lipid stores was probably due to an extremely high lipid output during the time of egg production (publication III). The production of large numbers of offspring, in turn, is one of the key features of a successful invasive species.

Physiological research can reveal the organismic prerequisites of species being successful invaders. Furthermore, the energetic impact of non-natives on their habitats can be evaluated (e.g. Baird et al. 2012). Around Helgoland, the population of *H. sanguineus* has a slightly larger demand for carbon and energy than the population of *C. maenas*, despite the population of *H. sanguineus* being still a lot smaller (publication II). In habitats, which are rich in macroalgae, mostly herbivorous *H. sanguineus* may not imply a detrimental impact as their primary food resource is unlimited. This, however, is probably the case in habitats like the Wadden Sea, which are usually poor in macroalgae.

Apparently, the success of *H. sanguineus* around Helgoland and in Europe is based on a combination of factors. First, the invasibility of habitats may be great as macroalgae-rich rocky shores dominate the European coastlines and thus food is not a limiting factor. Additionally, the availability of sheltered sites facilitates the invasion process as high-density populations occurring there might act as stepping stones for the invasion progression. Second, the invasiveness of *H. sanguineus* is high. This invader utilizes low-energy food and synthesizes high-energy lipid reserves, which may ultimately result in a high number of offspring. Mainly depending on macroalgae as a resource, the competition for food with the co-occurring *C. maenas* may be low.

Ultimately, continuous environmental monitoring for newcomers and following the progression of each case of potential invaders may be the most rewarding strategy to either be able to undertake actions like eradication or to gather more insights in the success of newcomers. More physiological case studies comparing invasive and native species in a certain habitat are required to examine the determining physiological features, which facilitate an invader’s success in a new habitat.

7.6 Conclusions

The Asian shore crab *Hemigrapsus sanguineus* was very successful in invading the intertidal habitat around the island of Helgoland and now co-occurs with the native European green crab *C. maenas*. The results of this thesis lead to several conclusions that ultimately point out that the abundance of *H. sanguineus* may increase in the future and *H. sanguineus* may colonize more new habitats:

- The abundance of *H. sanguineus* around Helgoland increased from 2009 to 2014, resembling numbers of *C. maenas* in 2014 (publication I).
- High hydrodynamic, yet wave-sheltered sites showed high local abundances possibly due to favoured settlement of *H. sanguineus* megalopa larvae (“gregarious settlement”). These local aggregations may act as ‘stepping stones’ for the further invasion (publication I).
- The competition for shelter between *H. sanguineus* and *C. maenas* around Helgoland may increase in the future, as abundances of *H. sanguineus* are expected to rise (publication I).
- The competition for food between the mostly herbivorous *H. sanguineus* and the omnivorous *C. maenas* may be low around Helgoland. In macroalgae-poor habitats like the Wadden Sea, this competition is probably stronger (publication III).

- Utilizing low-quality macroalgae to produce high number of high-quality eggs may be a key advantage of *H. sanguineus* in colonizing new habitats (publication III).
- High amounts of stored lipids and the pronounced seasonality may reflect a high lipid turnover in *H. sanguineus* as a result of its high reproductive efforts (publication III).
- *H. sanguineus* tolerates rapid changes in temperature and salinity, which may lead to a high survival rate, when transported to a new habitat. Likewise, the invasion of *H. sanguineus* may be expected to progress northwards along the North Sea shores and into the Skagerrak.
- Compared to *C. maenas*, *H. sanguineus* has a higher carbon and energy demand. This led to a higher annual energetic habitat impact of the *H. sanguineus* population around Helgoland. The availability of macroalgae is sufficient to compensate this impact around Helgoland. The community of the macroalgae-poor Wadden Sea may be more impacted as *H. sanguineus* may shift to a more omnivorous diet (publication II).
- Water temperatures around Helgoland are rising due to climate change and will continue to do so (Wiltshire and Manly 2004, Belkin 2009). Warming may accelerate growth, facilitate the reproductive output and increase the energetic habitat impact of *H. sanguineus*. Thus, the overall importance of *H. sanguineus* around the island of Helgoland and at the European coastlines may increase in the future.

8 Future perspectives

This thesis covered an important, yet underappreciated field of invasive species research. There are at least three different levels, at which further ecological and ecophysiological research is required.

The first level are interactions between species, as for instance between the two species investigated here, *Hemigrapsus sanguineus* and *Carcinus maenas*. Investigations on species interactions as for *H. sanguineus* and *C. maenas* are of global interest, as both species are successful invaders at coasts of several continents (see 'Introduction'). Both species co-occur in some of these habitats, which underlines the need for comparative studies. These include ecological studies of population developments in newly invaded areas (e.g. publication I), but also physiological studies. It remains, for instance, to be investigated if the dietary overlap is similarly low in habitats, which are poor in macroalgae (e.g. the Wadden Sea), compared to Helgoland, which is rich in macroalgae (publication III). Furthermore, the influence of temperature and shelter opportunities on the respiratory physiology needs to be investigated. Also the relation between body size, locomotive activity, and respiration in either species requires further consideration (publication II).

Comparative studies of *H. sanguineus* and *C. maenas* should also include the consequences of direct and indirect interactions. There is evidence that *H. sanguineus* is competitively superior in direct interactions over *C. maenas* (Jensen et al. 2002, Lohrer and Whitlatch 2002). This raises the question, whether *H. sanguineus* is also able to indirectly induce stress reactions in *C. maenas*, via chemical waterborne cues of the competitor (Trussell et al. 2003, 2006). For short-term investigations, behaviour and respiration rates might be a sufficient response variable. For long-term and habituation experiments, monitoring hemolymph metabolites related to stress may be suitable (e.g. Stentifort et al. 2001, Schock et al. 2010). Experiments should, for example, include individuals of a *C. maenas* population, which did not encounter *H. sanguineus* yet to evaluate the stress-response of *C. maenas* and potential habituation effects to rapidly increasing abundances and thus waterborne cues of *H. sanguineus*.

The second level of potential future investigations should be focused on the corporate ecophysiological properties, which favour the success of potential invasive taxa. Due to a high portion of invasive species, brachyuran crabs may be a promising taxon to investigate ecophysiological traits of invasive versus native species (Brockerhoff and McLay 2011). Both, invasive and native brachyurans can be found in almost every climatic region worldwide, which enables research on larger scales. Comparing invaders and natives, for instance, in different temperature regimes would reveal, if the same ecophysiological traits are responsible for the success of an invader and if the relative importance is shifting with environmental conditions, such as temperature. This would enable more precise predictions of the biotic and abiotic conditions, in which a potentially invading crab species would be successful and thus expansion limits might be better predictable (Crozier 2003, Kelley et al. 2013). Potential candidates for such comparative studies are for instance the nimble spray crab *Percnon gibbesi* in the Mediterranean and the snow crab *Chionoecetes opilio* around Spitsbergen. The aspects of this thesis and the ones mentioned above may be included in such studies but are not exhaustive.

To investigate ecophysiological properties of successful invasive species, taxa where closely related native and invasive species are co-occurring may be most suitable. Besides brachyuran crabs, such taxa are, for instance, caprellid amphipods, mytilid mussels or oysters (Braby and Somero 2006, Boos 2009, Pogoda 2012).

The third level takes into account overarching concepts of biological invasion research. Humanity is creating an increasing number of transportation vectors and the numbers of species introductions are increasing dramatically (Hulme 2009). Ecosystems worldwide have entered an ‘era of globalization’ and are getting more and more similar to each other (Hulme 2009). Theories like the ‘enemy release hypothesis’ or ‘biological resistance’ are thus probably hard to bring up as explanation background for the success or failure of biological invasions in the future. To be able to reconsider such hypotheses in the future, continuous data gathering, for instance of case studies (level 1) and investigating corporate ecological and ecophysiological properties (level 2) would be required.

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Acknowledgements

I am most grateful to Prof. Dr. Wilhelm Hagen that he gave me the opportunity to work in the Marine Zoology group of the University of Bremen. I was given lots of freedom, for instance in terms of my research and which courses I want to teach. Besides this very open working atmosphere, Willy was always available when needed, even on very short notices. Thank you very much for everything, Willy.

Likewise, I am most grateful to Dr. Reinhard Saborowski, who always supported me on various levels. He enabled me to conduct my research in the island of Helgoland as well as at the west coast of Sweden, he hired student co-workers who were processing plankton samples and took me to conferences with him. Reinhard put also a lot of effort in discussing results and in the development of manuscripts (‘manuscript ping-pong’). I learned a lot from your input. Thank you very much, Reinhard.

I would like to thank Prof. Dr. Christian Wild, who was so kind to review my dissertation and to join my examination committee. I am also grateful to Prof. Dr. Kai Bischof, Diana Martínez-Alarcón and Henrik Ellinghaus that they became members of my examination committee.

Thanks go to all members of the Marine Zoology working group at the University of Bremen, to the members of the Section Functional Ecology at the AWI Bremerhaven and to the members of the Biological Station Helgoland. All these people are thanked for their technical or administrative support, help in the laboratories, scientific discussions, cheerful conversations or other ways of helping me and contributing to the success of this thesis.

Discussions with Dr. Jan Beermann, crustacean-related or general science-talk, were always enlightening and interesting. Also, many thanks for all your support during the weeks I spent on Helgoland.

The German Academic Exchange Service (DAAD) supported me with funding for conference expenses and funding for hiring an intern. Thanks to the Graduate School GLOMAR, I was able to conduct a crab survey in Sweden. GLOMAR also provided some helpful training courses and some funding to cover conference expenses.

I am grateful to Dr. Matz Berggren, Rikard Karlsson, and Lars Storm for hosting me at the Kristineberg Marine Research Station, for help during the sampling at the Swedish west coast and for hosting me in Gothenburg, respectively.

I would like to thank my student co-workers Timothy Thomson and Hagen Buck-Wiese who were processing the Helgoland plankton samples.

The students I was co-supervising are thanked for choosing to work with me and for their interest in our shared projects: Patrick Bartolin and Lisa Kempka.

I am very grateful to all the efforts Morgan McCarthy expended to first become my intern, then Bachelor student, co-author and conference-buddy. Taking you as intern was one of the best decisions during these 3.5 years. Your friendship means a lot to me.

My dad Hubertus and his girlfriend Inge, my grandma Inge and my friends in my native area and in Bremen always motivated me with their interest in my research and my personal development.

My biggest thanks go to my three best friends, Nadine, Alex, and Dominik. Your friendship and love is what makes life colourful. It’s hard to express how thankful I am that we have met. I am looking forward to the long way we will go together.

Eidesstattliche Erklärung

Hiermit erkläre ich,

Simon Jungblut
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dass ich die Doktorarbeit mit dem Titel:

Ecology and ecophysiology of invasive and native decapod crabs in the southern North Sea

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen, 08. September 2017 ____

Simon Jungblut, MSc