The non-native crab *Hemigrapsus takanoi* in the south-western Baltic Sea: Population structure and sensitivity to salinity and temperature shifts

Dissertation

In fulfilment of the requirements for the degree “Dr. rer. nat”
of the Faculty of Mathematics and Natural Sciences
at the Christian-Albrechts-University of Kiel
Helmholtz Centre for Ocean Research GEOMAR

Submitted by

Ola Nour

Kiel, 2020

First referee: Prof. Dr. Martin Wahl

Second referee: Ass. Prof. Dr. Christian Pansch

Date of the oral examination: 14.08.2020

The non-native crab *Hemigrapsus takanoi* in the south-western Baltic Sea: Population structure and sensitivity to salinity and temperature shifts

Dissertation

In fulfilment of the requirements for the degree “Dr. rer. nat”
of the Faculty of Mathematics and Natural Sciences
at the Christian-Albrechts-University of Kiel
Helmholtz Centre for Ocean Research GEOMAR

Submitted by

Ola Nour

Kiel, 2020

Table of content

Summary

The Asian brush clawed shore crab *Hemigrapsus takanoi* is native to the north-western Pacific Ocean. Since the 1990s, *H. takanoi* is known to invade the intertidal zones of the European Atlantic and the North Sea coasts, where this species shares habitats with for example the native European green crab *Carcinus maenas*, and competition in habitat and food sources between both species was observed. *H. takanoi* was first detected in the Baltic Sea in summer 2014. Numerous juveniles and adults including ovigerous females were observed from its south-western region (most inner part of the Kiel Fjord). Gaining knowledge about population structure of a recently introduced species and its interactions with the biodiversity in its recipient habitat is crucial to assess the effects on the ecosystem. In addition, information of factors promoting its invasion and establishment success is important to predict future invasion potential and extension ranges. Thus, the main scope of this thesis is 1) to evaluate the current status of *H. takanoi* in south-western Baltic Sea Kiel Fjord, 2) to gain insights into the tolerance of different life history stages to salinity stress, 3) to assess larval responses to climate driven environmental variables, more specifically, on how tolerance to low salinity regimes is affected by increasing seawater temperature.

Up to 596 *H. takanoi* adult specimens were found from the inner Kiel Fjord during monitoring in 2017. Although males reached larger sizes than females, with sizes up to 29 mm compared to 25.5 mm for females, females were more abundant than males. Reproductive season started in June and lasted into August. The current study investigated that, the relatively large claws of males comparing to females, helped them to open mussels of *Mytilus edulis* up to a size of 18.1–21.0 mm, while the largest size opened by females was up to 12.1–15.0 mm only. In addition, males consumed twice as much as mussel biomass compared to females of similar sizes. Consumption rates for both sexes increased by increasing temperatures over seasonal changes.

Performance of *H. takanoi* towards a wide salinity gradient was different among the different life history stages. Early life history stages were more vulnerable than juveniles and adults. Fitness parameters, represented by consumption rates on *M. edulis*, of juveniles and adults, showed a significant positive correlation with increasing salinity. For adults, feeding was highest at a salinity of 25. However, larvae showed low resistance to lower salinities, and failure in development to megalopa at salinity below 16, which represents the current Kiel Fjord mean salinity conditions over the year. In general, survival to megalopa increased with increasing salinity under the examined salinity treatments (salinities of 0–35 and 10–25 during the 2017 and 2018 experiments, respectively). The highest numbers of megalopa were recorded at salinity 25.

The results of this thesis show that *H. takanoi* is able to complete its entire life cycle in the brackish Kiel Fjord, however, its sensitivity to low salinity might be a barrier for their further spread towards the Baltic Proper. In addition, the result revealed that intrapopulation variability, seen in the offspring of only one out of five females that succeeded to reach the megalopa at a salinity of 16, might potentially be the reason for *H. takanoi* population persistence in Kiel Fjord. *H. takanoi* early life history stages have been shown to suffer predicted near-future climate change and were affected by the tested multiple stressors (salinity and temperature). There were no interaction effects of temperature and salinity on larval survival and their time to reach the megalopa. Nevertheless, increased temperature accelerates their development and raised larval mortality at all salinity levels. At higher temperature, no larvae reach megalopa below salinity of 19.

In conclusion, *H. takanoi* successfully established a population in the south-western Baltic Sea (inner Kiel Fjord) and will most likely add more pressure on *M. edulis* compared to conditions before their arrival. The species showed an ontogenetic shift in response to salinity with development. Tolerance to low salinity is likely a key determinant for *H. takanoi*'s further spread throughout the whole Baltic Sea today and in future conditions.

Zusammenfassung

Die asiatische Strandkrabbe *Hemigrapsus takanoi* ist im nordwestlichen Pazifik beheimatet. Seit den 1990er Jahren ist bekannt, dass diese Art in die Gezeitenzonen der europäischen Atlantik- und Nordseeküste vorgedrungen ist, wo sie sich ihren Lebensraum mit zum Beispiel der einheimischen europäischen Strandkrabbe *Carcinus maenas* teilt, und wo schon eine Konkurrenz um Lebensraum und Nahrungsquellen zwischen beiden Arten beobachtet wurde. *H. takanoi* wurde erstmals im Sommer 2014 in der Ostsee nachgewiesen. In der südwestlichen Ostseeregion, dem innersten Teil der Kieler Förde, wurden zahlreiche Jungtiere und Adulte, darunter auch eiertragende weibliche Individuen, beobachtet. Die Gewinnung von Erkenntnissen über die Populationsstruktur einer relativ neu eingeführten Art und ihrer Wechselwirkung mit der Biodiversität des Habitats ist entscheidend um die Auswirkungen auf das Ökosystem beurteilen zu können. Um künftige Invasionsereignisse und Ausbreitungsgebiete abschätzen zu können, ist es darüber hinaus wichtig, Faktoren zu identifizieren, die ihre Invasion und ihren Etablierungserfolg begünstigen. Der Hauptgegenstand dieser Arbeit ist daher 1) die Bewertung des aktuellen Zustands von *H. takanoi* am ursprünglichen Fundort in der südwestlichen Ostsee, 2) die Gewinnung von Erkenntnissen über die Toleranz verschiedener Lebensstadien gegenüber Salzgehaltsstress, und 3) die Bewertung der Reaktion der Larven auf klimabedingte Umweltvariablen, im Besonderen die Toleranz gegenüber niedrigen Salzgehalten durch erhöhten Temperaturen.

Bis zu 596 erwachsene Exemplare von *H. takanoi* wurden 2017 während eines Monitorings in der inneren Kieler Förde gefunden. Obwohl die Männchen mit einer Größe von bis zu 29 mm, im Vergleich den Weibchen mit 25,5 mm, größer waren, waren die Weibchen in ihrer Anzahl häufiger als die Männchen. Die Fortpflanzungszeit begann im Juni und dauerte bis August an.

In der vorliegenden Studie wurde untersucht, dass die im Vergleich zu den Weibchen größeren Scheren der Männchen ihnen halfen, Muscheln der Art *Mytilus edulis* bis zu einer Größe von 18,1-21,0 mm zu öffnen, während die Größten von Weibchen geöffneten Muscheln nur 12,1-15,0 mm betrugen. Darüber hinaus verzehrten Männchen doppelt so viel Muschelbiomasse wie Weibchen gleicher Größe. Die Fraßraten für beide Geschlechter stiegen mit zunehmender Temperatur über jahreszeitlichen Veränderungen an.

Die Leistung von *H. takanoi* gegenüber Salzgehaltsveränderungen war in den verschiedenen Lebensstadien unterschiedlich. Die frühen Lebensstadien waren anfälliger als Jungtiere und Adulte dieser Art. Fitnessparameter, repräsentiert durch die Fraßraten, von Jungtieren und Adulten zeigten eine signifikant positive Korrelation mit zunehmendem Salzgehalt. Für Adulte

H. takanoi war die Nahrungsaufnahme bei einem Salzgehalt von 25 am größten. Die Larven zeigten jedoch eine geringe Widerstandsfähigkeit gegenüber niedrigeren Salinitäten und einen Misserfolg in der Weiterentwicklung zur Megalopa Larve bei einem Salzgehalt unter 16, was dem aktuellen jährlichen Mittelwert der Salinität in der Kieler Förde entspricht. Im Allgemeinen nahm die Überlebensrate bist zum Megalopa Stadium mit zunehmendem Salzgehalt unter den untersuchten Salinitätsbehandlungen zu (0-35 in 2017; 10-25 in 2018). Bei einem Salzgehalt von 25 wurden die höchsten Megalopazahlen verzeichnet.

Die aktuellen Ergebnisse zeigen, dass *H. takanoi* zwar den gesamten Lebenszyklus in der brackigen Kieler Förde vollziehen kann, aber die Empfindlichkeit gegenüber niedrigen Salzgehalten ein Hindernis für eine weitere Ausbreitung dieser Art in Richtung der nördlichen Ostsee darstellen könnte. Zudem konnte gezeigt werden, dass die Variabilität innerhalb der Population, bei der nur Larven von einem der fünf getesteten Weibchen bei einem Salzgehalt von 16 schlüpften und sich zum Megalopa-Stadium entwickelten, ein weiterer Grund für die Beständigkeit der *H. takanoi*-Population in der Kieler Förde sein könnte.

Die vorliegende Studie belegt, dass die frühen Lebensstadien von *H. takanoi* unter dem vorhergesagten Klimawandel leiden könnten und durch die getesteten multiplen Stressoren (Salzgehalt und Temperatur) beeinflusst werden. Es konnten keine Interaktionseffekte von Temperatur und Salzgehalt auf das Überleben der Larven und ihrer Entwicklungszeit bis zum Erreichen des Megalopa Stadium nachgewiesen werden. Dennoch beschleunigten wärmere Temperaturen die Entwicklung und erhöhten die Larvensterblichkeit in allen Salzgehaltsstufen. Bei Behandlungen mit einer Kombination aus erhöhten Temperaturen und einem Salzgehalt unter 19 erreichte keine der Larven das Megalopa Stadium.

Zusammenfassend kann gesagt werden, dass *H. takanoi* erfolgreich eine Population in der südwestlichen Ostsee (innere Kieler Förde) etablieren konnte und höchstwahrscheinlich mehr Fraßdruck auf *M. edulis,* im Vergleich zu den Bedingungen vor ihrer Ankunft, ausüben wird. Die Art zeigte zudem eine ontogenetische Verschiebung in ihrer Entwicklung als Reaktion auf Salinitätsveränderungen. Die Toleranz gegenüber niedrigen Salzgehalten ist wahrscheinlich eine Schlüsselkomponente für die weitere Ausbreitung von *H. takanoi* in der gesamten Ostsee, unter heutigen und unter zukünftigen Bedingungen.

1. General introduction

1.1 Biological invasions

Biological invasions rank among the most serious threats to world's biodiversity, and constitute a key component of global environmental change (Galil 2007; Simberloff et al. 2013). Species translocation rates and successful establishment beyond their native regions are increasing globally (Seebens et al. 2017). Even though only a small proportion of the introduced species becomes invasive in their recipient habitats (Blackburn et al. 2011), they can have a detrimental impact (Galil 2007). Bio-invasions studies increased as a demand to detect and understand these events, in addition, to comprehend the principles of invasion success (Simberloff 2011; Azurro et al. 2014a). The main common challenges that these studies face, are early detection of invasion events, proper identification, and the logistic problems of field works (Azurro et al. 2014b). Moreover, for full assessment of the invasion dynamics, researchers need to study ecological, biological, and behavioural aspects as well as continuously monitor the distribution of the invaders (Sax et al. 2005a).

A wide terminology is used in biological invasion studies, and yet, the relevant basic terminology of introduced and invasive species is clearly undetermined and used inconsistently among scientists and regulations (Boudouresque and Verlaque 2002; Colautti and MacIsaac 2004). In some studies they even contradict each other with regard to their used terminology (e.g. Blackburn et al. 2011; Guy-Haim et al. 2018). In order to facilitate discourse among scientists, policy and the management community, clarification of such terminology as a consensual set of terms and definitions is a matter of importance (Occhipinti-Ambrogi and Galil 2004). The terms used in this thesis and the most used terminology regarding biological invasions are illustrated in **Box 1.1.**

Box 1.1: Terminology used in biological invasions

Introduction of species: Deliberation or accidental transfer or release of organism by means of human activities across natural barriers of dispersal (Occhipinti-Ambrogi and Galil 2004).

Invasion: Any process of colonization and establishment beyond a natural range in which the species or population plays a conspicuous role in the recipient ecosystem (Occhipinti-Ambrogi and Galil 2004).

Natural dispersal: Accidental movement of organisms via winds, currents, and in or on animals**,** rather than by human actions (Ojaveer et al. 2018).

Native species (synonyms: indigenous, original): A species that occurs naturally in a given region or ecosystem, and that was not introduced via human actions (Falk-Petersen et al. 2006).

Non-native species (synonyms: alien, exotic, introduced, allochthonous, non-indigenous): A species that was moved outside its usual geographical range via anthropogenic actions (intentional or accidental), irrespective of its impact or effect on native species and the native ecosystem (Occhipinti-Ambrogi and Galil 2004).

Invasive species: A species which has established and spread outside of its natural distribution range, and which then threatens the invaded ecosystems, habitats and/or other species, potentially causing economic and/or environmental damage, or harm to human health (Occhipinti-Ambrogi and Galil 2004).

Cryptogenic species: A species that is not reliably demonstrated as being native or non-native to a specific region (Carlton 1996).

1.1.1 Effects on ecological and economical properties and on human-health

Rapid globalization, climate change, increasing trends of trade, travel and transport, pollution, and many other anthropogenic influences accelerated marine biological invasions by facilitating new introductions over that past decades (Ruiz et al. 2000, Occhipinti-Ambrogi 2007). Consequently, this might have fundamental ecological consequences by changing the structure and function of marine ecosystems (Galil 2007). Apparent effects are related to predation, competition for food and/or shelter, parasitism, disease transfection, as well as physical and chemical changes within the invaded habitats (Beisel and Lévêque 2010; Reise et al. 2017). The consequences may be drastic, leading to loss of biodiversity, impairment of ecosystem services (i.e. fishing and tourism), and an imbalance of the ecosystem (Ruiz et al. 2000; Occhipinti-Ambrogi and Galil 2010). In addition, environmental changes within a future ocean may stress and menace native species, while on the other hand benefiting non-native species (Alpert 2006; Sax et al. 2007).

Serious economic damaged are well documented for invaded species, with an annual damage of approximately €12 billion within the European Union (Kettunen et al. 2009; Shine et al. 2010), US$137 billion within the United States (Pimentel et al. 2000), and approximately CAD$7.5 billion in Canada (Dawson 2002). For instance, the invasion of the ctenophore *Mnemiopsis leidyi* in the Black Sea, led to the collapse of the US$250 million per annum anchovy (*Engraulis encrasicolus*) fishery for Europe (Zatisev 1992). The European green crab, *Carcinus maenas*, now causes an annually loss of US$K60 to K130 due to shellfish industry impacts, after introduction to the west coast of the United States. A further predicted loss is estimated to be US$1.2 million annually if its range expands northerly to Washington and Alaska (Grosholz 2011). The solitary tunicate *Styela clava* causes a loss of CAD$88.4 million per year to the aquaculture industry at the Atlantic coasts of Canada (Colautti et al. 2006).

While known negative effects of introduced species can be calculated to dollars, other factors especially those concerning human heath, may not have a monetary label. For example, the lethal poisonous pufferfish *Lagocephalus sceleratus*, which invaded the whole Levant coast (Galil et al. 2017), possesses a strong paralytic neurotoxin “tetrodotoxin” in its internal organs. An injury of humans by this species can cause respiratory and cardiac arrest, coma and death (Islam et al. 2011). The Chinese mitten crab *Eriocheir japonica* is the second intermediate host for the Oriental lung fluke, caused by the trematode *Paragonimus westermani*. Arriving of *E. japonica* to North America can therefore increase the potential of human infections by chronic inflammatory lung diseases (Lafferty and Kuris 1996).

1.1.2 Phases during the invasion process

The invasion process normally includes several stages, with one or more barriers that need to be overcome by the species or population within each stage (**Figure 1.1.2 A**). The process usually starts by one or more incidences of **introduction** of a species to its new region, after crossing a geographical barrier by **transportation**. Many species fail to survive during the journey, mainly due to unsuitable environmental conditions during transport (Blackburn et al. 2011). The individuals must then **establish** a self-sustaining population (i.e. founder population) through successful reproduction in their newly habitat, otherwise the population goes extinct. This founder population is considered as the initial vital point for population growth and increasing abundance, and the spread and **expansion** of the species in its new geographical range. Otherwise the population will remain small and local. Studies showed that non-native populations tend to have a **lag phase** before their exponential expansion phase starts, in which these are low in abundance and their effects are not clear. This lag time phase is unpredicted in duration and varies among species, and can range from few years (Armonies 2001) to decades (Thieltges et al. 2004). The expansion phase sooner or later comes to an end, or to what is named an **adjustment phase**, which could be one of three possible scenarios for population development: 1) permanent, if the population remains dominant, static, and unaffected by any enemies (Branch and Steffani 2004), 2) strongly fluctuating in abundance and population size (Reise et al. 2017a), or 3) breaking down in the population abundance and failing to recover after initial boom (Occhipinti-Ambrogi 2002). Typically, the process completes, and the non-native population receives the term of being "invasive" when it causes some sort of harmful impact to their new region.

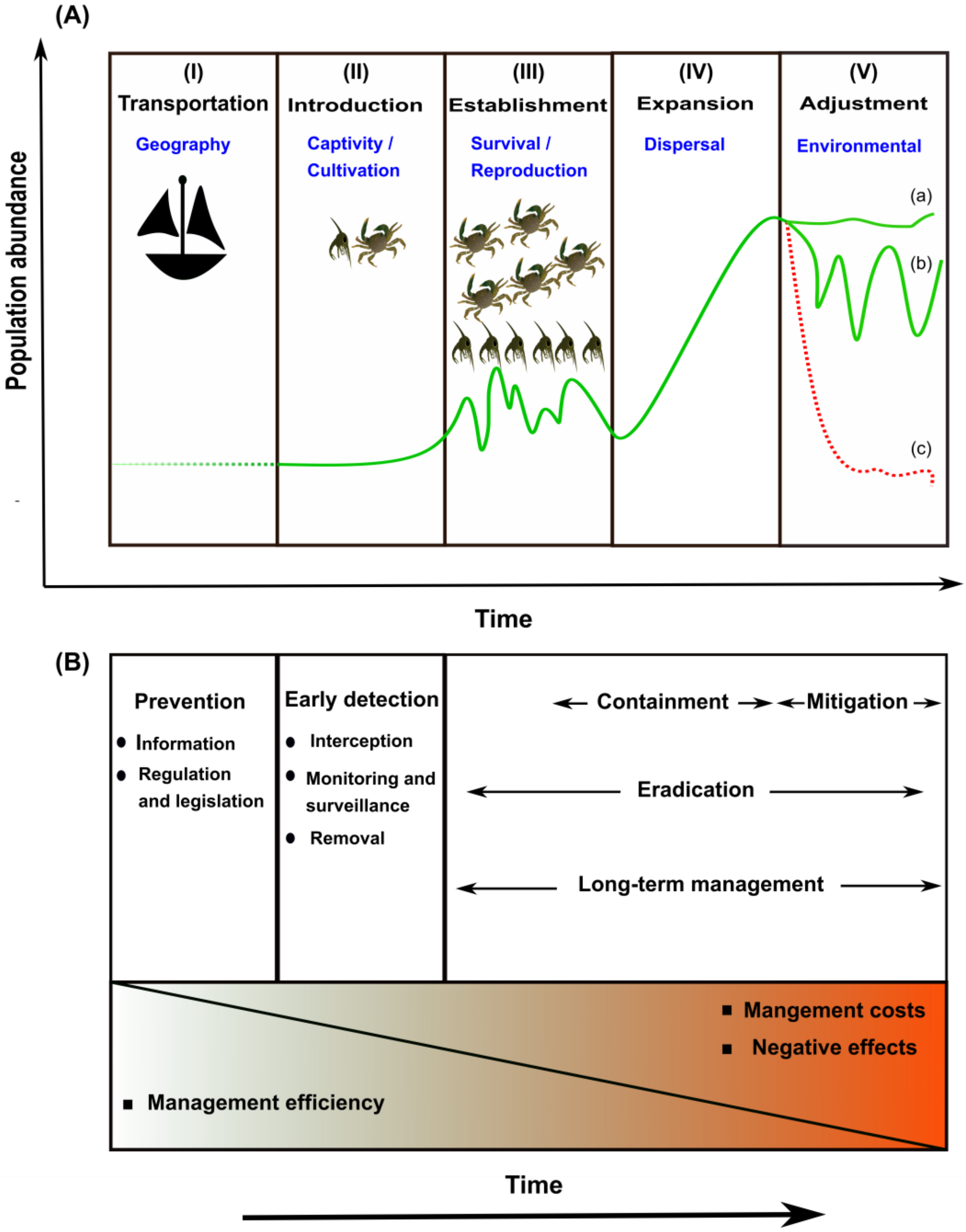

Figure 1.1.2: The invasion process of an introduced species. (**A).** The phases of invasion are highlighted in bold (I-V). In each step organisms must overcome one (or more) barriers or filters (highlighted in blue) to pass on to the next phase. Dominance may be maintained during the latter phase (a), strong fluctuations may be caused by environmental, intrinsic and enemy effects (b), or rarity prevails after boom and bust (c). green line: first appearance (dotted) and successful introduction (solid); red dotted line: failure of invasion. (modified from Boudouresque and Verlaque 2012; Reise et al. 2017b). Management strategy (**B)** against each invasive phase. Prevention before introduction is the optimum strategy and the most cost effective, and least disturbing to the environment. Management efficiency is decreasing, and management costs are increasing with time post introduction (modified from Simberloff et al. 2012).

1.1.3 Management of invasions

The Convention on Biological Diversity (2002) adopted guiding principles on invasive species, summarized in **Figure 1.1.2 B**: 1) **prevention** is manifested by constricting pathways, intercepting movement at boarders, and assessing the risk for intentional imports (Lovell et al. 2006). Even though some species may enter any jurisdiction, 2) **early detection** of the small population as a second step of management allows for cost-effective removal and can be improved by innovative tools and molecular approaches (Chown et al. 2008; Harris and Timmins 2009). In addition, 3) **eradication** and prompt removal, which is ecologically less risky than later interventions, can be applied through chemical or mechanical control methods, for example, shading algal species, chlorine treatment of marinas, or the physical smashing of individual snail hosts of an invasive parasite (Bax 1999; Culver and Kuris 2000; Dalton 2000). It is worth to mention that eradication of well-established populations could be risky as well by releasing another non-native previously suppressed species (Caut et al. 2009). So, before eradication is attempted, research is needed addressing the ecosystem role of a long-standing invasive population. Finally, public education through media and citizen sciences projects, supporting researchers and long-term monitoring, all aid fruitful contributions for successful managing measures.

1.1.4 Pathways, vectors, and propagule pressure of marine invasions

Introduction of new species to a new region may be intentional or unintentional through various vectors and pathways. Pathways are the means or tools (e.g. vessels, person), routes (e.g. canal), activities (e.g. mariculture, aquarium trade), or commodities (e.g. fisheries) by which non-native species are transported, while a vector is the specific mechanism by which a species is transferred within each pathway. Ballast water, biofouling and cargo are for example independent transport vectors within the shipping pathway, each necessitating a different managing approach (Minchini 2007). It is rather difficult to prove how a species had been transported since there are high levels of uncertainly associated with many of the pathways and vectors (Minchini et al. 2009). Moreover, after first arrival to its new location, which is called "primary introduction", non-native species could subsequently spread and expand its range to other new regions from the founder non-native habitat either by natural dispersal, human-associated transportation or even through additional vectors and pathways "secondary introduction" (Minchini et al. 2009). Nevertheless, proximity to known hubs for species introductions such as major ports, aquaculture farms or canals, and the inferential reasoning of the locality might help to put forth scientifically sound hypotheses (Ulman et al. 2017).

Propagule pressure, defined as the number of individuals introduced in an introduction event (propagule size) linked to the number of introduction events (propagule number) (Lockwood et al. 2005; Simberloff 2009), associated with pathways and transported vectors, are all playing a crucial role in determining the outcome of a particular biological invasion (Ruiz et al. 2000; Colautti et al. 2006). Increasing propagule size and number due to increasing ship traffic, increasing aquaculture efforts, opening of canals, and trading of species for aquaria, are all directly related to increasing invasion success (Lockwood et al. 2005). However, many other factors, like the duration of transportation between the donor and recipient region, could also influence the propagule pressure and the final success of a population (Williamson 2006).

1.1.5 Species-specific characteristics of invasion success

As mentioned before, even with a normally high number of introductions, only a small proportion (~10%) of species can successfully establish a population in their new recipient ecosystem (Kolar and Lodge 2001). This is mainly related to differences between taxa as well as physiological, ecological, and life-history traits.

Physiological tolerance often determines species ability to inhabit a new habitat. species must pass environmental ecological filters (including abiotic and biotic factors) in order to establish a sustainable population (Crowl et al. 2008). Within population, sex and growth phase differences in their tolerance could affect the populations invasion success (Pennoyer et al. 2016). In general, many studies support this and demonstrate the high resilience of non-native compared to native populations against stressful abiotic environmental conditions (Hammann et al. 2016; Schmidt et al. 2016).

Concerning life-history traits, reproduction success plays a fundamental role in determining whether a species successfully establishes and spreads. An *r*-selected breeding strategy, including early maturation, short generation time, several broods per reproductive season, high fecundity and rapid growth rate, all are valuable traits in most of the marine invaders (Sakai et al. 2001). Temporal shifts of breeding periods in some invasive crabs, for example *C. maenas* in Australia, could help by reducing competition between early juveniles of native crabs and invasive *C. maenas* (Garside et al. 2015). The common characteristic of many marine organisms having a planktonic larval stage is of great benefit for establishing a non-native population, due to an increased dispersal potential (Katsanevakis et al. 2013).

As a useful strategy for invasion, many invaders are more successful by having a broad diet and by being able to use a variety of food resources (Weis 2010). Being omnivorous with the ability to quickly adapt to changes, helps the invasive species to avoid food competition with native species (Blasi and O’Connor 2016). However, shifting of diet of native species is also known to be triggered by newly arriving species, which could be beneficial for the non-native species but detrimental for the native biota (Griffen et al. 2011).

1.1.6 Climate-driven changes and marine invasions

Aquatic system will be affected by climate change via warming, alternating stream flow patterns, and increasing storm events (Poff et al. 2002). Generally, climate change and marine invasions are linked in several ways (Smith et al. 2012): climate changes often increase the opportunity for non-native species to cross historical geographic barriers and spread into new region, while invasive species in themselves often change ecosystems to be more susceptible to climate changes (McNeely 2000). Profound effects of these changes are expected mainly on aquatic ecosystems productivity as well as species distribution and phenology (Parmesan 2006). Climate change and marine invasion impacts on aquatic systems are often considered independently (Rahel and Olden 2008), however, a strong likelihood that climate drive changes will interact with marine invasion process is likely (Kolar and Lodge 2000; Stachowicz et al. 2002). Moreover, climate change and invasive species effects could be synergistic. Warmer water temperatures in the northern latitude lakes; for example; may cause stressful conditions for cold water adapted native species, and on the other hand provide suitable thermal conditions for non-native warmer species to establish in these lakes exerting even more pressure on the native community (Sharma et al. 2007). Predation on native species by newly established species, and competition for food resources between native and invasive populations may also lead to a weakening of native population (Jackson and Mandrak 2002).

In terms of climate change, there is increasing evidence that global warming plays a critical role in invasion progresses of a diversity of species (Walther et al. 2002). Climate-related changes enhance the dispersal of thermophilic non-native species as for example demonstrated in the Mediterranean Sea (Occhipinti-Ambrogi 2007), where shifting of the distribution of many invasive thermophilic species has occurred (Perry et al. 2005).

One very special interactive effect of global warming and the invasion process is the “establishment” of a “sleeper” population (Simberloff and Gibbons 2004). This usually occurs when an invasive species arrives from warmer regions than their new habitats and the expansion phase of the population is inhibited by colder temperatures in the recipient habitat. An awakening of these ecological “sleepers” after decades is, thus, usually also enhanced by global warming (Crooks and Soulé 1999; Crooks 2005). For instance, a decline in numbers of the barnacle *Austrominius modestus*, after being very abundant between 1993-1995 in the intertidal zone of the island of Sylt (North Sea), was attributed to the harsh winter in 1995-1996. After more than 50 years with a period of several mild winters and warm summers, current exponential growth of this invasive barnacle was recorded since summer 2007. This species is even expected to further expand its range into the eastern North Sea with future climate changes (Witte et al. 2010).

According to Dukes and Mooney (1999), prediction of the climate change effect on invasions suggests that impacts of invasion on the ecosystem would be exacerbated. As the world is exposed to ongoing climate change, and with a growing world population, further studies are needed for better understanding the impacts of biological invasions on our ecosystem.

1.2 Study organism - *Hemigrapsus takanoi*

1.2.1 General information

The Asian shore brush-clawed crab *Hemigrapsus takanoi* (Brachyura, Grapsoidea, Varunidae) is a native species to the north-west Pacific Ocean. The species inhabits the intertidal zones along the four main islands of Japan from north Hokkaido to south Kyushu, and occurs along the adjacent waters of Russia (Sakhalin Island), Korea, northern China and Taiwan (Asakura and Watanabe 2005; Lee et al. 2013; Marin 2013; Makino et al. 2018; **Figure 1.2.1**). The species is known to prefer low hydrodynamic and sheltered regions of muddy flats, lagoons and estuaries, and is usually found in crevices, under stones and boulders and among other hard structures on rocky shores or slightly burrowed into soft sediments (Landschoff et al. 2013). The crab *H. takanoi,* was named and formally distinguished from its sibling, the most common Japanese intertidal species, *Hemigrapsus penicillatus* (De Haan 1835), only 15 years ago (Asakura and Watanabe 2005). The new species was defined based on some morphological aspects (i.e. size of setal patches on the male’s chela: Takano et al. 1997, egg size: Watanabe 2003, and pattern of spots along the body and morphology of male first pleopods: Asakura and Watanabe 2005), and confirmed by molecular studies (Yamasaki et al. 2011; Market et al. 2014).

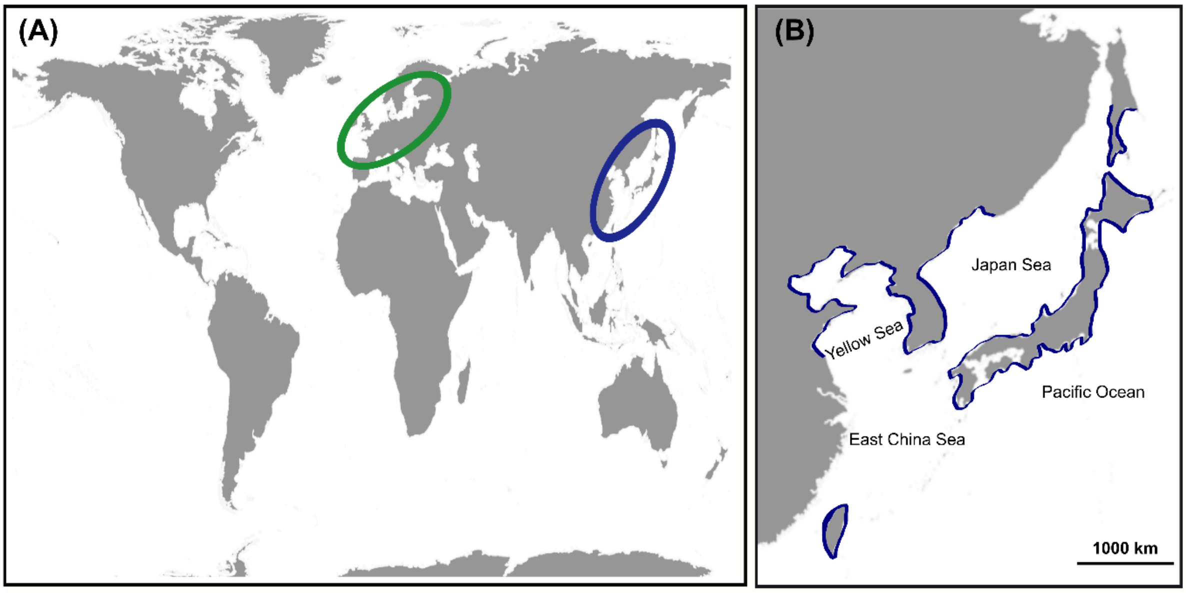

Figure 1.2.1: Worldwide distribution of *Hemigrapsus takanoi* (**A**): native (blue ellipse) and non-native (green ellipse) ranges distribution. Native Asian regions (**B**) are highlighted in blue. These maps were produced and adapted using the soft wares QGIS 3.12 and Inkscape 0.92.4.

1.2.2 Reproductive strategy and life cycle

The reproductive strategy of crab species normally depends on three main factors: 1) indeterminate or determinate growth patterns, 2) hard- or soft-shell mating, and 3) whether the location of the seminal receptacle “a pair of organs derived from the reproductive system, where females can retain and store sperms after mating to fertilize their eggs” is located dorsally or ventrally (McLay and Lopez Greco 2011). *H. takanoi*, and all members of the family Grapsoidae, follow the reproductive strategy with indeterminate growth, hard-shell mating and a ventrally located seminal receptacle. *H. takanoi*, with indeterminate growth pattern, can moult and grow throughout their lives, in contrast to species with determinate growth that are reproductively active after a terminal pubertal moult, but no longer growth in size, e.g. the native European crab *C. maenas*. Although as they grow, they can avoid some predators by crossing size thresholds, but regular moulting events make them generally more vulnerable to predation and damage during the soft-shelled stage. In species where mating is possible while the female integument is hard, female’s gonopores are either continuously open or covered by a temporary decalcified operculum.

H. takanoi has a complex biphasic life cycle (**Figure 1.2.2**), including a meroplanktonic larval phase and benthic juvenile and an adult phase. The larval phase comprises 5 zoeae stages and a single megalopa stage (Landeira et al. 2019).

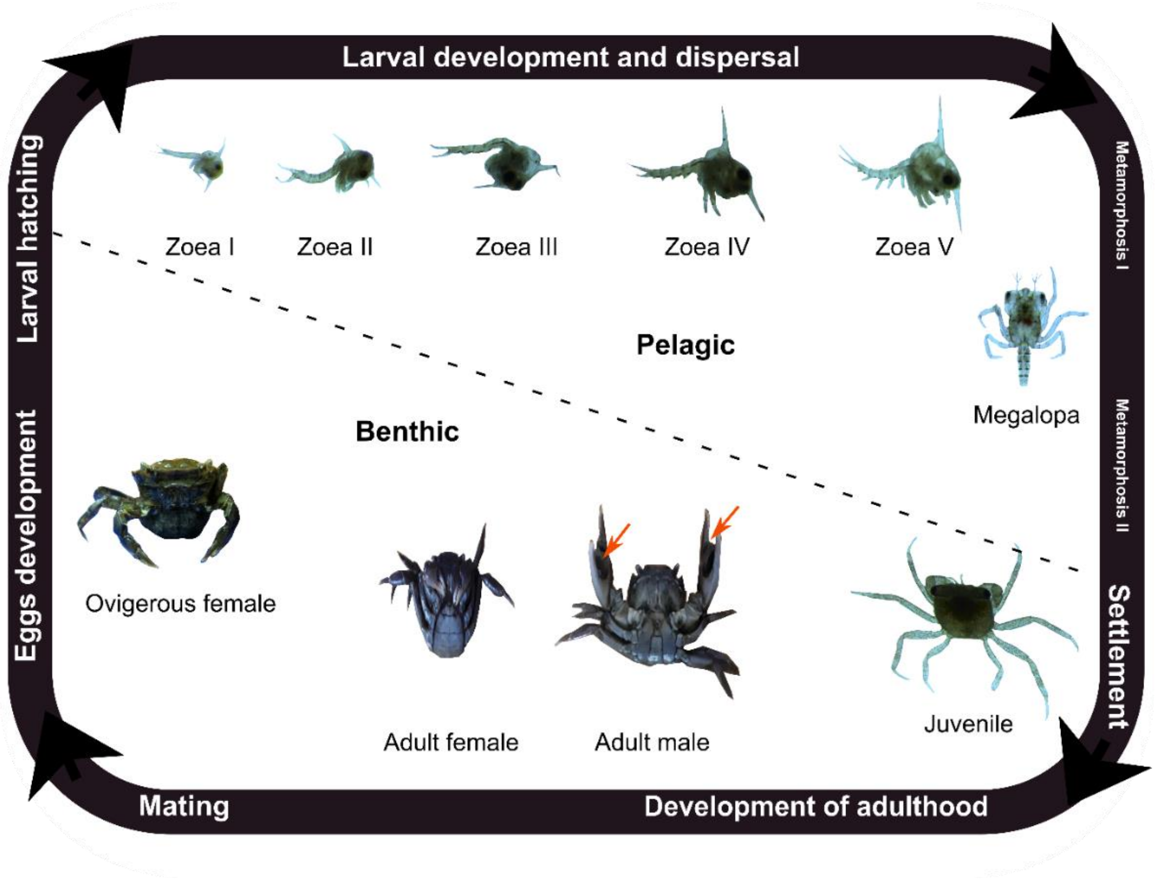

Figure 1.2.2: The life cycle of *Hemigrapsus takanoi* (pictures of the life history stages are not scaled). After mating with an adult male (orange arrows showing the secondary sexual characteristics: large chela with setal patches), females spawn fertilized eggs and attach them to their pleopods. After 5 embryonic developmental stages (van den Brink et al. 2013), the first pelagic Zoea I larvae hatch from the eggs and are released from the female into the water. The pelagic stage completes through 5 free swimming zoaea stages (ZI–ZV). The last zoea stage (ZV) metamorphoses into a semi benthic megalopa. The megalopa settles to a suitable habitats and metamorphoses into the benthic juvenile stage. Subsequently, moulting in juveniles occurs until reaching the mature adult stage at an age of approximately 11 months, and the cycle starts again (adapted by Spitzner 2018).

H. takanoi demonstrates a secondary sexual dimorphism between sexes. Male chelae are larger than those of females (Asakura and Watanabe 2005; Mingkid et al. 2006a), and only males have setal patches at the out- and inside of the chela propodus (Sakai 1976; **Figure 1.2.2**). The setal patches play no role in neither mating nor in agonistic interaction behaviour (like touching, grasping, jerking) during mating (Miyajima et al. 2012), however a function for chela protection is possible as males show to use chela more frequently than females during agonistic interactions (Miyajima et al. 2012).

1.2.3 Introduction history in Europe

Adults *H. takanoi* are considered an extremely eurythermal and eurohyline crustacean species, tolerating temperatures from freezing (-2°C) up to 30°C (Landschoff et al. 2013; Berggren and

Karlsoon 2017), and salinities from 7 to 35 (Mingkid et al. 2006a; Gittenberger et al. 2010; Soors et al. 2010). The strong plasticity in response to environmental fluctuations in terms of temperature and salinity, together with its rapid growth and early sexual maturity (~11 months after settlement; Okamoto and Kurihara 1987), its high fecundity (up to 60,000 egg per brood; Okamoto and Kurihara 1987), several broods per season (Pillay and Ono 1987), and a strong dispersal ability due to planktonic larval development (Landeira et al. 2019), are all considered to be advantageous requirements for this species to be a successful invader.

In European waters, *H. takanoi* was observed from the Atlantic coast of Europe since 1993 (Gollasch 1999; **Figure 1.2.3**, **Table 1.2.3**). The first record included only few individuals (2 males and 4 non-ovigerous females) from the ballast water during a German shipping study in Bremerhaven in 1993 (central Wadden Sea, Germany; Gollasch 1999). One year later, a reproductive population of *H. takanoi* was recorded at La Rochelle at the Atlantic coast of France (Noël et al. 1997). From here, the species distribution extended along the Bay of Biscay from its southern part Lareda (Spain) to its northern part Formentine (France; Noël et al. 1997). In 1999, *H. takanoi* was recorded in the English Channel at Le Havre harbour (Breton et al. 2002), from where an abundant population established along the French Opal coast of the English Channel to the southern part of the North Sea (Dauvin et al. 2009; Gothland et al. 2014).

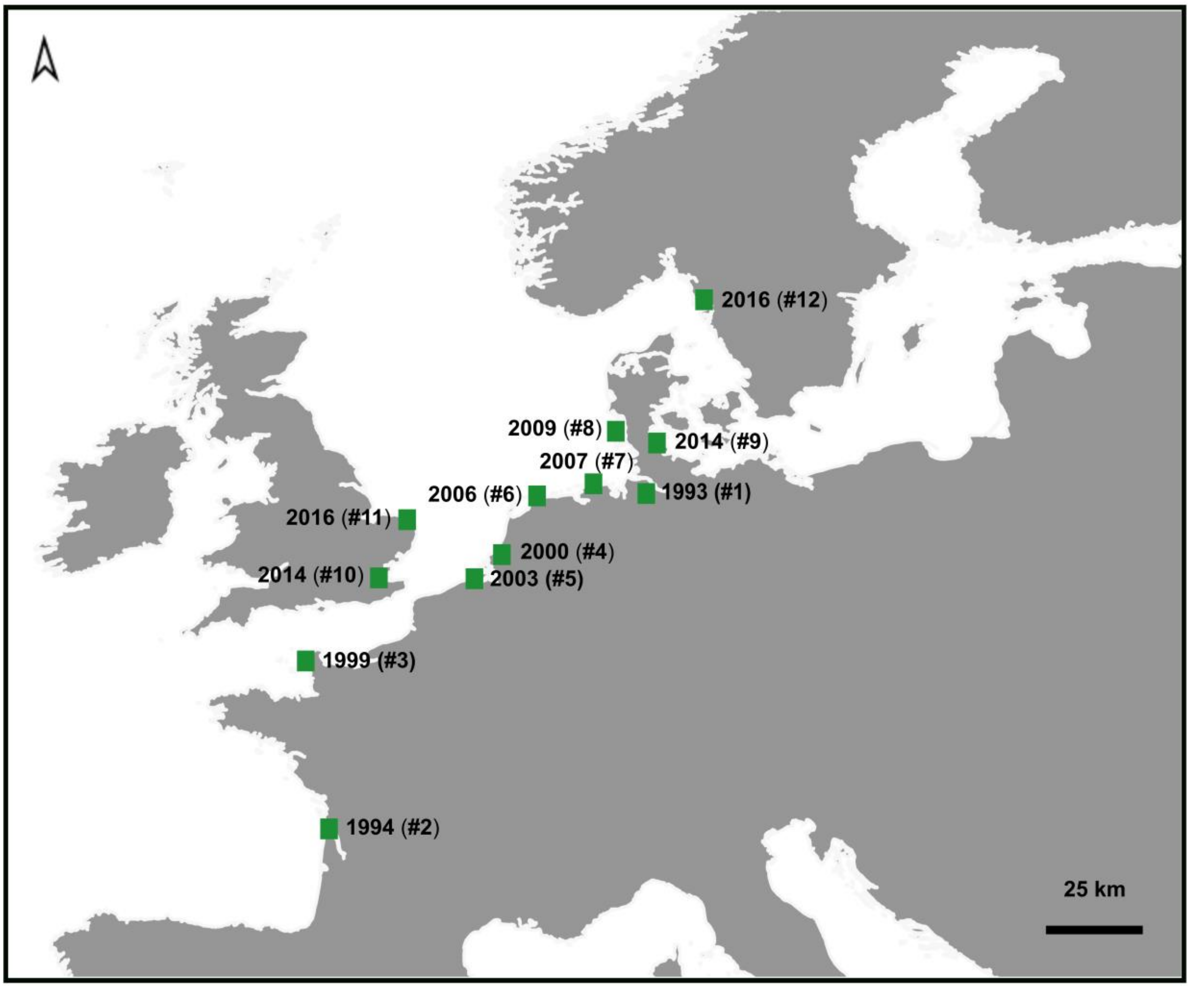

Figure 1.2.3: Non-native European regions invaded by *Hemigrapsus takanoi*. Numbers indicate the history of invasion, as first recorded species in the region, collected from different literatures. The map was produced and adapted using the soft wares QGIS 3.12 and Inkscape 0.92.4. Details about the location is illustrated in **Table 1.2.3**.

Table 1.2.3: Locations in Europe (related to **Figure 1.2.3**) where *Hemigrapsus takanoi* was recorded for the first time in a new region. Salinity measurements are recorded by the same study where *H. takanoi* is recorded (references), or by other studies on *H. takanoi* (*) or other species (†) from the same recorded region, or available online.

Location	Salinity regime	Reference
1 - Bremerhaven, central German Wadden Sea	18 – 32 data available on: https://marine.copernicus.eu/	Gollash 1999
2 - Bay of Biscay, France	13 – 35 (Gouillieus and Massé 2019) †	Noël et al. 1997
3 - Le Havre harbour, Bay of Seine, France	30 – 32	Breton et al. 2002
4 - Oosterschelde estuary, Netherlands	33 – 35 (van den Brink et al. 2013) *	Nijland and Beekman 2000
5 - Westerschelde Estuary, Belgium	0.5 – 17 (Soors et al. 2010) *	Kerckhof et al. 2007
6 - Dutch delta, Wadden Sea	12 – 32	Gittenberger et al. 2010

7 - Lower Saxony coast, German Wadden Sea	10 – 30 (Landschoff et al. 2013) *	Obert et al. 2007
8 - Sylt-Rømø Bight, north Wadden Sea	30 – 31	Landschoff et al. 2013
9 - Kiel Fjord, South-western Baltic Sea	8.4 – 24.7 (Nour et al. in preparation) *	Geburzi et al. 2015
10 - River Medway estuary, Kent, Great Britain	~28	Wood et al. 2015
11 - Orwell Estuary, Suffolk, Great Britain	31 – 32	Ashelby et al. 2017
12 - Southwestern coasts of Sweden	~30 – 34 (for example: Sjöboden Station, Rikard Karlsoon personal communication, data available on: https://www.weather.loven.gu.se/kristineberg/en/data.shtml)	Berggren and Karlsoon 2017

The species *H. takanoi* was first observed in the Oosterschelde (Netherland) in 1999 (Nijland and Beekman 2000), and at the coast of Belgium in 2003 (Kerckhof et al. 2007). In 2006 and 2007, establishing *H. takanoi* populations were recorded at the Dutch (Gittenberger et al. 2010) and at the German (along Lower Saxony coasts; Obert et al. 2007) Wadden Sea, respectively. By 2009 the species reached the Sylt-Rømø Bight between Germany and Denmark in the northern part of the Wadden Sea (Landschoff et al. 2013). Since 2014 *H. takanoi* is known to be present among some estuaries in Great Britain (Wood et al. 2015; Ashelby et al. 2017). In summer 2014, the species was first recorded from the Baltic Sea, form the inner Kiel Fjord (Geburzi et al. 2015). Since 2016, *H. takanoi* was recorded from the Swedish west coast (Berggren and Karlsoon 2017; Karlsoon et al. 2019).

1.2.4 Population genetics in native and non-native regions

H. takanoi populations from native regions showed a genetic differentiation between Japan (with exception of the Hokkaido population) and the Yellow Sea region, indicating very little gene flow between these native populations (Makino et al. 2018). Even though high similarity was found between the Hokkaido and the Yellow Sea populations, an absence of sharing the mitochondrial 16S haplotype between the two populations was detected (Makino et al. 2018). The current Hokkaido population could be a result of introgressive hybridization between Japanese population and Continental Asian population (Makion et al. 2018). Within European waters, the Bay of Seine population (northern France; **Figure 1.2.3, Table 1.2.3**) showed a genetic admixture from the native regions (Chinese, Korea and Japan), and a genetic dissimilarity to all remaining European populations, which are exclusively Japanese in their origin (Makino et al. 2018). Interbreeding between Japanese and Yellow Sea region populations in the Bay of Seine might be the reason for this genetically-unique Bay of Seine population (Makino et al. 2018). Generally, *H. takanoi* showed less genetic diversity in non-native European regions compared to native regions (Makino et al. 2018).

1.2.5 Possible pathways and vectors of the invasion to Europe

The recent study by Makino et al. (2018) strongly emphasis that shipping lines are the main vector, while oyster transportation, as suggested previously (Nöel et al. 1997; Dauvin and Delhay 2010), does probably not play a role in spreading of *H. takanoi* in Europe. Tokyo Bay for example including many major international ports in Japan, is known as a hot spot for marine invasion towards northern European waters (Seebens et al. 2013). The first live *H. takanoi* recorded in Europe (1993) was likely introduced via an automobile carrying vessel departing from Tokyo Bay port (Gollasch 1999).

In addition, spreading of *H. takanoi* along European coasts via secondary introduction is recently confirmed through genetic studies (Makino et al. 2018; Geburzi et al. under preparation). For example, the introduction of *H. takanoi* into the Baltic Sea, is mainly via larval and juveniles transportation in ships' ballast water coming in from the neighbouring North Sea (in particularly Wadden Sea; Geburzi et al. under preparation) or other Atlantic ports, or through juveniles and adults by ship hull fouling and very likely by recreational boating (Geburzi et al. 2015), which are believed to be effective vectors in secondary spread of non-native species (Clarke Murray et al. 2011). The artificial man-made Kiel Canal, the third most important route (after the Suez and the Panama canals), connecting North Sea to the Baltic Sea, is the main pathway for these vectors (ships and recreational boats), that benefits the introduction of *H. takanoi* (Geburzi et al. under preparation), and invasions in general (Leppäkoski et al. 2002), into the Baltic Sea.

1.3 Study region: The Baltic Sea and Kiel Fjord

The main vectors of species introduction to the Baltic Sea are shipping, including introduction through ballast water and hull fouling, as well as aquaculture (Leppäkoski et al. 2002). In addition, many geographical invasion corridors, including river systems and canals that connect the Baltic with the Black, the Caspian, and the White seas open into the Baltic Sea and its different sub-regions (Leppäkoski et al. 2002), providing connectivity and potential for invasions. Regions with a low species richness, like the Baltic Sea, are expected to be more susceptible to invasive species (Paavola et al. 2005). The species poverty of the Baltic sea is like due to the young age of the region being only 8000 years old, and only very few endemic species adapted to the low saline-brackish water conditions of the Baltic Sea (with the exception of macroalgae and fish species). On the other hand, 152 non-native and cryptogenic species were recorded up to now in the Baltic Sea (Casties et al. 2016; supplementary in Tsiamis et al. 2019).

The Baltic Sea is one of the largest brackish ecosystems worldwide, with a surface of 415,000 km^2, a volume of 21,700 km^3, and an average water depth of 58 m (Snoeijs-Leijonmalm et al. 2017). The semi-enclosed young post-glacial basin is connected to the North Sea only through the Danish Straits. This restriction of water exchange and inflow within the North Sea leads to a horizontal salinity gradient along the west to north-east Baltic Sea (Stigebrandt 2001), being ~30 at the entrance region of the Danish straits and down near freshwater conditions (~1–3) at the Gulf of Bothnia in the north and the Gulf of Finland in the northeast (Leppäranta and Myrberg 2009; Müller et al. 2016). This strong gradient in salinity is considered as the most ecologically relevant factor that determines the patterns of biodiversity in the Baltic Sea (Zettler et al. 2014). A decline of macroscopic species by an order of magnitude inside the transient zone (i.e. Danish straits) between the Kattegat and the Baltic Sea (Leppäkoski et al. 2002). Species diversity is generally higher in the south-western Baltic compared to the north-eastern Baltic Sea (Vuorinen et al. 2015), with the highest marine species recorded in the Kattegat, while the highest freshwater species richness is found in the Gulf of Finland (Zettler et al. 2014). The Baltic Sea is a highly variable and dynamic basin, which will be most likely be subject to strong future climate changes (Müller et al. 2016; Reusch et al. 2018). Notwithstanding, a prediction of increasing surface water temperature by 2 to 4°C and a decreasing in average surface salinity by about 2 units by 2100 was made (IPCC 2007; Gräwe et al. 2013; Holopainen et al. 2016). The continuous impact of both natural and multiple human-mediated stressors (warming, eutrophication, oxygen depletion, acidification) on the Baltic Sea have led to present-day conditions that can be expected for the future in other ocean habitats (Reusch et al. 2018).

Within the south-western Baltic Sea, in proximity to the Danish Straits, the Kiel Fjord is located as a part of the Kiel Bight. The Kiel Fjord is a narrow water body extending from north to south, with a depth of approximately 10 to 20 m. Salinity fluctuates between 8.5 at the surface to 24.5 at the bottom, with a mean of 15.9 at the surface and 18.5 at the bottom (Nour et al. in preparation). Many rivers and other water bodies drain in the area of the Kiel Fjord, leading to a highly atrophied habitat (Nausch et al. 2011; Franz et al. 2019b). In addition, conditions cause a strong stratification during summer, which is mainly driven by sedimented phytoplankton blooms (Hansen et al. 1999; Broecker 2003). This can lead to hypoxia in bottom waters (Hansen et al. 1999) and a strong increase in seawater $p\text{CO}_2$ and a drop in pH (Rabalais et al. 2002; Salisbury et al. 2008). When this water shoals due to wind events, temporary hypoxic and acidified conditions prevail in shallow-water habitats (Thomsen et al. 2010; Melzner et al. 2013).

2. Aim of the work

This thesis is divided into three chapters addressing 1) the prevalence and the potential impacts of *Hemigrapsus takanoi* in the south-western Baltic Sea inner Kiel Fjord, 2) salinity tolerance of the different life stages of *H. takanoi*, as well as 3) the effects of the future climate change scenarios (warming in combination with seawater freshening) on early life history stages of *H. takanoi*.

<u>The main research questions:</u>

1. What is the occurrence (current state) of the recently invaded crab Hemigrapsus takanoi in Kiel Fjord?

Detailed information on newly introduced species is crucial for understanding factors and mechanisms that influence its invasive success. *H. takanoi* was only recently recorded from the inner Kiel Fjord, south-western Baltic Sea (Geburzi et al. 2015). Even though, ovigerous females were observed during summer 2014 (Geburzi et al. 2015) and 2016 (personal observations), it is unclear whether *H. takanoi* is able to establish a self-sustaining population in this region, or whether egg-carrying females migrate to the inner Kiel Fjord for spawning, as is suggested for another invasive crab (*Eriocheir sinensis*) from the Baltic Sea (Ottos and Brandis 2011). During 2017, I monitored the *H. takanoi* inner Kiel Fjord population by bi-weekly assessments, including their abundance, sex ratio, biomass, claw size, and reproductive state through active and passive collection methods. The results of this study are presented in Chapter I of this thesis.

2. What are the potential impacts on local mussel populations among the different seasons?

Field and laboratory studies from non-native regions demonstrated strong negative effects of *H. takanoi* on the native European green crab *Carcinus maenas.* This includes reduction of recruitment intensity, competition for space and predation on juveniles *C. maenas* in the presence of adult *H. takanoi* (van den Brink et al. 2012; Geburzi et al. 2018). Apart from these first studies, the ecological role and effects of *H. takanoi* in the invaded ecosystem are not well understood and requires further research ranging from experimental approaches to field monitoring studies in non-native habitats to estimate its effect in the newly invaded ecosystem.In this thesis, I investigated *H. takanoi's* potential effects on Baltic Sea mussel

Mytilus edulis communities (*M. edulis* genotypes dominate the western Baltic Sea, while *M. trossulus* genotypes dominate the eastern Baltic Sea, and both species hybridize; Väinölä and Hvilsom 1991). I conducted repeated experiments on prey size selection during different seasons (spring: 8 ± 0.5 °C, summer: 19 ± 0.5 °C, and autumn: 13 ± 0.5 °C), under laboratory conditions, and for both sexes.

The results contributed to understanding the impacts of *H. takanoi* on *M. edulis* communities. In addition, I discuss, whether there is an overlap within the prey range and size preferences between *H. takanoi* and the native mussel predators, the crab *C. maenas* and sea star *Asterias rubens*. The results of this study are presented in Chapter I of this thesis.

3. Is salinity an obstacle for H. takanoi to further spread into the Baltic Sea?

Salinity is widely regarded as an abiotic stressor and a significant factor that limits invasion of less successful invaders (Paiva et al. 2018; Pauli and Briski 2018), determining species distribution in aquatic environments (Symth and Elliott 2016). Salinity is also the main factor that determines a species' invasion success in Baltic Sea habitats (Lehtiniemi et al. 2011). Early life history larval stages of marine decapods are often shown to be more vulnerable to salinity stress than juvenile and adult stages (Charmantier 1998; Anger 2003). Therefore, the aim of this study was to assess the environmental salinity range in which *H. takanoi* can successfully survive, develop and perform, and to detect the potential of this species to further spread into other less-saline Baltic Sea regions. I conducted a series of salinity gradient experiments along eight salinity levels from freshwater to fully marine conditions (0–35), for different life history stages of *H. takanoi*, in order to observe survival, and duration of larval development to complete metamorphosis to megalopa. Furthermore, fitness of juveniles and adults was measured by estimating the feeding rates on blue mussels, *M. edulis*.

4. What is the salinity threshold for H. takanoi larval development, and will future warming change the salinity tolerance window of this species?

The salinity treatments used to answer the previous question showed a constraint in larval development in salinities lower than 20, meaning that larvae of *H. takanoi* in Kiel Fjord are currently living at the border of their salinity tolerance window, and that there is a salinity threshold in which larvae would be able to complete their development in order to establish a population in the inner Kier Fjord. In order to define the salinity range in which larvae succeed to complete development, I conducted another experiment with a range of salinity treatments. Here, however, I narrowed the range of salinities tested, and in addition, since *H. takanoi* originates from warmer habitats (Asakura and Watanabe 2005) meaning that they likely cope better in warmer waters, I used temperature as additional environmental factor. This allowed assessing the combined effect of future warming and salinity shifts on larval development and consumption rates. A fully crossed combination of 2 temperatures (19 and 23°C) and 6 salinities (10–25, in 3 salinity unit steps) levels were tested. Results of this study are presented in Chapter III of this thesis.

3. Chapters and contributions of authors

Parts of this doctoral thesis have been published or will be submitted:

Chapter I: Population structure of the recent invader *Hemigrapsus takanoi* and prey size selection on Baltic Sea mussels

Authors: Ola Mohamed Nour, Meike Stumpp, Sonia C. Morón Lugo, Francisco R. Barboza, Christian Pansch

History: Published in Aquatic Invasions 15(2): https://doi.org/10.3391/ai.2020.15.2.06

Contributions: OMN, MS and CP designed the study. OMN and SML performed the sampling and run the experiments. OMN, FRB, SML, MS and CP analysed and visualized the data. OMN, FRB, MS and CP wrote the manuscript. All co-authors contributed to the final draft of the manuscript.

Chapter II: To invade or not to invade: Impaired larval development at low salinities could limit the spread of the non-native crab *Hemigrapsus takanoi* in the Baltic Sea

Authors: Ola Mohamed Nour, Christian Pansch, Mark Lenz, Martin Wahl, Catriona Clemmesen, Meike Stumpp

History: To be submitted.

Contributions: OMN, CP, MW and MS designed the study. OMN performed the experiments. OMN, CP, ML, and MS analysed and visualized the data. CC provided the long-term time series salinity data. OMN, CP and MS wrote the first draft of the manuscript. All co-authors contributed to the final draft of the manuscript.

Chapter III: Salinity and temperature impacts on survival and feeding of *Hemigrapsus takanoi* larvae: restrictions for its dispersal (secondary spread) along the Baltic Sea

Authors: Ola Mohamed Nour, Martin Wahl, Christian Pansch, Meike Stumpp

History: To be submitted

Contributions: OMN, MW, CP, and MS designed the study. OMN performed the experiments, analysed and visualized the data. All co-authors contributed to the first and final drafts of the manuscript.

Chapter I

Population structure of the recent invader *Hemigrapsus takanoi* and prey size selection on Baltic Sea mussels

Ola Mohamed Nour[1,2,] *, Meike Stumpp[3], Sonia C. Morón Lugo[1,4], Francisco R. Barboza[1] and Christian Pansch[1]

[1]GEOMAR Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

[2]Department of Biology and Geology, Faculty of Education, Alexandria University, 21526 Alexandria, Egypt

[3]Zoological Institute, Christian-Albrechts University, 24118 Kiel, Germany

[4]Departement des Sciences Fondamentales, Universite du Quebec a Chicoutimi 555, Chicoutimi, Quebec G7H 2B 1 Canada

*** Corresponding author:** e-mail: olasm.nour@gmail.com; GEOMAR, Düsternbrooker Weg 20, 24105 Kiel, Germany

Key words: invasion ecology, non-native species, Asian brush claw shore crab, prey size selection, *Mytilus edulis*, Kiel Fjord.

Abstract

The shore crab *Hemigrapsus takanoi* Asakura and Watanabe, 2005, native to the north-west Pacific, was recorded in European waters about 25 years ago and it was first found in the Baltic Sea in 2014. Information on population structure of invaders and their new niche is needed in order to understand their biological impact. Over one year, we assessed temporal changes in relative abundance, size-class and sex ratio, as well as breeding season of *H. takanoi* in the Kiel Fjord (Western Baltic Sea). In addition, prey size preference and consumption rates on mussels (*Mytilus edulis* Linnaeus, 1758) were experimentally assessed in spring, summer and autumn. A total of 596 individuals were collected with highest and lowest abundances in June and February, respectively. Females were dominant over males (sex ratio 1.4:1), but males grew to larger sizes. *H. takanoi* reproduced between June and August with ovigerous females representing 30% of the entire female abundance registered over the entire year. Males were able to open larger mussels (due to larger claws) and consumed twice as many mussels when compared to females of similar size. Consumption rates for males were 6 and 2 times higher in summer (seawater temperature of 19 °C) compared to spring (8 °C) and autumn (13 °C), respectively. Females consumed 3 times more mussels in autumn than in spring. *H. takanoi* is an active predator, capable of reproduction in stressful brackish water conditions. Due to large abundances and high feeding pressure, this recently introduced species could play a key role in structuring post-settlement population dynamics of the dominant habitat builder *M. edulis*.

Introduction

Worldwide, crustaceans represent one of the most successful group of invaders (Devin et al. 2005; Galil 2008; Karatayev et al. 2009). The vectors for their dispersal are diverse but can be mainly attributed to ship transport via ballast water (Gollasch 2006; Ricciardi 2006). Natural and artificial canal systems can also facilitate their introduction to new habitats (Galil 2009), as does aquaculture and stocking translocation (Savini et al. 2010). Advantageous biological and physiological characteristics, such as tolerance to environmental fluctuations, a broad variety of food choices, complex life-history traits, and modes of reproduction are generally thought to explain the invasion success of crustaceans (Hänfling et al. 2011). Crabs were described to largely contribute to the pool of invasions to the California Bay, the Laurentian Great Lake, as well as to the Baltic Sea (Cohen and Carlton 1998; Olenin and Leppäkoski 1999; Ricciardi and MacIsaac 2000).

The Baltic Sea is one of the largest brackish seas worldwide. This semi-enclosed basin, connected to the Atlantic Ocean only via narrow straits, is characterized by a significant salinity gradient, decreasing from south-west to north-east (Leppäkoski et al. 2002). According to their limited tolerance to low salinities, marine species are gradually replaced by freshwater species along this steep salinity gradient (Ojaveer et al. 2010). The Baltic Sea has a short geological age and is therefore thought to contain open niches for new species to arrive (Leppäkoski et al. 2002; Barboza and Franz 2016). Besides these natural environmental circumstances, human induced impacts, such as exploitation of living resources, land-based pollution, eutrophication and physical destruction impose a significant stress onto the Baltic Sea, an environment that is vulnerable to many invaders (Paavola et al. 2005; Ojaveer et al. 2016). To date, 152 non-native and cryptogenic species (34 species belong to crustaceans, among these 8 to decapods) have been recorded in the Baltic Sea (Casties et al. 2016; supplementary in Tsiamis et al. 2019). These non-native species compete for food with local native species (Kotta and Ólafsson 2003), and some of them have caused persistent restructuring and changed functioning of pelagic and benthic ecosystems (Ojaveer et al. 2015; Jormalainen et al. 2016; Tiselius and Møller 2017).

The Asian brush-clawed shore crab *Hemigrapsus takanoi* is a non-native species that recently arrived in the Baltic Sea. This species was recorded at the south-western coast of the Baltic Sea for the first time in 2014 (Geburzi et al. 2015). *H. takanoi* is native to the north-west Pacific, and was initially identified as *H. penicillatus*, later being declared as a separate, but closely related, sibling of *H. takanoi* (Asakura and Watanabae 2005; Yamasaki et al. 2011; Markert et al. 2014). In 1993, *H. takanoi* was first observed in Bremerhaven, Germany (Gollasch 1999), only one year after a reproductive population of *H. takanoi* was recorded at La Rochelle at the Atlantic coast of France (Noël et al. 1997). From here, the species distribution extended along the Bay of Biscay from its southern part Laroda (Spain) to its northern part Formentine (France; Noël et al. 1997). Today, the recorded distribution of *H. takanoi* along the Atlantic coast of Europe spans from the northern coast of Spain to the southern North Sea coast of Denmark (Dauvin and Delhay 2010). *Hemigrapsus* spp., may have strong impacts on native crab populations in the invaded ecosystem (Jensen et al. 2002; Dauvin et al. 2009; Gothland et al. 2014; Geburzi et al. 2018). In addition, significant ecological impacts on mussels (e.g. *Mytilus edulis*) were related to the occurrence of *H. sanguineus* after being introduced to the mid-Atlantic coast of North America, with likely consequences for the community structure (Gerard et al. 1999). However, to the best of our knowledge, no studies have been conducted on the predation impacts of *H. takanoi* on Baltic Sea mussel communities, and no information is available on seasonal differences in predation pressure.

In the present study, we assessed the population characteristics of *H. takanoi* in the Kiel Fjord (Western Baltic Sea). Biweekly semi-quantitative assessments of the relative abundance of this species were conducted over a full year cycle, which were used to determine size distribution, sex ratio and reproductive season of *H. takanoi*. In addition, feeding preferences (mussel-size selection) of both sexes of *H. takanoi* were investigated in repeated laboratory experiments in spring, summer and autumn. The results of this study provide valuable insights into possible ecological implications for the invaded habitat (Katsanevakis et al. 2013), and in particular insights into the potential impact of this non-native species on mussel (*M. edulis*) beds as well as mussels' size refuge from predation.

Materials and Methods

Study area

Field monitoring and samplings for feeding assays were carried out in the inner most part of the Kiel Fjord (54°32.9′N; 10°14.8′E; Supplementary material Figure S1), where several mussel beds and aggregations at walls and pontoons (mostly harbour constructions) of the blue mussel *M. edulis* exist. In this habitat, *H. takanoi* is now co-occurring with the two main native predators, the European green crab *Carcinus maenas* and the common sea star *Asterias rubens* (Reusch and Chapman 1997).

Field monitoring and crab sampling

Females of *H. takanoi* were found to be less active in movement than males (personal observation), and they are known to bury themselves in the sediment, spending more time grooming their eggs than feeding during their reproductive period (Turoboyski 1973; Sumpton and Smith 1990). Therefore, relative abundance and size distribution for *H. takanoi* were determined by biweekly samplings between January and December 2017, using two separate methods: (1) active manual scraping of mussel beds for 20 min and (2) passive collection using traps. Scraping was conducted at a depth of 1.5–2 m, with ~ 35 bottom samples taken at each time point, using a scraper of 0.008 m^2 surface area and a mesh size of 0.5 mm (covering a total area of ~ 0.28 m^2). The traps consisted of 50 × 50 × 20 cm PVC pipes covered entirely with a 1 mm mesh, with an entrance on one side (Figure S2). Traps were filled with crushed blue mussels as bait and deployed on the bottom (1.5–2 m water depth) keeping a minimum distance of 1 m between traps (n = 5 traps). The traps were retrieved after 24 h. The two sampling methods were applied at the same location during the entire sampling period (Figure S1). After sampling, crabs were transported immediately to the near-by laboratory facilities of GEOMAR, Kiel, Germany, and kept in well aerated seawater. Samples obtained by the two sampling methods were pooled (Figures S3 and S4 for a detailed comparison of the results obtained with each sampling method) as this presumably reduced the bias from using only one of the sampling methods.

All sampled *H. takanoi* with a size (carapace width) larger than 5 mm were counted, sexed (based on their abdominal structure and claw morphology; after Asakura and Watanabae 2005), and ovigerous females were recorded. Reproductive season was determined as the period in which ovigerous females appeared in the population (Pinheiro and Fransozo 2002). Carapace width was measured using Vernier callipers to the nearest 0.1 mm, as the distance between the two second (middle) antero-lateral teeth (Mingkid et al. 2006a). Size classes were defined in 2 mm intervals, ranging from 5.1–7.0 to 27.1–29.0 mm. To study weight-size relationships, wet weight of collected individuals was recorded after blotting dry each crab with tissue paper to the nearest 0.001 g. For this, only crabs with complete and nearly equally sized appendages were used. Collected crabs were released into the Kiel Fjord after sampling and size measurements or used for the prey-size selection assays.

In addition to the biological samples, seawater temperature and salinity (WTW Cond 3110, Tetracon 325, Weilheim, Germany) were recorded weekly around midday at the monitoring site.

Prey-size selection assays

Adult males and females of *H. takanoi* (size-based definition: carapace width > 10 mm after Geburzi et al. 2015) were used in the assays. Males were measured and grouped into one of six size classes defined every 2 mm from 15.1–17.0 to 25.1–27.0 mm carapace width (n = 5 crabs for each class). Females were grouped into one of five size classes, from 11.1–13.0 to 19.1–21.0 mm carapace width (n = 5). Size classes used corresponded to those found in the field in the respective season. Crabs were then placed individually into covered transparent plastic aquaria (20 × 13 × 12 cm) filled with 1.5 l of aerated seawater. Experiments were conducted three times during the year with representative water temperatures of 8 ± 0.5 °C in early spring (starting 05/04/2017), 19 ± 0.5 °C in summer (starting 03/08/2017), and 13 ± 0.5 °C in early autumn (starting 07/10/2017), in constant temperature rooms of GEOMAR, Kiel, Germany.

All assays were run under LED light and a photoperiod of 12:12 h. Crabs were acclimated for a period of 5 days and fed daily with fresh mussel flesh. Crabs were starved for 7 days before the feeding assays in order to standardize starvation levels (Elner and Hughes 1978; Jubb et al. 1983). All crabs used in the experiments had intact and equally grown claws. Because crabs stop feeding prior to moulting, and feed less while carrying eggs, ovigerous females as well as crabs that did not feed during the pre-experimental phase or moulted before or after the experiment, were excluded. Therefore, crabs were also monitored for moulting for a week after the feeding assay.

Individuals of *M. edulis* were collected by hand from the same site of crab collection, prior to each trial, and maintained in well aerated seawater under the same light and temperature conditions as the crabs. Only undamaged individuals were used as prey items. Mussels were cleaned from any epibionts prior to the experiment. The length of each mussel was measured to the nearest 0.1 mm using a Vernier calliper. Shell length was measured from the tip of the umbo to the edge of the margin at its greatest distance from the umbo. Mussels were then assigned into one of six size-classes defined every 3 mm from 3.1–6.0 to 18.1–21.0 mm. Each crab was offered 10 mussels of each size class. Since in a preliminary trial, none of the females were recorded opening mussels larger than 21.0 mm, the 18.1–21.0 mm size class was excluded in all female assays. After 24 h, shells of all mussels (eaten and non-eaten) were removed, tanks were cleaned and fresh seawater as well as new mussels were added. The assay lasted for 7 days. The mean number of each size class of mussels consumed by each size class of crabs was calculated for the overall experimental period.

In order to test for the effects of season and sex on the consumption rate, dry weights of mussels of all size classes consumed per day were calculated for males and females for spring, autumn and summer. Therefore, the number and shell length of mussels eaten by crabs were converted into dry weight. For this, a power function equation was calculated using data from Thomsen et al. (2013) to estimate the relationship between soft tissue dry weight and shell length of Kiel Fjord mussels (data obtained from Pangaea dataset: doi:10.1111/gcb.12109). Mean dry weight of mussels for the six size classes (3.1–21.0 mm) were 0.5, 2.3, 6.2, 12.8, 22.9, 37.2 mg, respectively. For consumed biomass, specific dry weight was multiplied with the number of eaten mussels in each size class.

To study the relation between crab size (carapace width) and claw height, crabs of both sexes, ranging in size from 11.5 to 20.2 mm, were used to calculate a claw height-crab size index (%):

Claw height - crab size index % $= \left(\frac{Mean\ claw\ height}{Mean\ carapace\ width} \times 100\right)$ (Equation 1).

Previous measurements have shown that both left and right chela of *H. takanoi* have a monochealeous structure (i.e. claws of more or less similar shape and size). Thus, only the left claw height (mm) was measured as the highest vertical distance along the propodus.

Statistical analyses

Predefined functions from the *stats* package of the statistical software R (version 3.4.0, R Development Core Team 2016) were used to perform the analyses.

Based on the collected field data, descriptive bar-plots were produced to depict the size-class distribution and seasonal trends of abundance for males, females and ovigerous females. In addition, the carapace width to wet weight relationship was adjusted using a logarithmic transformation of the allometric growth equation:

$\log \text{wet weight} = \log a + b \log \text{carapace width}$ (Equation 2).

Where b is the slope and log a is the intercept of the regression. Differences in the allometric growth of males, females and ovigerous females were assessed by using an analysis of covariance (ANCOVA). Before the analysis, normality and homogeneity of variance assumptions were tested using Shapiro-Wilk and Fligner-Killeen tests, respectively. In the ANCOVA, log wet weight was included as response variable, while sex and log carapace width were included as explanatory variables. Potential changes in the slope of the partial regressions adjusted for each sex were expressed as the interaction between sex and log carapace width.

The effects of carapace width, sex and season on the consumption rate and total biomass of *M. edulis* consumed by *H. takanoi* were evaluated using generalized linear models (GLMs) with Gamma distribution and identity link function. The assumptions of GLMs were evaluated by a detailed visual inspection of the residuals' plots. Separated ANCOVA (see applied tests for evaluating normality and homogeneity of variances above) for males and females were used to analyse the main effects of season and carapace width, and their interactive effects, on the prey size preference of the crabs. In order to explain the observed differential capacity of males and females to open large mussels (see details in the Results), the relationship between claw height and carapace width was analysed for both sexes. The relationship for females was adjusted using a simple linear regression:

$\text{Claw height } = a + b \text{ carapace width}$ (Equation 3).

Where b is the slope and a is the intercept of the regression. For males, after unsuccessfully trying different non-linear functions, a segmented regression (package *segmented*; Muggeo 2017) was used. In addition, an ANCOVA (see the applied tests for evaluating normality and homogeneity of variances above), using only males and females in the range of sizes from 15.3–20.1 and 11.5–20.2 mm, respectively, was performed to evaluate the potential differential increase in claw height and carapace width in males over females.

Results

Field monitoring and crab sampling

Abiotic conditions, occurrence of *H. takanoi* and reproductive season

The study area is characterized by a strong seasonality. Seawater temperature varied between ~ 2 °C in mid-February and ~ 19 °C during June and August, while salinity varied between 14.2 and 22.6 (monthly extremes) with highest values in winter (Figure 1). Strongest changes occurred during early spring, with small-scale fluctuations in summer and autumn (Figure 1), reflecting the highly dynamic physical-chemical conditions at this site. A total of 596 individuals of *H. takanoi* were collected during the study period. Males represented 41% and females 59% of the total relative abundance. The sex ratio showed a dominance of females over males for most of the year, except for December (Figure 2A). During end of spring and beginning of summer, an increase in density of *H. takanoi* was recorded, with 17% (101 individuals) of the total counts recorded in June. The abundance declined towards winter reaching 1% of all counts in February. The reproductive seasons of *H. takanoi* started in early

June, when ovigerous females were first observed, and ended in late August (Figure 1). A total of 106 ovigerous females was collected, representing 30% of the total female abundance registered over the entire year, with the highest abundance in July (48 individuals, 45% of the total of collected ovigerous females), when all females were found to be ovigerous (Figure 2A).

Biometric data of field-caught *H. takanoi*

Smallest male crabs had a size of 6.5 mm and the largest individual a size of 29 mm. For females, the smallest and largest sizes were 5.5 and 25.5 mm, respectively. Smallest ovigerous females had a size of 13.5 mm, while the largest a size of 22.7 mm. Most ovigerous females were recorded in the size class 17.1–19.0 mm (38 individuals, 10.8% of all collected females; Figure 2B).

Differences in *H. takanoi* catches between the two sampling methods (scraping *vs*. traps) are illustrated in Figures S3 to S5. In general, the two methods led to differences in both, numbers and sizes of crabs collected (Figures S3, S5). Scraping collected more females, while traps collected more males (Figure S3A, B). Scraping gathered more crabs and led to a better distribution of catches over the different size classes. Traps caught generally larger crabs compared to the scraping method. None of the small males (≤ 15.0 mm) and none of the large females or ovigerous females (≥ 21.1 mm) were collected by traps (Figures S3, S5).

The wet weight of crabs ranged from 0.11 to 11.41 g. There was a clear positive correlation between size and weight (males (n = 213): $R^2 = 0.91$, females (n = 234): $R^2 = 0.94$, ovigerous females (n = 100): $R^2 = 0.67$; Figure S6). Male crabs were heavier than both females and ovigerous females for a given size (ANCOVA: $F_{2,541} = 8.617$, $p < 0.001$; Table S1).

For individuals with sizes from 11.5 to 20.2 mm, a significant difference between claw height and size was observed between both sexes (ANCOVA: $F_{1,49} = 83.353$, $p < 0.001$; Table S2). Males had larger claws than females with a claw height-crab size index (39%) almost 2 times larger than that of females (21%). As shown by the segmented regression, the initial linear increase of claw height with body size for males reached a plateau (maximum claw height at about 9 mm) for individuals with a size larger than 20.5 mm (Figure S7). In the case of females, claw height increased linearly over the entire range of sizes registered (Figure S7). Considering only the range of sizes where both sexes overlap, the increase in claw height with body size was 3 times stronger for males than for females (Figure S7; Table S2).

Prey-size selection assays

Mussel size preference

When offering different size classes of mussels, *H. takanoi* males were recorded opening mussels of up to 18.1–21.0 mm during summer and autumn, while only mussels up to 15.1–18.0 mm were opened in spring (Figure 3A–C). In spring, no male crab fed on the smallest size class of mussels offered (3.1–6.0 mm). In contrast, all size classes of males opened the smallest mussels (3.1–6.0 mm) during summer and autumn, except for the largest size classes of crabs (21.1–23.0 and 25.1–27.0 mm) in autumn, in which crabs opened mussels larger than 6.1 mm (Figure 3A–C). All females opened mussels in the range of 3.1 to 12.0 mm, independently of their size and the season (Figure 3D, E). In general, female *H. takanoi* were not recorded opening mussels larger than 15 mm, and only females larger than 15.1 mm were recorded opening the largest mussels (12.1–15.0 mm; Figure 3D, E). Only small females of 11.0–13.1 mm failed to open mussels larger than 6.1–9.0 mm. Generally, crabs showed different optima in their mussel size class preference, depending on season and sex, which generally shifted towards larger mussel size classes with increasing crab size (Figure 3A–E).

The relation between *H. takanoi* size and shell length of mussels consumed showed that the chosen prey size increases with predator size (Figure 4A, B). The interaction effects between season and crab sizes showed no significant differences in the size of mussels opened by males in the different seasons (ANCOVA: $F_{2,74} = 2.929$, $p = 0.059$; Figure 4A; Table 1). However, the slope of the regression for females in autumn was significantly higher than that for females in spring (ANCOVA: $F_{1,41} = 7.529$, $p = 0.008$; Figure 4B; Table 1).

Effects of season and sex on consumption rates

Seasonality (and/or temperature) significantly affected consumption rates for both sexes ($p < 0.001$; Table 2). Mussels (biomass) were more consumed in warmer than in colder seasons (see the statistic of GLMs in Table 2; Figure 5A, B). Comparing only similar size classes of crabs over the different seasons, feeding rates of males (15.1–23.0 mm) were 2 and almost 6 times higher in summer (mean ± SD, 66 ± 1.773 mg day^{-1}) than in autumn (37 ± 6 mg day^{-1}) and spring (12 ± 2 mg day^{-1}), respectively. Feeding rates in autumn were 3 times higher than in spring. In addition, 3 times more mussel biomass was consumed by female crabs (13.1–21.0 mm) in autumn (14 ± 3 mg day^{-1}) than in spring (4 ± 1 mg day^{-1}). The interaction between season and crab size was statistically significant for both sexes (see statistics of GLMs Table

2). In addition, sex significantly affected feeding rates of crabs ($p < 0.001$; Table S3). When comparing male and female individuals of 15.1–21.0 mm in size, twice as much mussel biomass was consumed by males compared to females. Males consumed 33 ± 5 (mean ± SD) mg day^{-1} in spring and 12 ± 2 mg day^{-1} in autumn, while females consumed 5 ± 1 mg day^{-1} during spring and 17 ± 2 mg day^{-1} during autumn (see statistics of GLMs in Table S3; Figure S8A, B).

Discussion

This study aimed at elucidating the potential impact of the recent invader *H. takanoi* on a mussel-dominated benthic ecosystem, the Kiel Fjord, by describing its current relative field abundance and assessing the seasonality-dependent feeding pressure in laboratory-based experiments. Feeding rates were closely linked to season, likely due to seawater temperatures, and interrupted by the reproductive season in female individuals. Even though females were generally more abundant, males may have a stronger impact on the ecosystem as *per capita* feeding rates of males strongly exceeded those of females.

Kiel Fjord H. takanoi exhibited a shortened reproductive season

Female *H. takanoi* reached sexual maturity at a size (carapace width) of about 13.5 mm. Nevertheless, egg-carrying females as small as 10.2 mm were found in the Kiel Fjord during summer 2014 (Geburzi et al. 2015), which may be attributed to an unusual warm summer recorded during this particular year (see supplementary in Pansch et al. 2018; Pansch and Hiebenthal 2019), which might have led to early maturation at a smaller size in this non-native *H. takanoi* population. Long-term monitoring data, however, will be needed in order to correlate size at sexual maturity (and/or breeding season) with annual temperatures.

The duration of the reproductive period in brachyuran crabs may depend on latitude, and/or be related with seawater temperatures (McDermott 1998; Brousseau and McSweeney 2016). Ovigerous females were found in the Kiel Fjord over a period of three months, while in the native range the reproductive season lasts six months (Miyajima et al. 2012), and five months in the Dutch delta (van den Brink et al. 2013). In agreement with our study (with mean temperature of 15.4 °C), however, ovigerous females from the Oosterschelde estuary, Netherlands, have been observed in seawater temperatures above 15 °C (van den Brink et al. 2013). Consequently, the *H. takanoi* population from the Kiel Fjord might be constrained by low-salinity stress, which may compress their breeding season.

Generally, egg production and successful embryonic and larval development of many Baltic Sea invertebrates (Kinne 1971), and in particular brachyuran crabs (Otto and Brandis 2011), decreases drastically with decreasing salinity. In the Kiel Fjord, salinities can be as low as 12 (Nasrolahi et al. 2016) compared to native and non-native habitats of *H. takanoi* populations with often much higher salinity regimes (Shinji et al. 2009; van den Brink et al. 2013; Wood et al. 2015; Ashelby et al. 2017). Ovigerous *H. takanoi* from Kiel Fjord were found in salinities down to 14.8, suggesting the capacity to successfully reproduce in these salinities. Despite the occurrence of ovigerous females at lower salinities, successful larval development from megalopae to juveniles required salinities higher than 25 in the native range of *H. takanoi* (Mingkid et al. 2006b). This may suggest that Kiel Fjord *H. takanoi* have already adapted to the prevailing environmental conditions.

While H. takanoi females were more abundant, males reached a larger biomass

In the Kiel Fjord, the observed sex ratio (1.4 females to 1 male) is in contrast to similar studies from non-native ranges in Europe, which recorded almost equal sexes abundance for this species (Noël et al. 1997; Dauvin et al. 2009), albeit using different methods and a shorter sampling duration (two months *vs*. twelve months in the present study; Dauvin et al. 2009).

Overall, males reached larger sizes than females, likely explained by differential reproductive and energy demands between sexes (Angeletti and Cervellini 2015). Larger body size increases the success rate in intra-specific competition, courtship, handling as well as protection of females during copulation (Araújo et al. 2012). Individuals' weight increased with size for both sexes, but males were heavier than females at a given size, being a well-known pattern in brachyuran crabs (Saher et al. 2016; Glamuzina et al. 2017).

Mussel size preference and overall energy uptake differed between sexes of H. takanoi

According to the optimal foraging theory, predators choose their diet to maximize the net rate of energy intake per unit foraging time (Emlen 1968; Charnov 1976; Elner and Hughes 1978; Hughes 1979), assuming that predators are able to rank prey in order of their profitability (Enderlein et al. 2003). Males of *H. takanoi* from Kiel Fjord (15 to 27 mm carapace width), opened a broad range of *M. edulis* individuals with shell lengths between 3 and 21 mm. Females of slightly smaller size (11 to 21 mm) were restricted to smaller mussels (3 to 15 mm). Generally, optimal sizes were recorded around 6 to 15 mm and 3 to 12 mm, for males and females respectively. Males of *H. takanoi* (20–27 mm) from the native range (Japan) preferred to crush mussels (*Xenostrobus securis*) of 10 to 17 mm size, although these were occasionally found to open mussels up to 27 mm (Doi et al. 2009), as also observed in Kiel Fjord males. Smaller males (< 20 mm) preferred mussels between 5 and 12 mm while they were able to crush mussels of up to 17 mm (Doi et al. 2009).

Significant differences were present between sexes during autumn and spring, when males showed much higher feeding rates (energy uptake) than females. Elner (1980) explained this difference between sexes in *C. maenas* in the way that females rely more on other prey types than mussels. However, this aspect was not addressed within the present study. Claw size (propal height or gape) plays a critical role in prey size selection between crabs of similar sizes and/or weights (Yamada and Boulding 1998). In the present study, males of *H. takanoi* exhibited significantly larger claws than females of same sizes. Consequently, males of *H. takanoi* can feed on larger mussels than females of the same body size.

Seasonal temperature shifts determined feeding rates of H. takanoi

Feeding rates (and partly preferred prey size classes) for males and females of *H. takanoi* changed strongly with season. This might be directly linked to prevailing seawater temperatures during respective seasons when the assays were conducted, being highest in late summer (~ 19 °C) and early autumn (~ 13 °C), and lowest in spring (~ 8 °C). Observed increased feeding at higher temperatures has been described for other crustaceans as well. For example, *C. maenas* consumed about twice as much food at 24 °C than at 10 °C (0.2 times increased food uptake per °C; Wallace 1973). Moreover, consumption rates of *C. maenas* upon the cockle *Cerastoderma edule* increased by about 4-fold with increasing temperatures from 9.5 to 15.5 °C (0.6 times incresaed food uptake per °C; Sanchez-Salazar et al. 1987). This shows the direct influence of seasonal temperature changes on feeding behaviour of crabs, which is likely related to an increase in metabolic rates and behavioural activity at warmer conditions (Thomas et al. 2000; Kemp and Britz 2008; Wang et al. 2016).

Interestingly, similar to *C. maenas* (Elner 1980; Sanchez-Salazar et al. 1987), equally-sized males of *H. takanoi* were unaffected by temperature and/or season when choosing their prey. In contrast, prey preference in females of equal sizes was significantly different between seasons. Females during spring chose to open smaller mussels than females of the same size in autumn. Female crabs require large amounts of energy to produce their eggs (Ruiz-Tagle et al.

2002). Presumably, females needed to accumulate energy during spring for the upcoming reproductive investment.

Possible ecological consequences

Overall, our data demonstrate that *H. takanoi* is an important predator particularly on small individuals of *M. edulis*, and especially so during the warmer seasons. Mussel recruitment occurs mainly from the end of May to September (Thomsen et al. 2010), with high biomass accretion during summer in this eutrophic area (Thomsen et al. 2013). However, growth of *M. edulis* is reduced in the presence of its two main predators: the green crab *C. maenas* and the sea star *A. rubens* (Dobretsov and Wahl 2001), and likely also in the presence of the recent invader *H. takanoi*, at the observed high abundances.

Adults of *C. maenas* in the western Baltic Sea show a preference for mussels smaller than 40 mm (Enderlein et al. 2003). Adults of *A. rubens* prey on mussels smaller than 33 mm in Kiel Fjord (Reusch and Chapman 1997). While in the Wadden Sea, *H. takanoi* and *C. maenas* individuals of the same size were recently shown to prey upon similar sized mussel prey (David Thieltges *personal communication*), *H. takanoi* exerts a significant pressure on smaller mussels (< 15 mm shell length) in the Kiel Fjord area. In turn, this might affect *M. edulis* recruitment patterns in the future. Once mussels reach a size of approximately 20 mm in the Kiel Fjord, they might be too large for *H. takanoi*, but continuing to be within the predation window of adult *C. maenas* or *A. rubens*. To what extent this pressure on smaller prey size will affect recruitment dynamics remains to be investigated by experimental and field studies on early post-settlement *M. edulis* individuals smaller than those examined in the present study. The results presented provide the first insights towards an understanding of the effects of *H. takanoi* predation on Western Baltic Sea mussel beds. Still, it remains to be addressed by what extent *H. takanoi* can further populate the Baltic proper. Adults and juveniles can tolerate salinities as low as 7 to 9 (Mingkid et al. 2006a; Gittenberger et al. 2010; Soors et al. 2010), and abrupt short-term changes in salinity regimes are not stressful to adult *H. takanoi*, unless salinities reach freshwater conditions (Shinji et al. 2009). This is considered an advantage to its invasion success and it is assumed that their current distribution is rather limited by cold temperature, not by low salinities (van den Brink et al. 2013). In contrast, higher salinities are required for *H. takanoi* larvae to develop (Mingkid et al. 2006b), and knowledge on the salinity tolerance of all life-history stages of Baltic Sea populations is needed to understand *H. takanoi's* invasion potential into the wider Baltic Sea.

Conclusions

Despite strong differences in feeding rates between males and females of *H. takanoi*, and a clear difference in prey size selection, *H. takanoi* will likely have a persistent impact on western Baltic Sea mussel populations, with likely implications for mussel-associated communities (Koivisto and Westernbom 2012). The direct interactions with *C. maenas* and *A. rubens* and resulting changes in feeding pressure on mussel beds are not yet understood. The presence of *H. takanoi* will, however, most certainly increase predation pressure on small-sized mussels, with potential consequences for overall population dynamics. Clearly, the breeding season of Kiel Fjord *H. takanoi* is constrained, likely related to the prevailing seawater temperatures and/or the low-salinity regime. Therefore, a further spread of *H. takanoi* into the wider Baltic Proper might be restricted by low salinity, but be supported by favourable biological traits for dispersal (i.e. high fecundity, storing sperm for several broods, and high dispersal), all of which contribute to the overall invasion success of this species (Weis 2010). As both sexes recorded higher consumption rates in warmer temperatures, the impacts on the ecosystems as well as a potentially on-going invasion process will likely be favoured by future local and global warming trends.

Data availability

Data used in the present study are available at PAGAEA:

https://doi.org/10.1594/PANGAEA.905576

Acknowledgements

We thank Laura Hennigs and Claudia Bommarito for their help during crab collection, Prof. Dr. Martin Wahl and Dr. Frank Melzner for their helpful advice, Björn Buchholz for technical assistance, Markus Franz for helping with map preparation and for carefully proof reading of the manuscript, and Dr. Mark Lenz for statistical advice. OMN acknowledges the financial support of the German Academic Exchange Service (DAAD) through the project German Egyptian Research Long-term Scholarship Programme (GERLS) 2015/16 (57147166). FRB acknowledges the financial support of the German Academic Exchange Service (DAAD) through the project Doctoral Programmes in Germany 2015/16 (57129429). CP acknowledges financial support by Helmholtz, by GEOMAR and by the Cluster of Excellence "The Future Ocean". "The Future Ocean" is funded within the framework of the Excellence Initiative by the Deutsche Forschungsgemeinschaft (DFG) on behalf of the German federal and state governments. We thank the associated editor Dr. Philippe Goulletquer, the thematic editor Dr. Charles Martin, and three anonymous reviewers for their valuable comments and advice, which greatly improved the manuscript.

References

Angeletti S, Cervellini P (2015) Population structure of the burrowing crab *Neohelice granulata* (Brachyura, Varunidae) in a southwestern Atlantic salt marsh. *Latin American Journal of Aquatic Research* 43: 539–547

Araújo MS, Barreto AV, Negromonte AO, Schwamborn R (2012) Population ecology of the blue crab *Callinectes danae* (Crustacea: Portunidae) in a Brazilian tropical estuary. *Anais Da Academia Brasileira De Ciencias* 84: 129–138

Asakura A, Watanabae S (2005) *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25: 279–292

Ashelby CW, Sewell J, Rostron J, Shrubsole R, Child T, Clark PF (2017) Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90: 695–708

Barboza FR, Franz M (2016) Die Biodiversität der Ostsee: Erkenntnisse der Vergangenheit und Perspektiven für die Zukunft. In: Lozán JL, Breckle S-W, Müller R, Rachor W (eds), Warnsignal Klima: Die Biodiversität. Universität Hamburg, pp 277–283. doi:10.2312/warnsignal.klima.die-biodiversitaet.45.

Brousseau DJ, McSweeney L (2016) A comparison of reproductive patterns and adult dispersal in sympatric introduced and native marine crabs: implications for species characteristics of invaders. *Biological Invasions* 18: 1275–1286

Casties I, Seebens H, Briski E (2016) Importance of geographic origin for invasion success : A case study of the North and Baltic Seas versus the Great Lakes – St. Lawrence River region. *Ecology and Evolution* 6: 8318–8329

Charnov EL (1976) Optimal foraging, the marginal value theorem. *Theoretical Population Biology* 9: 129–136

Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. *Science* 279(5350): 555–558

Dauvin JC, Delhay JB (2010) First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern cotentin, Normandy, western English channel. *Marine Biodiversity Records* 3: e101

Dauvin JC, Rius AT, Ruellet T (2009) Recent expansion of two invasive crabs species *Hemigrapsus sanguineus* (de Haan 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal coast, France. *Aquatic Invasions* 4: 451–465

Devin S, Bollache L, Noël PY, Beisel JN (2005) Patterns of biological invasions in French freshwater systems by non-indigenous macroinvertebrates. *Hydrobiologia* 551: 137–146

Dobretsov S, Wahl M (2001) Recruitment preferences of blue mussel spat (*Mytilus edulis*) for different substrata and microhabitats in the White Sea (Russia). *Hydrobiologia* 445: 27–35

Doi W, Iinuma Y, Yokota M, Watanabe S (2009) Comparative feeding behavior of invasive (*Carcinus aestuarii*) and native crabs (*Hemigrapsus takanoi*). *Crustacean Reserach* 38: 1–11

Elner RW (1980) The influence of temperature, sex and chela size in the foraging strategy of the shore crab, *Carcinus maenas* (L.). *Marine Behaviour and Physiology* 7: 15–24

Elner RW, Hughes RN (1978) Energy maximization in the diet of the shore crab, *Carcinus maenas*. *Journal of Animal Ecology* 47: 103–116

Emlen JM (1968) Optimal choice in animals. *American Naturalist* 102: 385–389

Enderlein P, Moorthi S, Röhrscheidt H, Wahl M (2003) Optimal foraging versus shared doom effects: interactive influence of mussel size and epibiosis on predator preference. *Journal of Experimental Marine Biology and Ecology* 292: 231–242

Galil BS (2008) Alien decapods in the Mediterranean Sea – which, when, where, why? *Hydrobiologia* 606: 105–116

Galil BS (2009) Taking stock: inventory of alien species in the Mediterranean Sea. *Biological Invasions* 11: 359–372

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

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

Gerard VA, Cerrato RM, Larson AA (1999) Potential impacts of a western Pacific grapsid crab on intertidal communities of the northwestern Atlantic Ocean. *Biological Invasions* 1: 353–361

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

76

Glamuzina L, Conides A, Mancinelli G, Dobroslavić T, Bartulović V, Matić-Skoko S, Glamuzina B (2017) Population dynamics and reproduction of Mediterranean green crab *Carcinus aestuarii* in Parila lagoon (Neretva estuary, Adriatic Sea, Croatia) as fishery management tools. *Marine and Coastal Fisheries* 9: 260–270

Gollasch S (1999) The Asian decapod *Hemigrapsus penicillatus* introduced in European waters : status quo and future perspective. *Helgolander Meeresunters* 2: 359–366

Gollasch S (2006) Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60: 84–89

Gothland M, Dauvin JC, Denis L, Dufossé F, Jobert S, Ovaert J, Pezy JP, Tous Rius A, Spilmont N (2014) Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142: 41–49

Hänfling B, Edwards F, Gherardi F (2011) Invasive alien crustacea: dispersal, establishment, impact and control. *Biological Control* 56: 573–595

Hughes RN (1979) Optimal diets under the energy maximization premise: the effects of recognition time and learning. *American Naturalist* 113: 209–221

Jensen GC, McDonald PS, Armstrong DA (2002) East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp*. *Marine Ecology Progress Series* 225: 251–262

Jormalainen V, Gagnon K, Rothäusler E (2016) The invasive mud crab enforces a major shift in a rocky littoral invertebrate community of the Baltic Sea. *Biological Invasions* 18: 1409–1419

Jubb CA, Hughes RN, Rheinhallt T (1983) Behavioural mechanisms of size-selection by crabs, *Carcinus maenas* (L) feeding on mussels, *Mytilus edulis* (L). *Journal of Experiemntal Marine Biology and Ecology* 66: 81–87

Karatayev AY, Burlakova LE, Padilla DK, Mastitsky SE, Olenin S (2009) Invaders are not a random selection of species. *Biological Invasions* 11: 2009–2019

Katsanevakis S, Gatto F, Zenetos A, Cardoso AC (2013) How many marine aliens in Europe? *Management of Biological Invasion*s 4: 37–42

Kemp JOG, Britz PJ (2008) The effect of temperature on the growth, survival and food consumption of the east coast rock lobster *Panulirus homarus rubellus*. *Aquaculture* 280: 227–231

Kinne O (1971) Salinity, animals: invertebrates. Environmental factors. In a comprehensive, integrated treatise on life in oceans and coastal waters. *Marine Ecology* 1: 821–995

Koivisto M, Westerbom M (2012) Invertebrate communities associated with blue mussel beds in a patchy environment : a landscape ecology approach. *Marine Ecology Progress Series* 471: 101–110

Kotta J, Ólafsson E (2003) Competition for food between the introduced polychaete *Marenzelleria viridis* (Verrill) and the native amphipod *Monoporeia affinis* Lindström in the Baltic Sea. *Journal of Sea Research* 50: 27–35

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

Markert A, Raupach MJ, Segelken-Voigt A, Wehrmann A (2014) Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatu*s (De Haan, 1835) versus *Hemigrapsus takano*i Asakura & Watanabe 2005. *Organisms Diversity and Evolution* 14(4): 369–382

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

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

Mingkid WM, Yokota M, Watanabe S (2006b) Salinity tolerance of larvae in the penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). *La Mer* 44: 17–21

Miyajima A, Fukui Y, Wada K (2012) Agonistic and mating behavior in relation to chela features in *Hemigrapsus takanoi* and *H. sinensis* (Brachyura,Varunidae). *Crustacean Research* 41: 47–58

Muggeo VM (2017) segmented:Regression models with break-points/change-points estimation. R package version:0.5-3.0. Available: https://CRAN.R-project.org/package = segmented

Nasrolahi A, Havenhand J, Wrange A, Pansch C (2016) Population and life-stage specific sensitivities to temperature and salinity stress in barnacles. *Scientific Reports* 6: 32263, doi: 10.1038/srep32263

Noël PY, Tardy E, d'Udekem d'Acoz C (1997) Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? *Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Science* 320: 741–745

Ojaveer H, Jaanus A, Mackenzie BR, Martin G, Olenin S, Telesh I, Zettler ML, Zaiko A (2010) Status of biodiversity in the Baltic Sea. *PLoS ONE* 5: e12467

Ojaveer H, Galil BS, Lehtiniemi M, Christoffersen M, Clink S, Florin A, Gruszka P, Puntila R, Behrens JW (2015) Twenty five years of invasion : management of the round goby *Neogobius melanostomus* in the Baltic Sea. *Management of Biological Invasions* 6: 329–339

Ojaveer H, Olenin S, Narščius A, Florin A, Ezhova E, Gollasch S, Jensen KR, Lehtiniemi M, Minchin D, Normant-Saremba M, Strāke S (2016) Dynamics of biological invasions and pathways over time : a case study of a temperate coastal sea. *Biological Invasions* 19: 799–813

Olenin S, Leppäkoski E (1999) Non-native animals in the Baltic Sea: alteration of benthic habitats in coastal inlets and lagoons. *Hydrobiologia* 39: 233–243

Otto T, Brandis D (2011) First evidence of *Eriocheir sinensis* reproduction from Schleswig-Holstein, northern Germany, western Baltic Sea. *Aquatic Invasions* 6 (Suppl 1): 65–69

Paavola M, Olenin S, Leppäkoski E (2005) Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuarine, Coastal and Shelf Science* 64(4): 738–750

Pansch C, Hiebenthal C (2019) A new mesocosm system to study the effects of environmental variability on marine species and communities. *Limnology Oceanography: Methods* (Supplementry) doi: 10.1002/lom3.10306

Pansch C, Scotti M, Barboza FR, Al-Janabi B, Brakel J, Briski E, Bucholz B, Franz M, Ito M, Pavia F, Saha M, Sawall Y, Weinberger F, Wahl M (2018) Heat waves and their significance for a temperate benthic community : a near-natural experimental approach. *Global Change Biology* 24: 4357–4367

Pinheiro MAA, Fransozo A (2002) Reproduction of the speckled swimming crab *Arenaeus cribrarius* (Brachyura: Portunidae) on the Brazilian coast near 23°30′S. *Journal of*

Crustacean Biology 22: 416–428

R Core Team (2016) R: a language and environment for statistical computing. https ://www.R-project.org/

Reusch T, Chapman ARO (1997) Persistance and space occup by subtidal blue mussel patches. *Ecological Monographs* 67: 65–87

Ricciardi A (2006) Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions* 12: 425–433

Ricciardi A, MacIsacc HJ (2000) Recent mass invasion of the north American Great Lakes by Ponto-Caspian speceis. *Trends in Ecology and Evolution* 15: 62–65

Ruiz-Tagle N, Fernández M, Pötner HO (2002) Full time mothers: daily rhythms in brooding and nonbrodding behaviors of brachyuran crabs. *Journal of Experimental Marine Biology and Ecology* 276: 31–47

Saher NU, Qureshi NA, Aziz U (2016) Allometric growth pattern, sexual dimorphism and size at the onset of sexual maturity in *Opusia indica* (Brachyura: Ocypodoidea: Camptandriidae) from mangrove areas of Pakistan. *International Journal of Aquatic Biology* 4: 224–232

Sanchez-Salazar ME, Griffiths CL, Seed R (1987) The effect of size and temperature on the predation of cockles *Cerastoderma edule* (L.) by the shore crab *Carcinus maenas* (L.). *Journal of Experimental Marine Biology and Ecology* 111: 181–193

Savini D, Occhipinti-Ambrogi A, Marchini A, Tricarico E, Gherardi F, Olenin S, Gollasch S (2010) The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology* 26 (Suppl 2): 1–7

Shinji J, Strussmann CA, Wilder MN, Watanabe S (2009) Short-term responses of the adults of the common Japanese intertidal crab, *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsoidea) at different salinities: osmoregulation, oxygen consumption, and ammonia excretion. *Journal of Crustacean Biology* 29: 269–272

Soors J, Faasse M, Stevens M, Verbessem I, de Regge N, Van den Bergh E (2010) New crustacean invaders in the Schelde estuary (Belgium). *Belgian Journal of Zoology* 140: 3–10

Sumpton WD, Smith GS (1990) Effect of temperature on the emergence, activity and feeding of male and female sand crabs (*Portunus pelagicus*). *Australian Journal of Marine and Freshwater Research* 41: 545–550

Tiselius P, Møller LF (2017) Community cascades in a marine pelagic food web controlled by the non-visual apex predator *Mnemiopsis leidyi*. *Journal of Plankton Research* 39: 271–279

Thomas CW, Crear BJ, Hart PR (2000) The effect of temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, *Jasus edwardsii*. *Aquaculture* 185: 73–84

Thomsen J, Gutowska MA, Saphörster J, Heinemann A, Trübenbach K, Fietzke J, Hiebenthal C, Eisenhauer A, Körtzinger A, Wahl M, Melzner F (2010) Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* 7: 3879–3891

Thomsen J, Casties I, Pansch C, Körtzinger A, Melzner F (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology* 19: 1017–1027

Tsiamis K, Palialexis A, Stefanova K, Gladan ZN, Skejić S, Despalatović M et al.(2019) Non-indigenous species refined national baseline inventories: a synthesis in the context of the European Union's Marine Strategy Framework Directive. *Marine Pollution Bulletin* 145: 429–435

Turoboyski K (1973) Biology and ecology of the crab *Rhithropanopeus harrisii* ssp. *tridentatus*. *Marine Biology* 23: 303–313

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

Wallace JC (1973) Feeding, starvation and metabolic rate in the shore crab *Carcinus maenas*. *Marine Biology* 20: 277–281

Wang G, Robertson LM, Wringe BF, McGaw IJ (2016) The effect of temperature on foraging activity and digestion in the American lobster *Homarus americanus* (Milne Edwards, 1837) (Decapoda: Nephropsidae) feeding on blue mussels *Mytilus edulis* (Linnaeus, 1758). *Journal of Crustacean Biology* 36: 138–146

Weis JS (2010) The role of behavior in the success of invasive crustaceans. *Marine and Freshwater Behaviour and Physiology* 43: 83–98

Wood CA, Bishop JDD, Davies CJ, Delduca EL, Hatton JC, Herbert RJH, Clark PF (2015)

Hemigrapsus takanoi Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4: 109–113

Yamada SB, Boulding EG (1998) Claw morphology, prey size selection and froaging effecincy in generalist and specialist shell-breaking crabs. *Journal of Experimental Marine Biology and Ecology* 220: 191–211

Yamasaki I, Doi W, Mingkid WM, Yokota M, Strüssmann CA, Watanabe S (2011) Molecular-based method to distinguish the sibling species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura:Varunidae). *Journal of Crustacean Biology* 31: 577–581

Figures

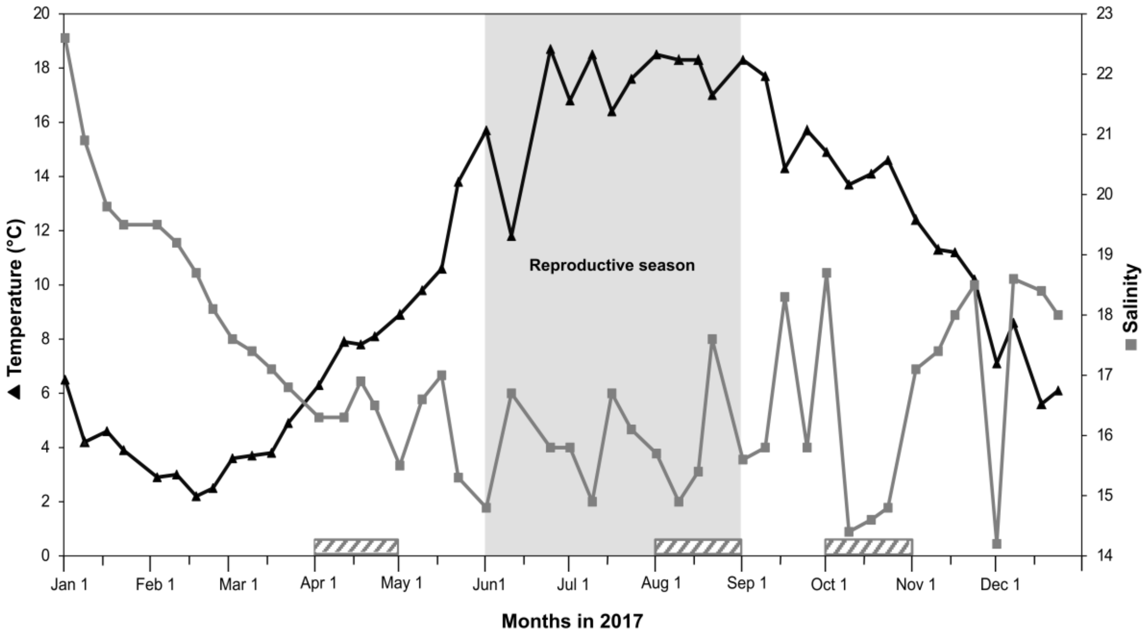

Figure 1. Kiel Fjord seawater temperature (°C; black triangle) and salinity (grey square) at a depth of 1 to 1.5 m during 2017. The grey shaded area indicates the reproductive season of *Hemigrapsus takanoi* (occurrence of ovigerous females) in the Kiel Fjord. The striped areas show when feeding assays were performed in spring (April), summer (August) and autumn (October).

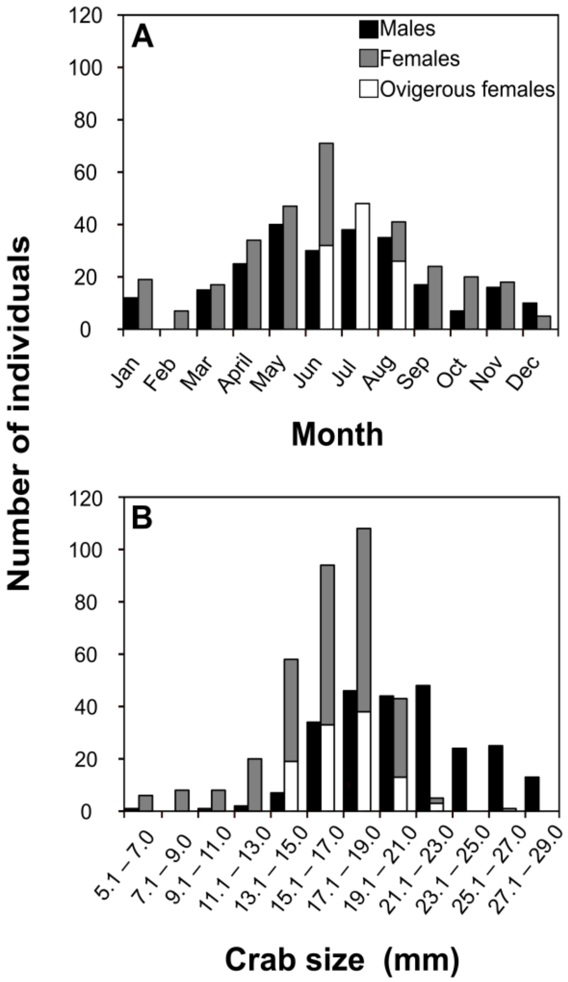

Figure 2. Relative abundance (**A**) and size (mm; **B**) of males (black), females (grey) and ovigerous females (white) of *Hemigrapsus takanoi*, sampled from January to December 2017 in the Kiel Fjord. Samples were obtained by scraping and traps (pooled in these plots).

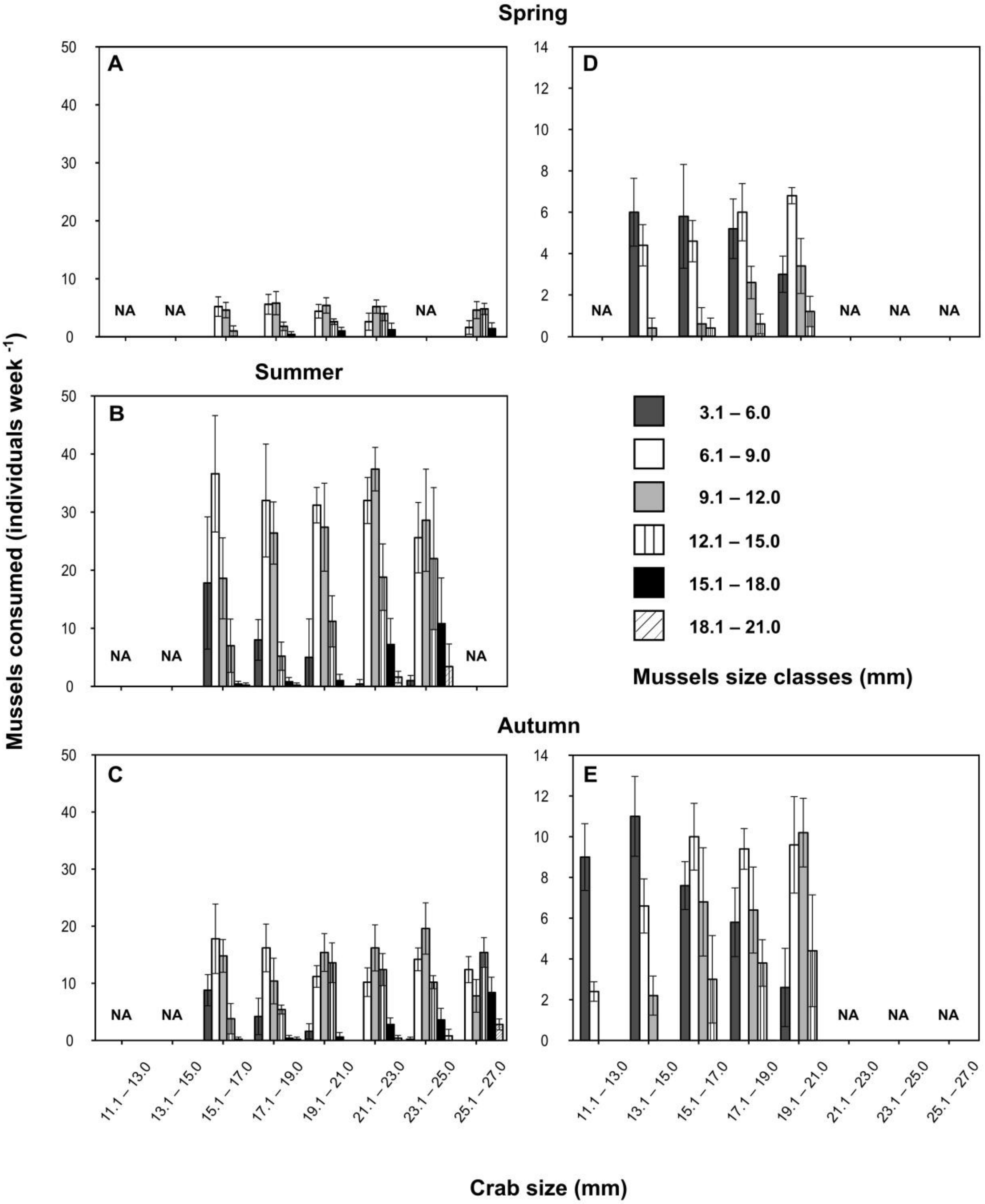

Figure 3. Mean consumption rates of mussels (*Mytilus edulis*) by different size classes of males (**A**, **B** and **C**) and females (**D** and **E**) of *Hemigrapsus takanoi* in different seasons (sums over 7 days). NA indicates size classes of crabs not found in the field when feeding assays were performed. Note that preferred mussel size classes can be observed within separate crab size classes. Error bars represent 95% confidence intervals.

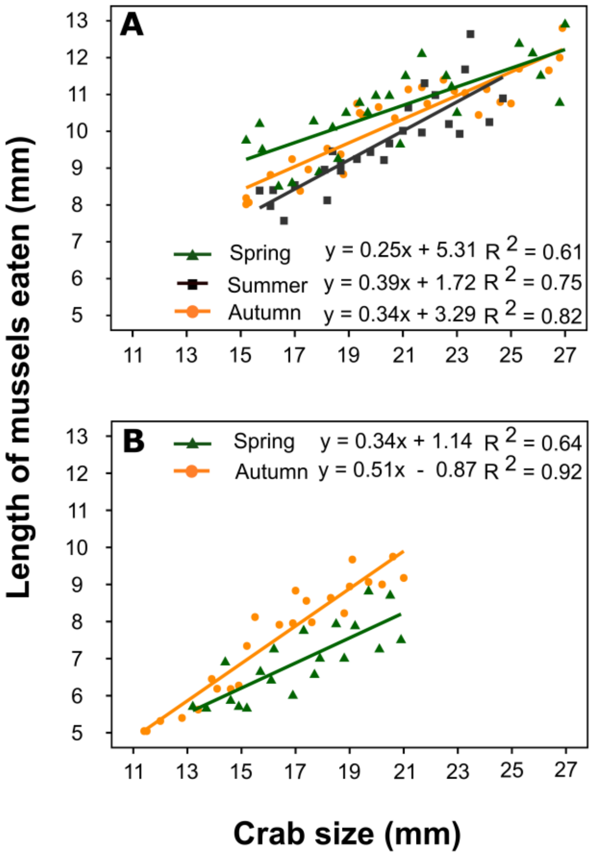

Figure 4. Relation between the mean length of mussels consumed and crab size for males (**A**) and females (**B**) of *Hemigrapsus takanoi* in different seasons (spring: green triangles, summer: black squares, autumn: orange circles). The slopes of all adjusted regressions were significantly different from zero. See statistical details in Table 1.

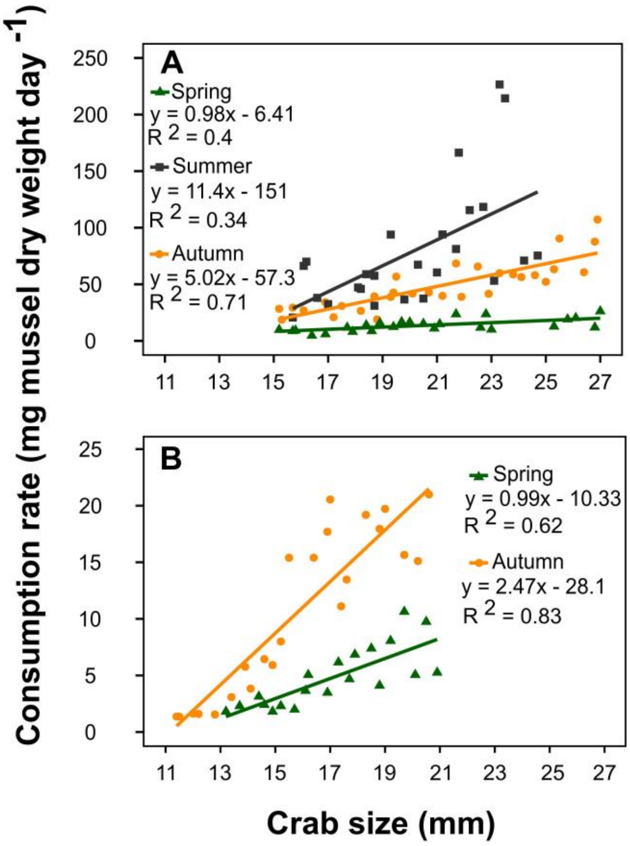

Figure 5. Dry weight of mussels eaten per day by males (**A**) and females (**B**) of *Hemigrapsus takanoi* in different seasons (spring: green triangles, summer: black squares, autumn: orange circles). The slopes of all adjusted regressions were significantly different from zero. See statistical details in Table 2.

Tables

Table 1. Analysis of covariance (ANCOVA) testing the main and interactive effects of season and crab size on the length (mm) of *Mytilus edulis* eaten by males and females of *Hemigrapsus takanoi*. Significant results ($p \leq 0.05$) are highlighted in bold.

Dependent variable	Source of variation	SS	df	MS	F-value	p-value
Mussel length consumed by males	Season	11.972	2	5.986	14.033	**<0.001**
	Crab size	88.942	1	88.942	208.505	**<0.001**
	Season: Crab size	2.499	2	1.25	2.929	0.059
	Error	31.566	74	0.427		
Mussel length consumed by females	Season	2.975	1	2.975	10.264	**0.002**
	Crab size	66.873	1	66.873	230.694	**<0.001**
	Season: Crab size	2.183	1	2.183	7.529	**0.008**
	Error	11.885	41	0.29		

Table 2. Generalized linear models (GLMs) testing the main and interactive effects of season and crab size on the dry biomass of *Mytilus edulis* daily eaten by males and females of *Hemigrapsus takanoi*. Significant results ($p \leq 0.05$) are highlighted in bold.

Dependent variable	Source of variation	SS	df	F-value	p-value
Mussels consumed by males	Season	29.277	2	116.173	**<0.001**
	Crab size	4.750	1	37.702	**<0.001**
	Season: Crab size	4.823	2	19.141	**<0.001**
Mussels consumed by females	Season	5.939	1	56.599	**<0.001**
	Crab size	15.520	1	147.917	**<0.001**
	Season: Crab size	4.604	1	43.909	**<0.001**

Supporting information for chapter I:

Supplementary figures

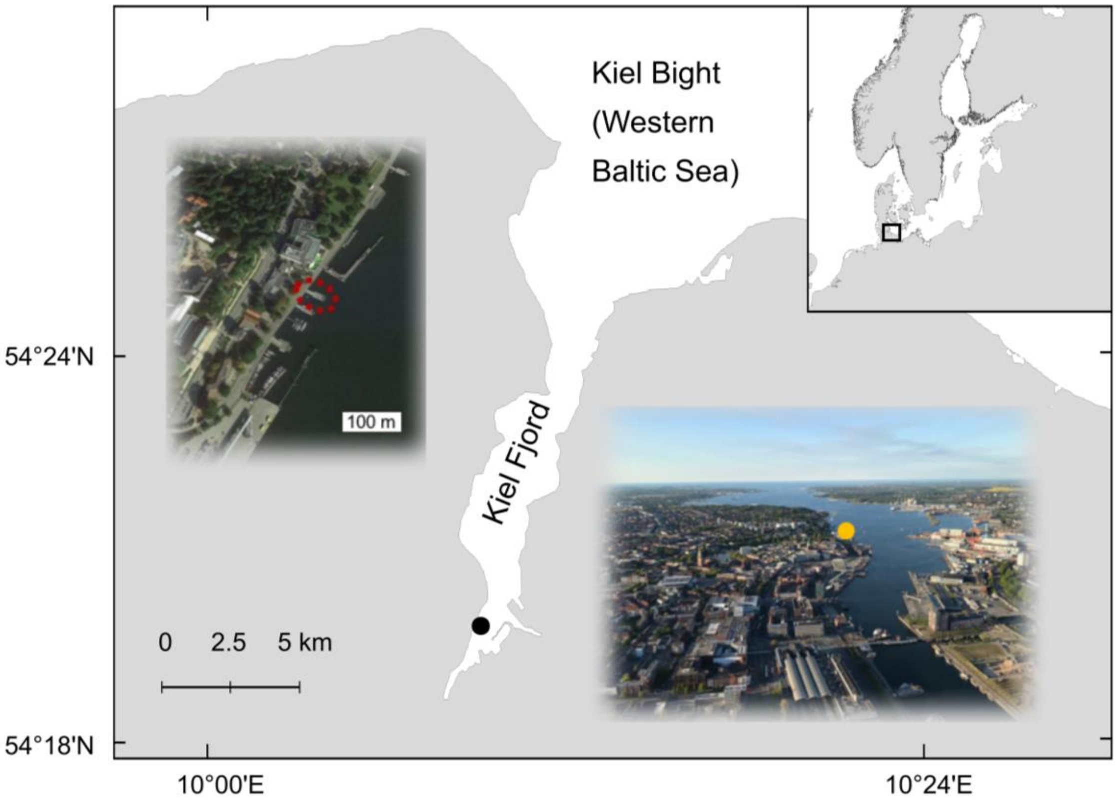

Figure S1. Sampling site in the Kiel Fjord from which all *Hemigrapsus takanoi* individuals were collected during the sampling campaign in 2017 and for the feeding assays (black and yellow solid circles). The Kiel Fjord is a small sheltered bay within the larger Kiel Bight in the south-west of the Baltic Sea. The Kiel Fjord is an industrialized and inhabited area characterized by man-made harbour structures (bottom right). A satellite image (®Google Earth, top left) illustrates the precise location at which the sampling occurred.

Figure S2. Trap design for catching crabs, consisting of 50 × 50 × 20 cm PVC pipes covered entirely with a 1 mm mesh, allowing mussel predators to enter on both sides. The traps were regularly filled with crushed *Mytilus edulis* mussels.

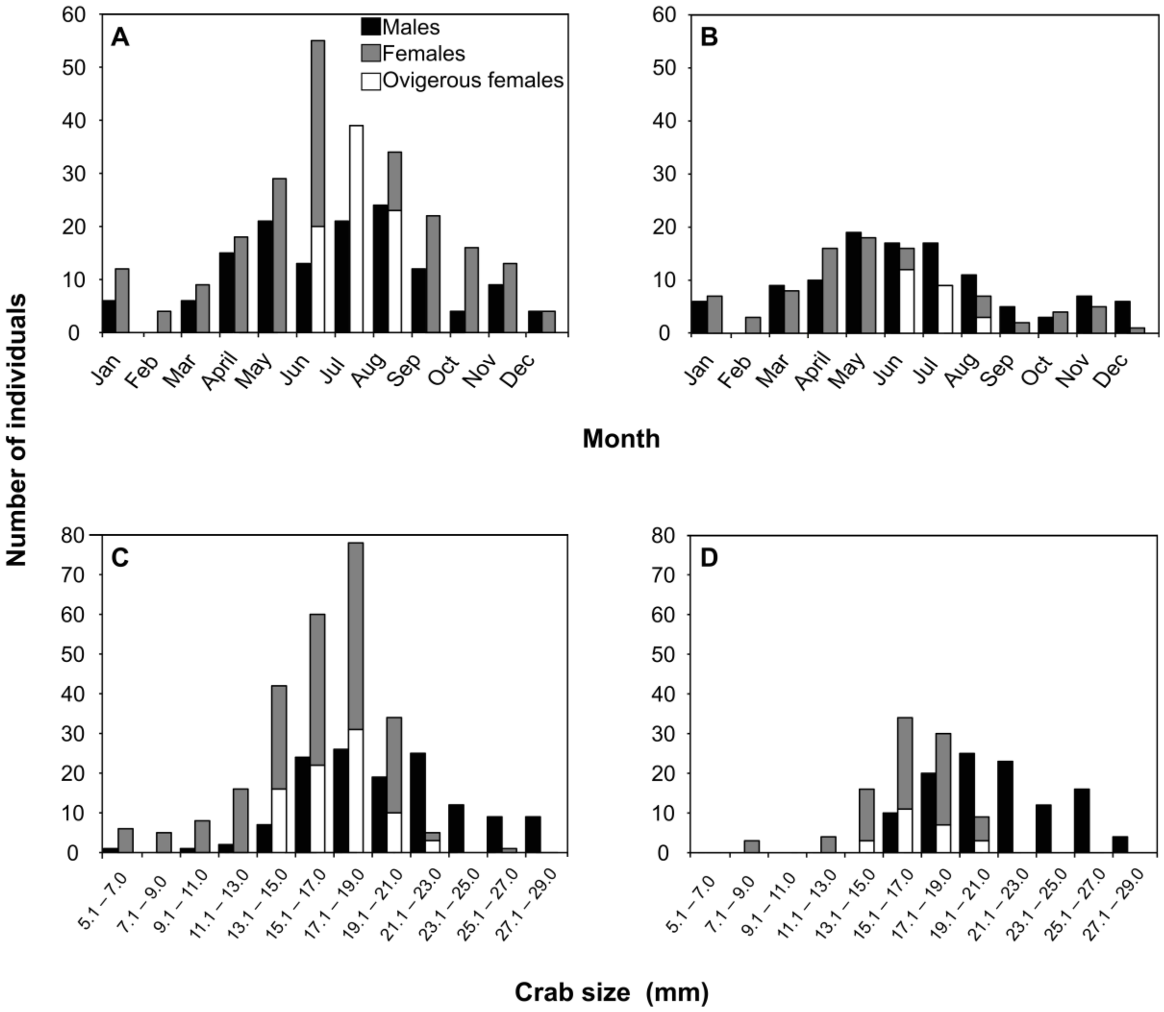

Figure S3. Relative abundance (**A** and **B**) and size (**C** and **D**) for males (black), females (grey) and ovigerous females (white) of *Hemigrapsus takanoi* collected by scraping (**A** and **C**) and using traps (**B** and **D**) in the Kiel Fjord during 2017.

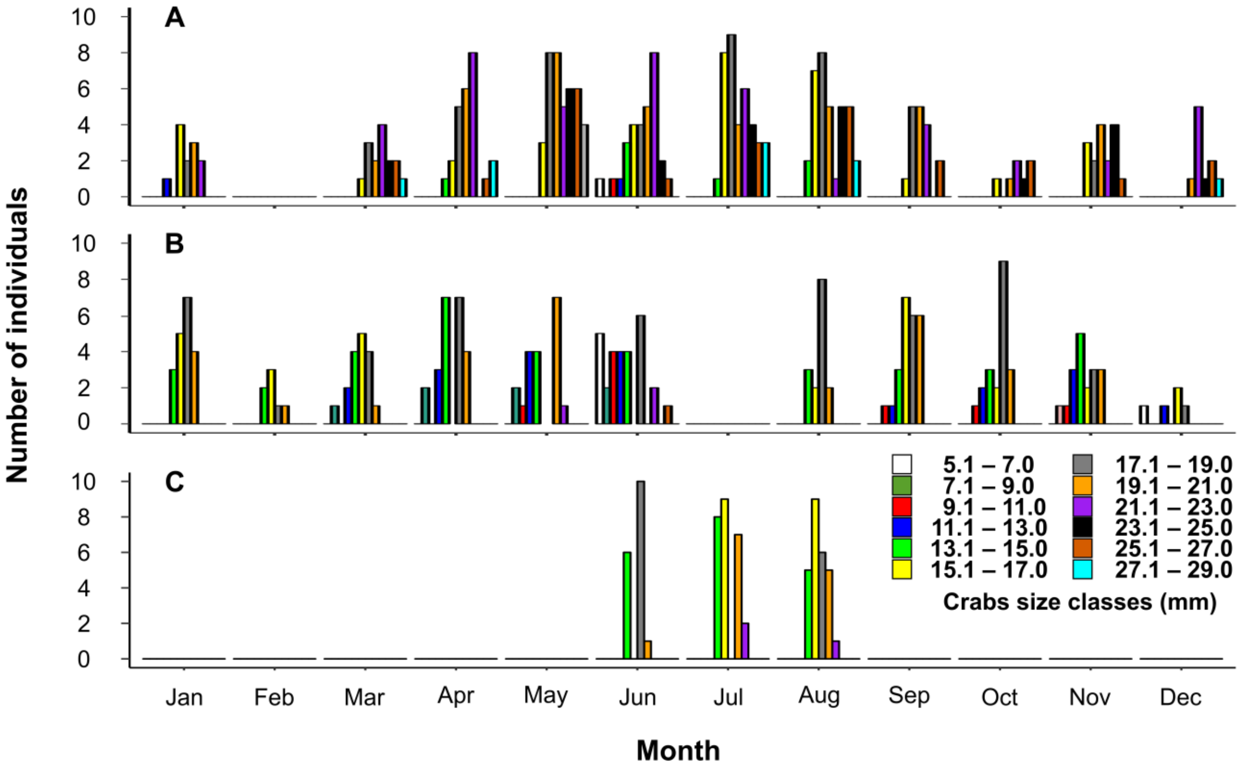

Figure S4. Size class (mm) distribution of males (**A**), females (**B**) and ovigerous females (**C**) of *Hemigrapsus takanoi* collected in the Kiel Fjord during 2017. Samples were obtained by scraping and using traps (pooled in these plots).

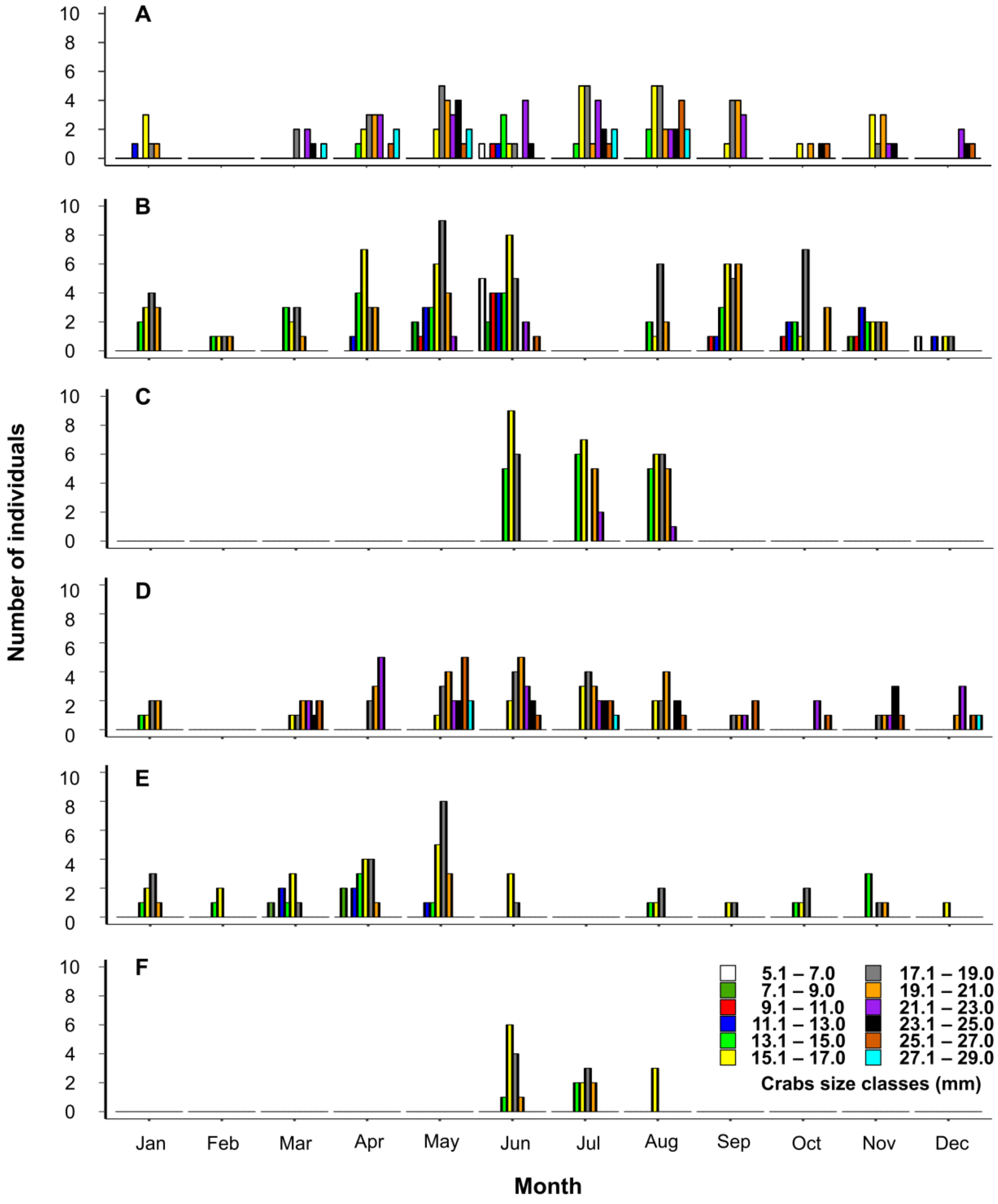

Figure S5. Size class (mm) distribution of males (**A** and **D**), females (**B** and **E**) and ovigerous females (**C** and **F**) of *Hemigrapsus takanoi* collected by scraping (**A**-**C**) and using traps (**D**-**F**) in the Kiel Fjord during 2017.

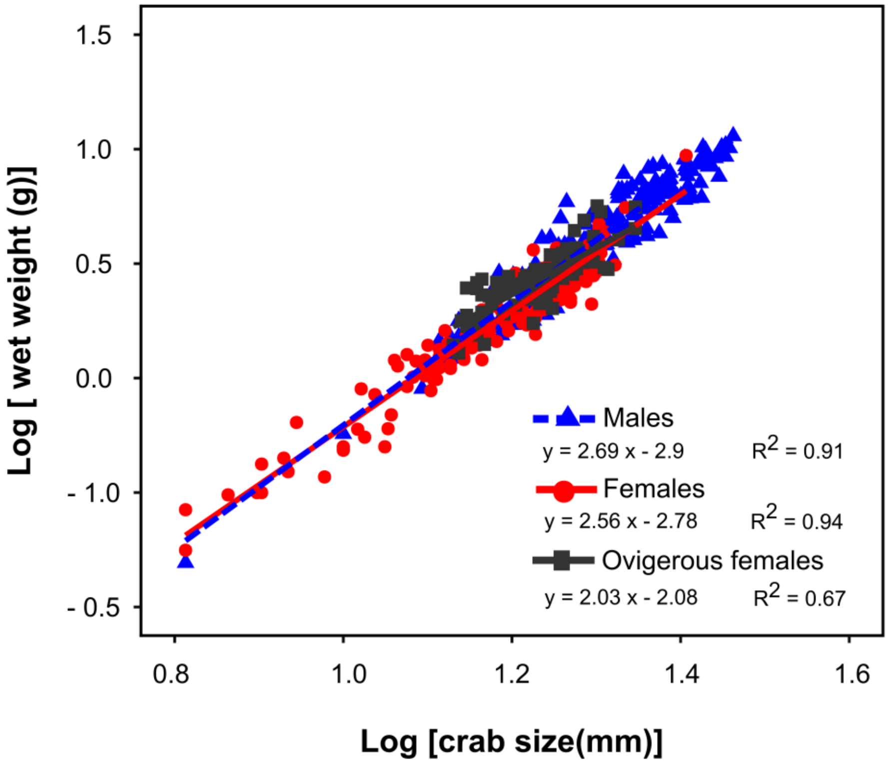

Figure S6. Relationship between size (carapace width) and wet weight of males (blue), females (red) and ovigerous females (black) of *Hemigrapsus takanoi* caught in the Kiel Fjord during 2017. The slopes of all adjusted regressions were significantly different from zero. See statistical details in Table S1.

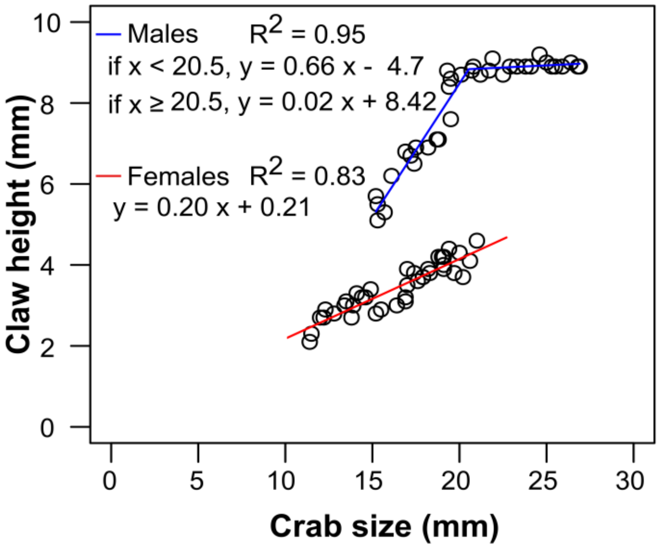

Figure S7. Relationship between size and claw height of males (blue) and females (red) of *Hemigrapsus takanoi* caught in the Kiel Fjord during 2017. Notice the segmented regression applied in the case of males. See statistical details in Table S2.

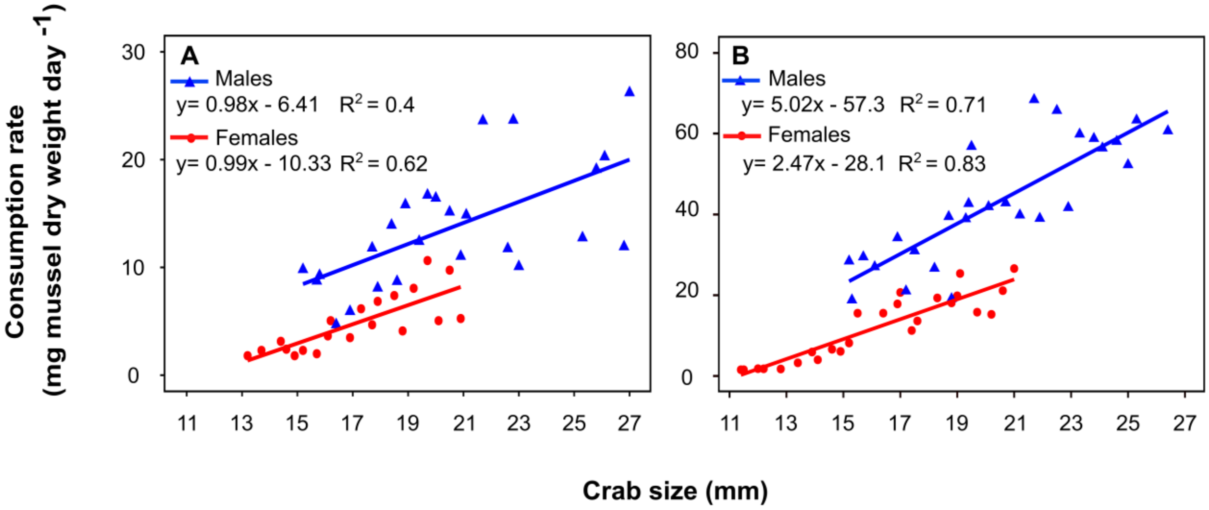

Figure S8. Dry weight of mussels consumed per day by males (blue triangles) and females (red circles) of *Hemigrapsus takanoi* in spring (**A**) and autumn (**B**). The slopes of all adjusted regressions were significantly different from zero. See statistical details in Table S3.

Supplementary tables

Table S1. Summary of the ANCOVA table showing the main and interactive effects of sex and carapace width on the body weight of males, females and ovigerous females of *Hemigrapsus takanoi*. Significant results ($p \leq 0.05$) are highlighted in bold.

Source of variation	SS	df	MS	F-value	p-value
Sex	12.725	2	6.362	1149.337	**<0.001**
Log carapace width	32.279	1	32.279	5831.132	**<0.001**
Sex : Log carapace width	0.095	2	0.048	8.617	**<0.001**
Error	2.995	541	0.006		

Table S2. Summary of ANCOVA table showing the main and interactive effects of sex and carapace width (< 20.5) on the claw height of males and females of *Hemigrapsus takanoi*. Significant results ($p \leq 0.05$) are highlighted in bold.

Source of variation	SS	df	MS	F-value	p-value
Sex	145.229	1	145.229	1451.549	**<0.001**
Carapace width	21.212	1	21.212	212.016	**<0.001**
Sex : carapace width	8.34	1	8.340	83.353	**<0.001**
Error	4.902	49	0.100		

Table S3. Generalized linear models (GLMs) testing the main and interactive effects of sex and crab size on the dry biomass of *Mytilus edulis* daily consumed by males and females of *Hemigrapsus takanoi* in spring and autumn. Significant results ($p \leq 0.05$) are highlighted in bold.

Dependent variable	Source of variation	SS	df	F-value	p-value
Mussels consumed in spring	Sex	4.275	1	47.080	**<0.001**
	Crab size	5.773	1	63.575	**<0.001**
	Sex: Crab size	0.089	1	0.984	0.327
Mussels consumed in autumn	Sex	6.4905	1	79.078	**<0.001**
	Crab size	19.069	1	232.339	**<0.001**
	Sex: Crab size	1.140	1	13.895	**<0.001**

Chapter II

To invade or not to invade: Impaired larval development at low salinities could limit the spread of the non-native crab *Hemigrapsus takanoi* in the Baltic Sea

Ola Mohamed Nour [1, 2*], Christian Pansch [1, 3], Mark Lenz [1], Martin Wahl[1], Catriona Clemmesen[1], Meike Stumpp [4]

[1] GEOMAR Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

[2] Department of Biology and Geology, Faculty of Education, Alexandria University, 21526 Alexandria, Egypt

[3] Environmental and Marine Biology, Åbo Akademi University, Artillerigatan 6, 20520 Åbo, Finland

[4] Zoological Institute, Christian-Albrechts University, 24118 Kiel, Germany

*** Corresponding author:** e-mail: olasm.nour@gmail.com; GEOMAR, Düsternbrooker Weg 20, 24105 Kiel, Germany

Key words: Salinity, larval development, non-native species, Kiel Fjord, Asian brush-clawed shore crab, invasion potential

Abstract

The Asian shore crab *Hemigrapsus takanoi* was recently recorded from the Kiel Fjord (western Baltic Sea). In laboratory experiments, we assessed the salinity tolerance of *H. takanoi*, to deflect its future potential to invade the brackish Baltic Sea, across eight salinity levels (0 to 35) and across three life-history stages (larvae, juveniles and adults). Larval development and growth at the different salinities were monitored from hatching to the megalopa stage, while survival and feeding of juveniles and adults were assessed over 17 days. Larvae of *H. takanoi* were capable to complete their development to the megalopa stage at salinities ≥ 20 and the time needed from hatching to reaching the megalopa stage was not different between these salinities. At a salinity of 15, larvae still reached the last zoea stage (Zoea V), but further development to megalopa was not completed. Juveniles and adults showed 100% survival at all salinities higher than freshwater conditions. Feeding rates in juveniles and adults increased with increasing salinity along the entire salinity range, while for adults feeding was highest at a salinity of 25. Our results indicate that both, juveniles and adults of *H. takanoi*, are euryhaline and can tolerate a wide range of salinity conditions, at least for the time period tested (2 weeks). However, larval development was impaired at salinities lower than 20, what will likely prevent the spread of *H. takanoi* into the Baltic Proper.

Introduction

Introduction of non-native species is amongst the most serious threats to the conservation of biodiversity and to ecosystem integrity worldwide (Molnar et al. 2008; Occhipinti-Ambrogi and Galil 2010). Many factors currently facilitate the expansion of species beyond their natural habitats. Among them, the most important are the increasing globalization of international trade, tourism, travel as well as population growth (Lenda et al. 2014; Canning-Clode 2016).

Non-native species are considered to be invasive when they proliferate, establish new populations, reproduce successfully and affect their new environment (Stephenson et al. 2009). In order to establish a self-sustaining population in a new area, a species must pass through a series of successive steps (Lockwood and Somero 2011): (1) an establishment phase that is characterized by low abundances of the introduced species, (2) an expansion phase, which makes the introduced species a dominant component with strong effects on the invaded system, and (3) an adjustment phase, which starts when the introduced species shows behavioural and evolutional adaptation to the abiotic and biotic conditions of its new habitat (identified and described by Reise et al. 2006). If the population of the new invader remains unaffected by enemies or diseases, the adjustment phase turns into a (4) permanent situation.

European waters are severely affected by introduced species (Galil et al. 2014), and the Baltic Sea as one of the largest brackish water bodies in the world has to date received 152 non-native and cryptogenic species (Casties et al. 2016; supplementary in Tsiamis et al. 2019). The Baltic is characterized by a strong salinity gradient ranging from 24 down to 2 (Leppäkoski et al. 2002), which spans from the southwest to the northeast. Furthermore, it is amongst the fastest warming seas worldwide (IPCC 2007, Reusch et al. 2018). This coincides with a reduction in mean sea surface salinity (IPCC, 2007, 2014; Gräwe et al. 2013). This generally strong environmental fluctuations of the Baltic Sea system, which is caused by natural conditions such as the strong salinity-temperature gradient, and by diverse human impacts from overfishing and eutrophication, makes the Baltic Sea extremely susceptible to invasions by new (Paavola et al. 2005), and in particular by euryhaline and eurythermal species (Lehtiniemi et al. 2011). Here, accelerated warming is expected to further increase future invasions, as most invaders originate from warmer latitudes (Paavola et al. 2005).

Many known invasive species often show physiological tolerance towards different environmental stressors, such as warming, de-salination, reduced light availability, hypoxia, and pollution, which is assumed to be an important prerequisite for the invasion success (Piola and Johnston 2006; Lenz et al. 2011; Nagar and Senkar 2016; Paiva et al. 2018). Salinity is one of the most important environmental factors that control survival, reproduction and growth in aquatic organisms as well as their geographical distribution, particularly so in marginal seas (Kinne 1971; Anger 2003; Thomsen et al. 2018). Salinity is also the main factor that determines a species' invasion success in Baltic Sea habitats (Lehtiniemi et al. 2011), and tolerance to low salinity can therefore be used as a tool for assessing the potential invasion threat of a species into the Baltic Sea area, and into other comparable habitats (Hudson et al. 2018; Reusch et al. 2018).

Some species possess a very broad salinity tolerance mediated by physiological acclimation mechanisms (Bulger et al. 1993; Charmantier et al. 2002; Wolf et al. 2009), while others developed a narrow tolerance window, and are therefore not able to thrive in brackish water habitats (Whitfield et al. 2012). In addition to this strong species-specific tolerance (Kumlu and Jones 1995; Kumlu et al. 2001), tolerance to low salinities can differ among ontogenetic stages of the same species (Kinne 1971; Charmantier 1998; Anger et al. 2008). Embryos and larvae of marine decapod crustaceans, for example, are often more sensitive, because they are less tolerant to osmotic pressure, when compared to juveniles and adults (Bas and Spivak 2000). Therefore, adaptations to extreme salinity regimes at all life-history stages are necessary for the successful establishment of a species in a brackish environment (Charmantier 1998).

A crustacean that has recently been introduced to the brackish Baltic Sea is the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Asakura and Watanabae 2005). This species, which is native to the north-west Pacific Ocean, inhabits muddy and rocky shores as well as sheltered harbours and estuaries (Gollasch 1999), but can also be found on soft sediments in sub-tidal regions (Asakura and Watanabae 2005). *H. takanoi* is an euryhaline species, tolerating a broad salinity range from 7 – 35 (Mingkid et al. 2006a; Soors et al. 2010). The first record of *H. takanoi* in Europe was in 1993 in the German North Sea, based on several individuals found on ship hulls at Bremerhaven harbour (Gollasch 1999).The species subsequently extended its range and reached the coast of France, Spain and Belgium (Noël et al. 1997; Kerckhof et al. 2007), as well as the Dutch and German Wadden Sea (Obert et al. 2007; Gittenberger et al. 2010). Today it is also known from the western part of the English channel (Dauvin and Delhay 2010) and from Great Britain (Wood et al. 2015; Ashelby et al. 2017). In the south-western Baltic Sea (Kiel Fjord), *H. takanoi* was first recorded in summer 2014 (Geburzi et al. 2015).

In the western Baltic Sea, *H. takanoi* might alter the existing ecosystem (Geburzi et al. 2015), by competing with the two main benthic invertebrate predators, the starfish *Asterias rubens* and the shore crab *Carcinus maenas,* for the same food source, i.e. the blue mussel *Mytilus edulis*

(Nauen 1978; Reusch and Chapman 1997). However, in order to predict whether *H. takanoi* can establish a stable population in the western Baltic Sea, as well as whether it can spread further into the Baltic Proper, precise knowledge of its salinity tolerance across the full reproductive cycle is essential. Although only limited information is available for northern Europe (but see van den Brink et al. 2012), juveniles and adults of *H. takanoi*, in their native range, have been observed in waters with salinities as low as 7 to 9 (Mingkid et al. 2006a; Gittenberger et al. 2010). This is surprising, since laboratory experiments only showed successful development from megalopa into a juvenile at salinities ≥ 25 in individuals from a *H. takanoi* population from Tokyo Bay (Mingkid et al. 2006b).

The present study determined the influence of a wide range of salinities on survival, development and feeding on different life-history stages of *H. takanoi*. We monitored the development of larvae to the megalopa stage and investigated feeding rates of juveniles and adults. Individuals from a population that was recorded only recently (~4 years ago) in Kiel Fjord (salinities around 15) were exposed to eight levels of salinity, from freshwater (0) to fully marine conditions (35) to elucidate the species' potential to establish a stable population in this region, and to further invade the Baltic Sea Proper. In order to evaluate the relevance of the salinity treatments, a 13-years salinity dataset from Kiel Fjord was used to analyse salinity ranges and variability in this region.

Materials and Methods

Survival and development of larvae

Ovigerous females of *Hemigrapsus takanoi* were collected by scraping mussel aggregates from pilings and from the bottom of the inner Kiel Fjord (54°32.9′N; 10°14.8′E) in mid-July to early August 2017. In order to identify females that were close to egg hatching, a small patch of eggs (20 – 30 eggs) was isolated carefully from each female individual using a fine sterilized tweezer. The patches were then investigated under a dissecting stereomicroscope (Olympus SZ51). Four females with carapace widths of 17.3 ± 0.8 mm (mean ± SD), which had near-to-hatch embryos with almost 10% yolk, prominent eye spots, and a fully developed zoeae near to hatch (van den Brink et al. 2013), were used to study larval development under different salinity regimes. Each female was reared in an individual 2 L plastic aquarium filled with aerated seawater that had the same salinity as the Kiel Fjord by the time of collection (15 – 16.7), at a temperature of 20 ± 0.5°C, and a photoperiod of 12h:12h (light: dark). Water was exchanged daily and females were offered crushed mussels *ad libitum* twice per week until larval hatching. The duration from capturing the female crabs to the hatching of the embryos never exceeded two weeks.

Eight salinity levels (0, 5, 10, 15, 20, 25, 30, and 35) were adjusted using filtered (0.2 µm Millipore) and aerated North Sea water (salinity ~33). Salinities between 5 and 30 were obtained by diluting the seawater with appropriate amounts of de-ionized tap water (Epifanio et al. 1998; Bas and Spivak 2000), while for the highest salinity (35) artificial sea salt (SeequaSal, Münster, Germany) was added. Although diluting seawater may lead to a reduction in nutrients concentration mainly at low salinity values, this is a common method used in salinity assays on Brachyura (e.g. Charmantier et al. 2002; Anger et al. 2008) and should not have an effect on the crabs. For fresh water (salinity = 0), we also used de-ionized tap water. For salinities from 5 to 30, the seawater was slightly more acidic due to the decreased bicarbonate levels of the added de-ionized water. We adjusted pH_{NBS} to a value of ~7.9, resembling Kiel Fjords' pH_{NBS} during the time of crab collection (see also Melzner et al. 2013, Thomsen et al. 2015 for Kiel Fjord carbonate chemistry), using 1M sodium bicarbonate solution (Ravi and Manisseri 2010; Thomsen et al. 2018). pH_{NBS} values were measured with a WTW 3310 pH-meter equipped with a SenTix 81 pH electrode. Salinity was adjusted using a WTW Cond 3110 equipped with a Tetracon 325 electrode.

Immediately after hatching, actively swimming Zoea I larvae were transferred into individual 100 mL culture vials filled with 80 mL of seawater using wide-bore pipettes. At each salinity, ten 100 mL vials, each containing an initial number of 10 Zoea I larvae, equivalent to 125 larvae per L seawater were used. This setup was repeated for each of the four female crabs. In total, the experiment included 320 vials (8 salinities x 4 females x 10 vials). Larvae were cultured from Zoea I to the megalopa stage without aeration, but with open lids (Simith et al. 2012), at 20 ± 0.5 °C, pH 7.9 ± 0.1, and a photoperiod of 12h :12h (light : dark). The water in each of the vials was exchanged daily. On these occasions, larvae were checked for mortality as well as for moults and were provided freshly hatched (<100 µm) brine shrimp *Artemia spp.* nauplii (Great Salt Lake Brand), at a density of ~10 nauplii per mL seawater (Anger et al. 2008). In order to avoid undesired changes in the experimental salinities, *Artemia spp.* were rinsed with water of the same salinity level prior to adding them to the culture vials (Lárez et al. 2000; Simith et al. 2012). Moulting was checked by inspecting the vials for exuviae (for Zoeae II to V) and was confirmed by microscopy (for Zoeae IV and V) based on their morphology (Online Resource Fig. S1 a–b; after Landeira et al. 2019). Megalopae could be observed without any microscopic inspection due to their large size and distinct morphology (Online Resource Fig. S1c). The experiment was conducted until the last zoea larva at each salinity level had either died or moulted into the megalopa. Overall survival was determined by counting all megalopae in each vial. The time (in days) that was needed by most of the larvae for the development from the Zoea I to the megalopa stage at each salinity level was used as an indicator for zoeae development.

Survival and feeding rates of juveniles and adults

Juveniles (5.8 – 6.9 mm carapace width) and female adults (16.7 – 23.2 mm carapace width) of *H. takanoi* were collected in November 2018 in the same way as described above, and were kept under Kiel Fjord seawater conditions present at the time of collection, in the laboratory at 12 ± 0.5°C, a salinity of 20 ± 0.5, and a photoperiod of 10 h :14 h (light : dark). Water was exchanged daily and crabs were fed with crushed mussels *ad libitum*. In addition to mussel food, frozen isopods *Gammarus spp.* and shrimps were added for the juveniles.

Salinity levels (0, 5, 10, 15, 20, 25, 30, and 35) were adjusted and kept well-aerated as described above. Two weeks after acclimation to laboratory conditions a total of 40 juveniles (n = 5 crabs per salinity level) and 24 adults (n = 3 crabs per salinity level) with intact appendages were selected for the experiment. Juveniles and adults had carapace widths of 6.45 ± 0.32 (mean ± SD) and 19.98 ± 1.65 mm, respectively. Each juvenile was placed separately in a 1 L Kautex bottle (filled with 0.5 L of water), while each adult was placed separately in a 2 L plastic aquarium (filled with 1 L of water). Seawater conditions in the experimental units were then adjusted from 20 ± 0.5 (acclimation conditions) to the respective target salinities in a stepwise manner at 1.25 salinity units per hour. After the target salinities were reached, the experiments were started and lasted for 17 days.

In a pilot study, we observed that juvenile crabs cannot open living mussels by themselves. However, to ensure a comparable experimental set-up with the same food source as used in the experiments with adult individuals (see below), three opened mussels (*Mytilus edulis*; 11.0 – 11.9 mm) were offered to each individual juvenile crab every other day. Before feeding, all faecal particles were manually removed using disposable transfer pipettes, while the seawater including the remaining mussel shells and soft tissues was filtered through pre-weighted tea filters. Following, the wet filters, including the mussel shells and soft tissues, were washed quickly with distilled water to eliminate adhesive salt, and placed on pre-weighted and numbered aluminium plates. The filters were then dried in a furnace at 60 °C for 48 h and dry weight was quantified to the nearest 0.001 g. We assessed the food consumption rates of the crabs by measuring the inverse of the food uptake (Urbina et al. 2010), i.e. the mass of the mussel shells and soft tissues that was left after 48 h. This method assumes that the average wet weights of the mussels, which were offered to the crabs, were the same across individuals, as only individuals of a similar size were offered.

Adults of *H. takanoi* were offered 20 closed mussels ranging from 6 to 9 mm. Every other day, all mussels were collected, all opened mussels were counted, and 20 new mussels were added (see details in Nour et al. 2020). Once a mussel was opened by adults of *H. takanoi,* this was consumed immediately (personal observations), assuming that all soft tissue was ingested by the individual crab.

During the 17-days of the experiment, water was exchanged every other day, while survival was monitored daily. Lack of movement even after stimulation with a glass rod, absence of beating activity of scaphognathites (i.e. lack of gill chamber ventilations), and abdomen opening and relaxation (Novo et al. 2005) were considered as indicators of the death of an individual. Feeding was assessed every other day, while feeding rates were calculated as the mean food dry weight (g) that was left after the trial for the juveniles, and as the mean number of mussels opened and consumed over the entire experimental period for the adults.

Kiel Fjord salinity measurements

Salinity in Kiel Fjord was recorded on mostly weekly cruises of the research vessel FK Polarfuchs from 2005 to 2018 using a CTD48M probe (Sea and Sun technologies, Trappenkamp, Germany) at a station in front of the GEOMAR Pier (“Wittlingskuhle”, Position: 54°19’69 N, 10°09’06 E).

Statistical analysis

All analyses and statistical procedures were performed using the statistical software R (version 3.6.0, Development Core Team 2016). To test whether survival rates differed between salinities, the Log-Rank test, in case of proportional hazards, or the Peto-Wilcoxon test for non-proportional hazards were used. Survival rates were visualized by Kaplan-Meier curves. Pair-wise comparisons of survival rates were done for relevant survival and salinity combinations and the p-values were adjusted for multiple testing using the Benjamini-Hochberg (BH) correction. Analyses were performed using the "survival" and “survminer” packages in R (Therneau 2015, Kassambara et al. 2019).

Furthermore, we used a linear model (LM) by fitting a 2nd degree polynomial to test whether salinity significantly affected the number of larvae reaching megalopae, time to moult, and feeding rates of both, juveniles and adults. Since larvae reached the megalopa stage only in salinities from 20 to 35, we included these four salinities and the level 15, as the highest salinity under which the megalopa stage was not reached, in the analyses. Since both, juveniles and adults, died at a salinity of 0, we obtained no feeding rates for this level. We checked whether the assumptions of the LMs were met by the inspection of residual plots. In addition, the salinities at which the response variables were at maximum (i.e. larval survival, feeding rates of juveniles and adults) or minimum (i.e. time needed to reach megalopa), were identified with the quadratic polynomial curves for all cases in which salinity significantly affected the examined response variables. Furthermore, we verified whether the optimum point (maximum or minimum point) was in the range of salinity levels (0–35) used in this study by using the following equation (Koram 2019):

General quadratic equation:

$$y = ax^2 + bx + c \text{ (Equation 1)}$$

Where a and b are the slopes of x^2 and x; respectively, and c is the intercept of the regression line.

$$\text{x-coordinate of the vertex (optimum point)} = -b/2a \text{ (Equation 2)}$$

Results

Survival of larvae, juveniles and adults

Larvae of *Hemigrapsus takanoi* completed development until the megalopa stage only when reared in salinities ≥ 20 (Fig. 1a). Survival rates were significantly different between salinities (Peto-Wilcoxon test: $\chi^2 = 540$, df = 7, $p \leq 0.001$; Fig. 1a), the median time to death was 1, 7, 19, and 33.5 days for the salinities 0, 5, 10, and 15, respectively. In the salinity range 0 – 15, survival steadily declined with decreasing salinity (15 > 10 > 5 > 0) and was clearly different from survival in the salinity range between 20 and 35.

Juveniles and adults of *H. takanoi* survived under all salinities for a minimum of 17 days. The only exception was the fresh water condition with a salinity of 0, under which no individual survived and the median time to death was 2 days for juveniles (Log-Rank test: $\chi^2 = 39$, df = 7, $p \leq 0.001$; Fig. 1b) and 4 days for adults (Log-Rank test: $\chi^2 = 28$, df = 7, $p \leq 0.001$; Fig. 1c).

Development of larvae to the megalopa stage

Although survival rates did not differ between the experimental groups that covered the salinity range between 20 and 35 (Pair-wise Peto and Peto tests; Online Resource Table S1), the percentage of larvae that reached the megalopa stage within 50 days after hatching significantly changed with salinity (2nd degree polynomial regression: F (2,197) = 124, $p \leq 0.001$, $R^2 = 0.55$). The highest proportions of larvae, which successfully developed into the megalopa, were observed at salinities of 25 and 30 (median survival of 50% and 40%; respectively; Figs. 2a and 3), while development success was lower (median survival of 20%) at salinities of 20 and 35 (Figs. 2a and 3). The maximum time larvae of *H. takanoi* needed from hatching to reaching the megalopa stage was 34 days (median time to megalopa, at salinities 20 and 25), 38 days (salinity 30), and 33.5 days (salinity 35). However, different from the percentage of larvae reaching the megalopa stage, these time spans did not change significantly with salinity (2nd degree polynomial regression: F (2,149) = 2, p = 0.13, $R^2 = 0.03$; Figs. 2b and 3).

In general, the highest percentages of survivors across the different zoea stages were recorded at the salinities 25 and 30; respectively, followed by 20 and 35 (Fig. 3 and Online Resource Fig. S2a–e; Online Resource Table S2). In this regard, the optimum salinity was ~26.5. At salinity 0, the Zoea I suffered from abrupt mortality during the first 24 hours and no larva moulted to the next stage (Fig. 3 and Online Resource Fig. S2a). At salinity 5, only 4.7 ± 3% (mean ± SD) of larvae succeeded to moult to Zoea II, but none of them reached the Zoea III stage (Fig. 3 and Online Resource Fig. S2b–c). Zoea III was the highest zoea stage recorded at a salinity of 10, while Zoea V was the highest zoea stage observed at a salinity of 15 (Fig. 3 and Online Resource Fig. S2b–d).

Feeding rates of juveniles and adults

There were significant effects of salinity on the feeding rates in juveniles and adults of *H. takanoi*. Feeding rates in juveniles increased significantly with increasing salinity (as seen in the inverse-feeding response curve; 2nd degree polynomial regression: F (2, 32) = 29, $p \leq 0.001$,

R^2 = 0.64; Fig. 4a). In adults, the optimum salinity for feeding was 25.5, while food intake generally increased with increasing salinities and reached a maximum at salinities between 15 and 35 (2nd degree polynomial regression: F (2, 18) = 27, $p \leq 0.001$, $R^2 = 0.75$; Fig. 4b).

Prevailing salinity regimes in Kiel Fjord

Median (mean) salinity in Kiel Fjord over the entire dataset was 16.8 (17.0), ranging between 8.4 (min) and 24.7 (max; Fig. 5). Median (mean) salinity was 15.7 (15.9) in surface waters down to 1 m, 16.5 (16.9) in 7 m and 18.1 (18.4) in 18 m water depths, respectively. Among seasons, salinity varied and was lowest in spring and summer when larvae of *H. takanoi* occur in Kiel Fjord, while it was highest in autumn and winter when juveniles are found (Fig. 5d).

Discussion

Our experiments showed that salinity differently affects the different ontogenetic stages of *Hemigrapsus takanoi,* a species that recently arrived in the south-western Baltic Sea. Although, larvae of this crab species were more sensitive to low-salinity stress than adults, they successfully developed to the megalopa stage as long as salinities were equal to or above 20. Neither the duration of development to megalopae, nor the probability to reach this stage differed between these higher (≥20) salinities. Yet, survival and success in development were highest at a salinity of 25. In contrasts to larvae, juveniles and adults were more robust to reduced salinity levels and showed 100% survival in response to salinities between 5 and 35. Feeding rates, however, also differed between salinity levels. Generally, feeding increased with increasing salinity. In adults, however, feeding rates remained constant at salinities ≥15.

Generally, increasing salinity favoured larval development of *H. takanoi* from Kiel Fjord. All larvae died, however, before moulting to the megalopa stage when they were reared at salinities below 20. Controversially, Mingkid et al. (2006b), who tested a salinity gradient from 5 to 35 (5 salinity unit steps) in Tokyo Bay showed that 1.4 and 30% of *H. takanoi* larvae successfully moulted into megalopae at salinities as low as 10 and 15, respectively.

The discrepancy in the lower salinity thresholds between the present study (≥20) and Mingkid et al. (2006b) (≥15) indicates that *H. takanoi* larvae have a narrower salinity range in their new habitat compared to their native range. Supporting this, Kiel Fjord larvae survived and developed best at salinities between 25 and 30. In contrast, Mingkid et al. (2006b) demonstrated best development to megalopae at a salinity of 20. Seemingly, Kiel Fjord *H. takanoi* larvae prefer higher salinities than larvae tested from a native-range population (Mingkind et al. 2006a). A similar pattern was observed for gammarid crustaceans (Pauli and Briski 2018). Between-population differences in salinity tolerance were also shown for different crustacean taxa (Ojaveer et al. 2007). *Carcinus maenas* for example, from the Baltic Sea, has a higher capacity to hyper-osmoregulate than individuals from a North Sea population (Theede 1969). This potential shift in the salinity preference might be a result of local adaptation of a population to different local environmental conditions as a study on 22 populations of eight gammarid species from different regions suggests, or may have occurred during the invasion process of a species (Paiva et al. 2018).

Within Kiel Fjord, *H. takanoi* must tolerate salinities between 8 and 25, while salinities range between 7 and 35 in the native range of *H. takanoi*, e.g. the Tokyo Bay (Mingkid et al. 2006a). Salinity tolerance thresholds and performance maxima of 20 and 25, respectively, indicate the invasion process rather than adaptation to local environmental conditions to be the driving force of selection in the Kiel Fjord *H. takanoi* population. As Baltic Sea *H. takanoi* populations are genetically very similar to those from the North Sea (J. Geburzi pers. comm.), one possible scenario for the arrival of *H. takanoi* to the Baltic Sea could be the transportation of larvae in the ballast water of ships that came from North Sea or European Atlantic ports (Geburzi et al. 2015) or by advection from the Kattegat due to strong westerly winds pushing high saline water into the Baltic Sea (Lehmann and Javidpour 2010). North Sea populations could have adapted to the fully marine conditions for >25 years, while the Kiel Fjord population potentially only had ~5 years to adapt to local environmental conditions. However, detailed studies on the osmoregulation capacity of *H. takanoi* larvae from populations along the invasion gradient would be needed to confirm this concept.

We found larval duration to be only marginally impacted by osmotic stress at salinities of 20 and higher. This has also been found in other crab species such as the mangrove crab *Ucides cordatus* from the Caeté Estuary northern Brazil (Diele and Simith 2006) and the mud crab *Scylla olivacea* from estuaries and creeks in Thailand (Jantrarotai et al. 2002). Kiel Fjord *H. takanoi* larvae in the present experiment developed generally slower (larval duration of 35 – 37 days) than individuals of the same species from its native range (~16 – 21 days; Mingkid et al. 2006b). Diele and Simith (2006) suggest that variations in the time needed for development in *U cordatus* could be due to diet. In our study, we fed larvae with *Artemia sp.* nauplii only, while, Mingkid et al. (2006b) provided rotifers as a food until the Zoea III stage was reached, after which a mixture of *Artemia sp.* nauplii and rotifers were offered. However, the differences between the two studies can potentially also be explained by differences in the temperature that was applied during incubation of the larvae, i.e. 20°C herein and 24°C in Mingkid et al. (2006b) (see also Anger 2001; Baylon and Susuki 2007 for temperature effects on crustacean larval development).

In contrast to larvae, juveniles and adults of Kiel Fjord *H. takanoi* tolerated a wide range of salinities from 5 to 35, at least for the two weeks these individuals were exposed to the stress gradient. In contrast to this, freshwater conditions led to 100% mortality in juveniles and adults within two and four days, respectively. Shinji et al. (2009) exposed adult *H. takanoi* individuals from the native range (Tokyo Bay) to salinities from 0 to 40 for 8 hours to study their immediate physiological responses. Although no mortality was observed over the short incubation period at any of the salinities, oxygen consumption and ammonia excretion levels were remarkably increased at a salinity of 0, while no significant differences were detected among all other salinity levels (Shinji et al. 2009). Data from the congeneric *H. sanguineus* showed a higher survival probability at a salinity of 5 compared to a salinity of 1, mainly explained by the ability to maintain internal haemolymph osmolality under osmotic stress at a salinity of 5 for several days (Hudson et al. 2018). Since osmoregulation is an energy demanding process (Anger 1999), future research is required to study the consequences of osmotic stress on *H. takanoi* in the long-term.

Feeding rates in both, juveniles and adults of *H. takanoi*, were strongly influenced by salinity, yet in different ways. In juveniles, feeding decreased linearly with decreasing salinity. These findings are in line with observations of other juvenile crabs (*Portunus trituberculatus;* Shentu et al. 2015). Feeding rates in adults, however, were not impacted across the wide salinity range from 15 to 35 and showed a peak at a salinity of 25. This might be explained by optimal osmoregulation, as the iso-osmotic point of adult *H. takanoi* haemolymph was recorded at a salinity of ~24.5 (Shinji et al. 2009). Contrary to our results, Urbina et al. (2010) showed that feeding rates in adult *H. crenulatus* decreased with increasing salinities. They interpreted this as a compensatory mechanism to face the increased energy costs from excretion and respiration during the osmoregulation activity. In general, a decrease in feeding rates as a reaction to environmental stress has been observed in different marine invertebrate taxa, such as cnidarians (Houlbrèque et al 2015; Podbielski et al. 2016), crustaceans (Curtis 2009; Appelhans et al. 2012), molluscans (Sutton et al. 2012; Zhang et al. 2016), and echinoderms (Stumpp et al. 2012; Hu et al. 2018).

While adults of *H. takanoi* tolerated the applied salinity range of 5 to 35, our results suggest a clear bottleneck in larval development. The critical threshold for *H. takanoi* larvae to complete their full life cycle at 20°C (common summer Kiel Fjord temperatures) was found to be between 15 and 20. In the western Baltic Sea Kiel Fjord salinities range between 8 and 25 (Fig. 5). The salinity regimes recorded in different water depths between 2005 and 2018 would therefore allow juveniles and adults of *H. takanoi* to strive. In a monitoring effort in 2017, ovigerous females appeared in late June, in salinities higher than 15, and larval development probably continued into September and October, when salinities were still above 17 (Nour et al. 2020). In line with this, juveniles massively occurred in November 2018, when salinities were around 19 (O. Nour, pers. observ.). During the reproductive season, when hatching and development of larvae takes place (June to October), the median salinity (surface to a water depths of 7 m) in Kiel Fjord between 2005 and 2018 ranged from 8.4 to 22 (Fig.5). This indicates that Kiel Fjord salinity conditions can allow successful larval development, at least during years with higher salinities. However, to indicate years in which successful development can occur more precisely, experiments investigating the salinity range between 15 and 20 are necessary. Salinity threshold on larval development has been observed in other species of the Kiel Fjord (*Eriocheir sinensis*: Anger 1991; Ottos and Brandis 2011; *Carcinus maenas*: Cieluch et al. 2004; *Asterias rubens*: Casteis et al. 2015).

H. takanoi larvae are planktonic organisms and likely move towards the water surface immediately after hatching (Landeira et al. 2019). In the western Baltic Sea, the surface seawater is generally less saline than the water in deeper layers (Fig. 5), therefore, in Kiel Fjord, *H. takanoi* larvae are facing a dilemma: On the one hand, surface waters show a higher phytoplankton abundance and consequently also a higher abundance of small zooplankton organisms (Anger 2003), which could serve as a food source for the larvae. On the other hand, the salinity regimes in surface waters potentially restrict successful development. However, it remains to be investigated, if *H. takanoi* larvae can adjust their behaviour to match their salinity requirements by actively migrating through the water column.

The findings of the present study suggest that *H. takanoi* populations are limited in distribution to the western Baltic Sea, mainly due to their larval salinity sensitivity. Future monitoring of the spatial and temporal distribution of *H. takanoi* larvae along the salinity gradient from the western Baltic Sea into the Baltic Proper will reveal whether this assumption proofs to be correct. Whether the prolongation of larval development observed in this study is an advantage for *H. takanoi* dispersal and population establishment in the further Baltic Sea is unclear.

Extension of larval developmental duration can have important consequences on recruitment success (Sukin and Mckeen 1994), by increasing natural mortality risk due to such factors as predation (Morgan 1995). Neverthless, a longer developmental period increases the probability for the larvae to spread and reach appropriate substrates prior to metamorphosis (Jackson and Strathmann 1981), which could allow the species to colonize other new areas and even enhance gene flow among local populations (Díaz and Bevilacqua 1986). Since juvenile and adult stages of *H. takanoi* have the potential to invade the central Baltic Proper with bottom salinities of 8 to 6 (HELCOM 2018), the whole reproductive cycle of *H. takanoi* may take place in this lower salinity region, if the species manages to locally adapt to this condition. However, physiological responses of this species towards salinity stress still need to be investigated in studies that cover a longer part of its lifetime.

Conclusions

Previous studies showed that non-native species are generally tolerant to a wide range of salinities, and that they tend to be more resistant to stressful conditions than native species (Lenz et al. 2011; Lejeusne et al. 2014; Kumar et al. 2018). Herein, we show a strong influence of salinity on the earliest stages of the recent invader *H. takanoi*. The development *of H. takanoi* larvae was only completed in salinities between 20 and 35, which contrasts with the salinity tolerance of larvae from the native range of this species (Mingkid et al. 2006b). Reasons for this discrepancy are unspecified, but could potentially relate to adaptation to full marine conditions during the invasion process, when harbours in fully marine environments served as steppingstones for the dispersal of this species. Juveniles and adults can tolerate salinities as low as 5, but their feeding rates mostly declined at low salinities with possible long-term implications for their fitness. Although field observations showed that the Western Baltic Sea is a suitable habitat for *H. takanoi* (Nour et al. 2020), salinity likely represents a strong boundary towards a further invasion into the eastern and central Baltic Sea, in particular for the larval stages of *H. takanoi*. According to future climate scenarios, Meier et al. (2012), which predict warming and a reduction in salinity across the Baltic Sea by the end of the century, Kiel Fjord will most likely experience a decline in salinity by 2 units on average (Gräwe et al. 2013), which could lead to more frequent and longer phases throughout the year when salinities are outside the tolerance boundaries of larvae of this species. However, the question remains whether the species will be able to complete its development in the future, if it cannot adapt to the changes that have been predicted for this region.

Data availability

All data used in this study will be made publicly available on PANGAEA (https://www.pangaea.de) following acceptance as required by GEOMR Helmholtz Centre for Ocean Research Kiel regulations.

Ethical statement

This study was carried out and funded by GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, with no conflict of interest. All applicable international, national, and/or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed. Applying methods and protocols approved by the regulatory committee on the ethic of animal experiments, of Schleswig-Holstein Germany under the permit number: 1101, and in strict accordance to the relevant regulations and guidelines. All possible actions were taken to reduce animals suffering and stress during handling and sampling.

Acknowledgements

We thank Dr. Gabriela Torres for giving advice for larvae rearing techniques and Björn Buchholz for technical assistance. OMN acknowledges the financial support of the German Academic Exchange Service (DAAD) through the project German Egyptian Research Long-term Scholarship Programme (GERLS) 2015/16 (57147166).

References

Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). Mar Ecol Prog Ser 72:103–110

Anger K (2001) The biology of decapod crustacean larvae, crustacean issues. Lisse, The Netherlands: A A Balkema Publishers

Anger K (2003) Salinity as a key parameter in the larval biology of decapod crustaceans. Inver Reprod Dev 43(1):29–45

Anger K, Spivak E, Luppi T, Bas C, Ismael D (2008) Larval salinity tolerance of the South American salt-marsh crab, *Neohelice* (*Chasmagnathus*) *granulata*: physiological constraints to estuarine retention, export and reimmigration. Helgol Mar Res 62:93–102

Asakura A, Watanabae S (2005) *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). J Crustac Biol 25:279–292

Appelhans Y, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. Mar Ecol Prog Ser 459:85–97

Ashelby CW, Sewell J, Rostron J, Shrubsole R, Child T, Clark PF (2017) Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. Crustaceana 90:695–708

Bas CC, Spivak ED (2000) Effect of salinity on embryos of two southwestern Atlantic estuarine grapsid crab species in vitro. J Crustac Biol 20(4):647–656

Baylon J, Suzuki H (2007) Effects of changes in salinity and temperature on survival and development of larvae and juveniles of the crucifix crab *Charybdis feriatus* (Crustacea: Decapoda: Portunidae). Aquaculture 269:390–401

Bulger AJ, Hayden BP, Monaco MF, Neslon DM, McCormick-Ray MG (1993): Biologically-based estuarine salinity zones derived from multivariate analysis. Estuar Coast 16(2):311–322

Canning-Code J (2016) Biological invasions in changing ecosystems:vectors, ecological impacts, management and predictions. De Gruyter Open, pp474

Casties I, Clemmesen C, Melzner F, Thomsen J (2015) Salinity dependence of recruitment success of the sea star *Asterias rubens* in the brackish Western Baltic Sea. Helgol Mar Res 69 (2):169–175

Casties I, Seebens H, Briski E (2016) Importance of geographic origin for invasion success : A case study of the North and Baltic Seas versus the Great Lakes –St. Lawrence River region. Ecol Evol 6:8318–8329

Charmantier G (1998) Ontogeny of osmoregulation in crustaceans: a review. Invert Reprod Develop 33:177–190

Charmantier G, Giménez L, Charmantier-Daures M, Anger K (2002) Ontogeny of osmoregulation, physiological plasticity , and larval export strategy in grapsid crab *Chasmagnathus granulata* (Crustacea , Decaopda). Mar Ecol Prog Ser 229:185–194

Cieluch U, Anger K, Aujoulat F, Buchholz F, Charmantier-Daures M, Charmantier G (2004) Ontogeny of osmoregulatory structures and functions in the green crab *Carcinus maenas* (Crustacea, Decapoda). J Exp Biol 207:325–336

Curtis DL (2009) Integrated responses of crustaceans inhabiting estuaries to the challenges of feeding and digestion in low salinity. Dissertaion. University of Nevada Las Vegas

Dauvin JC, Delhay JB (2010) First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern cotentin, Normandy, western English channel. Mar Biodiver Rec 3: e101, doi:10.1017/S1755267210000928

Díaz H, Bevilacqua M (1986) Larval development of *Aratus pisonii* (Milne Edwards) (Brachyura, Grapsidae) from marine and estuarine environments reared under different salinity conditions. *J. coast. Res.* 2: 43–49

Diele K, Simith DJB (2006) Salinity tolerance of Northern Brazilian mangrove crab larvae, *Ucides cordatus* (Ocypodidae): necessity for larval export ? Estuar Coast Shelf Sci 68:600–608

Epifanio CE, Dittel AI, Park S, Schwalm S, Fouts A (1998) Early life history of *Hemigrapsus sanguineus* a non-indigenous crab in the Middle Atlantic Bight (USA). Mar Ecol Pro Ser 170:231–238

Galil BS, Marchini A, Occhipinti-Ambrogi A, Minchin D, Narščius A, Ojaveer H, Olenin S (2014) International arrivals: wide spread bioinvasions in European Seas. Ethol Ecol Evol. doi:10.1080/03949370.2014.897651

Geburzi JC, Graumann G, Köhnk S, Brandis D (2015) First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. BioInvasions Rec 4:103–107

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

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

Gräwe U, Friedland R, Burchard H (2013) The future of the western Baltic Sea: two possible scenarios. Ocean Dynam 63:901–921

HELCOM (2018) State of the Baltic Sea–Second HELCOM holistic assessment 2011–2016. Baltic Sea Environment Proceedings, p. 155

Houlbrèque F, Reynaud S, Godinot C, Oberhänsli, Rodolofo-Metalpa R, Ferrier-Pagès (2015) Ocean acidification reduces feeding rates in the scleractinian coral *Stylophora pistillata*. Limnol Oceanogr 60:89–99

Hu MY, Lein E, Belich M, Melzner F, Stumpp M (2018) Trans-life cycle acclimation to experimental ocean acidification affects gastric pH homeostasis and larval recruitment in the sea star *Asterias rubens.* Acta Physiol 223: e13169

Hudson DM, Sexton DJ, Wint D, Capizzano C (2018) Physiological and behavioral response of the Asian shore crab, *Hemigrapsus sanguineus*, to salinity : implications for estuarine distribution and invasion. PeerJ 6. doi:10.7717/e5446

IPCC (2007) Topic 1: Observed Changes and their Causes. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, Miller HL et al (Eds.), Climate change 2007. The physical science basis: Working group I contribution to the fourth assessment report of the IPCC. Cambridge, UK: Cambridge University Press

IPCC (2014) Mitigation pathways and measures in the context of sus- tainable development. In Core Writing Team, R. K. Pachauri & L. A. Meyer (Eds.), Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergov- ernmental panel on climate change. Geneva, Switzerland: IPCC

Jackson GA, Strathmann RR (1981) Larval mortality from offshore mixing as a link between precompetent and competent periods of development. *The American Naturalist* 118: 16–26

Jantrarotai P, Taweechuer K, Pripanapong S (2002) Salinity levels on survival rate and development of mud crab (*Scylla olivacea*) from zoea to megalopa and from megalopa to carb stage. Kasetsart J Nat Sci 36:278–284

Kassambara A, Marcin Kosinski M, and Przemyslaw Biecek P(2019) Survminer: Drawing survival curves using 'ggplot2'. R package version 0.4.6. http://www.sthda.com/english/rpkgs/survminer/

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

Kinne O (1971) Salinity. In Otto Kinne (ed) Marine ecology, Vol. 1, Part 2. J. Wiley and Sons, London, pp 821–995

Koram SS (2019) Key concept of quadratic functions and Inequalities-First Edition: Springer Series in Geomechanics and Geoengineering 13(1): 9–22

Kumar AB, Schofield PJ, Raj S, Satheesh S (2018) Salinity tolerance of non-native suckermouth armoured catfish (Loricariidae: *Pterygoplichthys Sp*.) from Kerala, India. Manag Biol Invasion 9(1):49–57

Kumlu M, Eroldogan OT, Saglamtimur B (2001) The effects of salinity and added substrates on growth and survival of *Metapenaeus monoceros* (Decapoda: Penaeidae) post-larvae. Aquaculture 196:177–188

Kumlu M, Jones DA (1995) Salinity tolerance of hatchery-reared postlarvae of *Penaeus indicus* H. Milne Edwards originating from India. Aquaculture 130:287–296

Landeira JS, Cuesta JA, Tanaka Y (2019) Larval development of the brush-clawed shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae). J Mar Biol Assoc UK 99(5):1153–1164

Lárez MB, Palazón-Fernández JL, Bolaños CJ (2000) The effect of salinity and temperature on the larval development of *Mithrax caribbaeus* Rathbun, 1920 (Brachyura: Majidae) reared in the laboratory. J Palnkton Resrach 22:1855–1869

Lehmann A, Javidpour J (2010) Potential pathways of invasion and dispersal of *Mnemiopsis leidyi* A. Agassiz 1865 in the Baltic Sea. Hydrobiologia 649:107–114

Lehtiniemi M, Lehmann A, Javidpour J, Myrberg K (2011) Spreading and physico-biological reproduction limitations of the invasive American comb jelly *Mnemiopsis leidyi* in the Baltic Sea. Biol Invasions 14:341–354

Lejeusne C, Latchere O, Petit N, Rico C, Green AJ (2014) Do invaders always perform better ? comparing the response of native and invasive shrimps to temperature and salinity gradients in South-West Spain. Estuar Coast Shelf Sci 136:102–111

Lenda M, Skórka P, Knops JMH, Moroń D, Sutherland WJ, Kuszewska K, Woyciechowski M (2014) Effect of the internet commerce on dispersal modes of invasive alien species. PLoS ONE 9: e97786

Lenz M, da Gama BA, Gerner NV, Gobin J, Frederike G, Harry A, Jenkins SR et al (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. Environ Res 111:943–952

Leppäkoski E, Gollasch S, Gruszka P, Ojaveer H, Olenin S, Panov V (2002) The Baltic - a sea of invaders. Can J Fish Aquat Sci 9:1175–1188

Lockwood, BL, Somero GN (2011) Invasive and native blue mussels (genus *Mytilus*) on the California coast: The role of physiology in a biological invasion. J Exp Mar Biol Ecol 400 (1–2):167 –174

Meier HEM, Hordoir R, Andersson HC, Dieterich C, Eilola K, Gustafsson BG, Höglund A, Schimanke S (2012) Modeling the combined impact of changing climate and changing nutrient loads on the Baltic Sea environment in an ensemble of transient simulations for 1961 – 2099. Clim Dyn 39:2421–2441

Melzner F, Thomsen J, Koeve W, Oschlies A, Gutowska MA, Bange HW, Hansen HP, Körtzinger A (2013) Future ocean acidifcation will be amplified by hypoxia in coastal habitats. Mar Biol 60(8):1875–1888

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

Mingkid WM, Masashi Yokota M, Watanabe S (2006b) Salinity tolerance of larvae in the penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). La mer 44:17–21

Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Front Ecol Environ 6:485–492

Morgan SG (1995) Life and death in the plankton: larval mortality and adaptation. In McEdward L. (ed.) Ecology of marine invertebrate larvae. Boca Raton, FL: CRC Press, pp. 279–321

Nagar LR, Shenkar N (2016) Temperature and salinity sensitivity of the invasion ascidian *Microcosmus exasperatus* Heller, 1878 Aquat Invasions11(1):33–43

Nauen CE (1978) The growth of the sea star, *Asterias rubens*, and its role as benthic predator in Kiel Bay. Kiel Meeresforsch Sonderheft 4:68–81

Noël PY, Tardy E, d'Udekem d'Acoz C (1997) Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Science 320:741–745

Nour OM, Stumpp M, Morón SC, Barboza FR, Pansch C (2020) Population structure of the recent invader *Hemigrapsus takanoi* and prey size selection on Baltic Sea mussels. Aquat Invasions 15(2):297–317

Nour OM, Stumpp M, Morón SC, Barboza FR, Pansch C (2020) Population structure of the recent invader *Hemigrapsus takanoi* and prey size selection on Baltic Sea mussels. Aquat Invasions 15(2):297–317

Novo MS, Miranda RB, Bianchini A (2005) Sexual and seasonal variations in osmoregulation and ionoregulation in the estuarine crab *Chasmagnathus granulatus* (Crustacea, Decapoda). J Exp Mar Biol Ecol 323:118–137

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

Occhipinti-Ambrogi A, Galil B (2010) Marine alien species as an aspect of global change. Adv Oceanogr Limnol 1:199–218

Ojaveer H, Gollasch S, Jaanus A,Kotta J, Laine AO, Minde A, Normant M; Panov VE (2007) Chinese mitten crab *Eriocheir sinensis* in the Baltic Sea–a supply-side invader? Biol Invasions 9:409–418

Otto T, Brandis D (2011) First evidence of *Eriocheir sinensis* reproduction from Schleswig-Holstein, Northern Germany, Western Baltic Sea. Aquat Invasions 6 (Supplement 1): S65–69

Paavola M, Olenin S, Leppäkoski E (2005) Are invasive species most successful in habitats of low native species richness across European brackish water seas? Estuar Coast Shelf Sci 64(4):738–750

Paiva F, Barco A, Chen Y, Mirzajani A, Chan FT, Lauringson V, Baltazar-Soares M et al (2018) Is salinity an obstacle for biological invasions? Glob Change Biol 24(6):2708–2720

Pauli NC, Briski E (2018) Euryhalinity of Ponto-Caspian invader in their native and introduced regions. Aquat Invasions 13:439–447

Piola RF, Johnston EL (2006) Differential tolerance to metals among populations of the introduced bryozoan Bugula neritina. Mar Biol 148:997–1010

Podbielski I, Bock C, Lenz M, Melzner F (2016) Using the critical salinity (Scrit) concept to predict invasion potential of the anemone *Diadumene lineata* in the Baltic Sea. Mar Biol 163(11):1–15

R Core Team (2016) R: a language and environment for statistical computing. https://www.Rproject.org

Ravi R, Manisseri MK (2010) Survival rate and development period of the larvae of *Portunus pelagicus* (Decapoda, Brachyura, Portunidae) in relation to temperature and salinity. Fish Aquacult J 3(1):2–12

Reise K, Olenin S, Thieltges DW (2006) Are aliens threatening European aquatic coastal ecosystems? Helgol Mar Res 60:77–83

Reusch T, Chapman ARO (1997) Persistence and space occupancy by subtidal blue mussel patches. Ecol Monogr 67: 65–87

Reusch TBH, Dierking J, Andersson HC, Bonsdorff E, Carstensen J, Casini M et al (2018) The Baltic Sea as a time machine for the future coastal ocean. Sci Adv 4: eaar8195.doi: 10.1126/sciadv.aar 8195

Shentu J, Xu J, Ding Z (2015) Effects of salinity on survival, feeding behavior and growth of the juvenile swimming crab, *Portunus trituberculatus* (Miers, 1876). Chin J Oceanol Limnol 33(3): 679–684

Shinji J, Strussmann CA, Wilder MN, Watanabe S (2009) Short-term responses of the adults of the common Japanese intertidal crab, *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsoidea) at different salinities: osmoregulation, oxygen consumption, and ammonia excretion. J Crustac Biol 29 (2):269–272

Simith DJB, de Souza AS, Maciel CR, Fernando M, Abrunhosa FA, Diele K (2012) Influence of salinity on the larval development of the fiddler crab *Uca vocator* (Ocypodidae) as an indicator of ontogenetic migration towards off shore waters. Helgol Mar Res 66: 77–85

Soors J, Faasse M, Stevens M, Verbessem I, de Regge N, Van den Bergh E (2010) New crustacean invaders in the Schelde estuary (Belgium). Belg J Zool 140:3–10

Stephenson EH, Steneck RS, Seeley RH (2009) Possible temperature limits to range expansion of non-native Asian shore crabs in Maine. J Exp Mar Biol Ecol 375:21–31

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. Aquat Toxicol 110–111:194–207

Sukin SD, Mckenn G (1994) Influence of temperature on larval development of four co-occuring species of the brachyuran genus *Cancer*. Mar Biol *118:593*–600

Sutton AE, Yankson K, Wubah DA (2012) The effect of salinity on particle filtration rates of the west African mangrove Oyster. J Young Investig 24(4):55–59

Theede H (1969) Einige neue Aspekte bei der Osmoregulation von Carcinus maenas. Mar Bio 2:114–120

Therneau TM (2015) A package for survival analysis in S. version 2.38. Available: https://CRAN.R-project.org/package=survival

Thomsen J, Haynert K, Wegner KM, Melzner F (2015) Impact of seawater carbonate chemistery on the calcification of marine bivalves. Biogeosciences 12:4029–4220

Thomsen J, Ramesh K, Sanders T, Belich M, Melzner R (2018) Calcification in a marginal sea-influence of seawater [Ca^{2+}] and carbonate chemistry on bivalve shell formation. Biogeosciences 15:1469–1482

Tsiamis K, Palialexis A, Stefanova K, Gladan ZN, Skejić S, Despalatović M, Cvitović I et al (2019) Non-indigenous species refined national baseline inventories: a synthesis in the context of the European union’s marine strategy framework directive. Mar Pollut Bull 145: 429–435

Urbina M, Paschke K, Gebauer P, Chaparro OR (2010) Physiological energetics of the estuarine crab *Hemigrapsus crenulatus* (Crustacea: Decapoda: Varunidae): responses to different salinity levels. J Mar Biol Associ UK 90(2):267–273

van den Brink A, Godschalk M, Smaal A, Lindeboom H, McLay C (2013) Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). J Mar Biol Associ UK 93:189–196

van den Brink AM, Wijnhoven S, McLay CL (2012) Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. J Sea Res 73:126–136

Whitfield AK, Elliot M, Basset A, Blaber SJM, West RJ (2012) Paradigms in estuarine ecology-a review of the Remane diagram with a suggested revised model for estuaries. Estuar Coast Shelf Sci 97:78–90

Wolf B, Kiel E, Hagge A, Krieg HJ, Feld CK (2009) Using the salinity preferences of benthic macroinvertebrates to classify running water in brackish marshes in Germany. Ecol Indic 9: 837–847

Wood CA, Bishop JDD, Davies CJ, Delduca EL, Hatton JC, Herbert RJH, Clark PF (2015) *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. BioInvasions Rec 4:109–113

Zhang H, Shin PKS, Cheung SG (2016) Physiological responses and scope for growth in a marine scavenging gastropod, *Nassarius festivus* (Powys, 1835), are affected by salinity and temperature but not by ocean acidification. ICES J Mar Sci 73(3):814–824

Figures

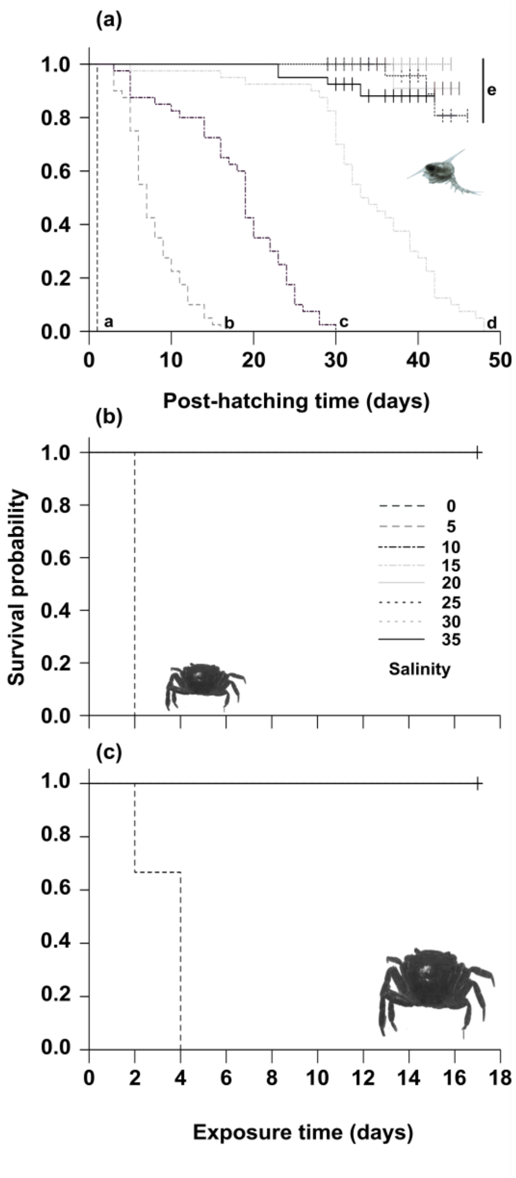

Figure 1. Influence of salinity (0 – 35) on survival rates in different life stages of *Hemigrapsus takanoi* from Kiel Fjord. Initial numbers of individuals were (**a**) 400 larvae (100 larvae per salinity level * 4 females), (**b**) 5 juveniles and (**c**) 3 adults per salinity level. Larvae were monitored until they reached the megalopa stage. Juveniles and adults were monitored over a period of 17 days. Marks (|) indicate censored data, i.e. animals reared at the respective salinity levels survived until the end of the experiment. Different lower-case letters indicate significant differences (pairwise Peto and Peto tests, $p \leq 0.05$) between experimental groups.

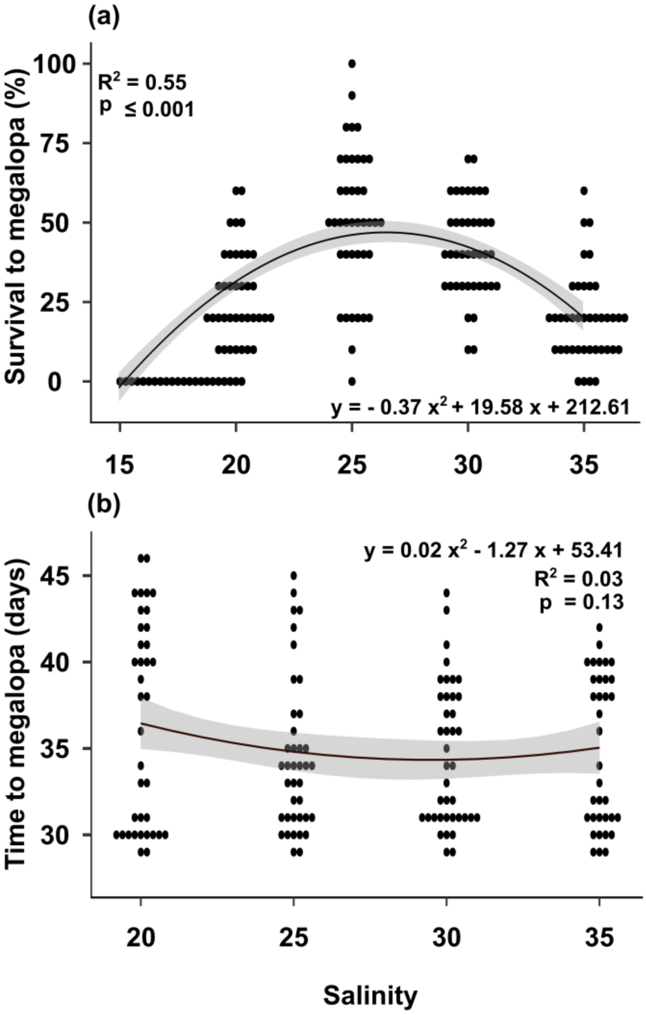

Figure 2. Influence of salinity (0 – 35) on (**a**) the percentage of larvae that reached the megalopa stage, and (**b**) the time needed to reach this stage in *Hemigrapsus takanoi* from Kiel Fjord. Salinity levels at which larvae did not reach the megalopa stage (i.e. salinities 0 to 15) were removed from the analysis of the time needed to reach megalopa, while for modelling the survival data, the salinity of 15 is included. Each black dot represents the proportion of survivors in a single vial with 10 larvae or the mean time needed to reach the megalopa stage in a single vial. 2nd degree polynomials were fitted to the data. Grey areas represent the 95% confidence intervals of the fitted lines.

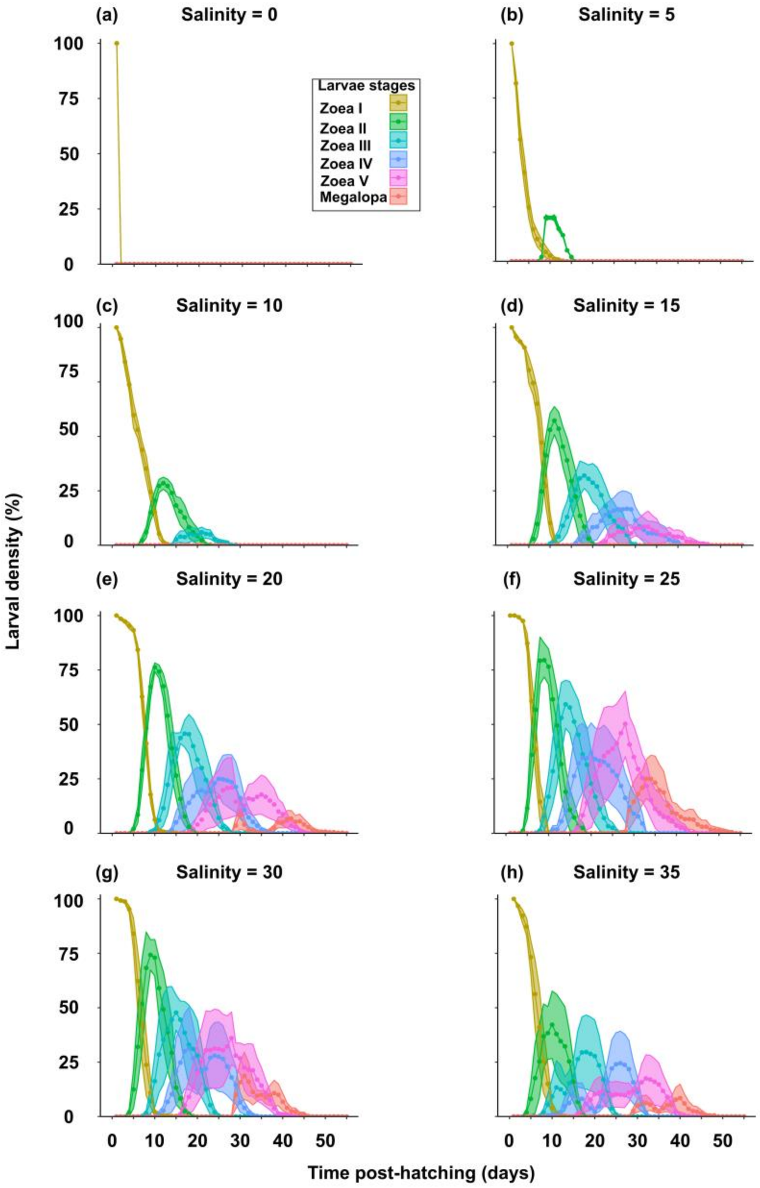

Figure 3. Influence of salinity (0 – 35, **a** – **h**) on the percentage of larvae, which reached the next larval stage, over time. Percentages of Zoea I that reached Zoea II refer to initially 10 larvae per vial. For the remaining observations (from Zoea II to the megalopa stage), the percentages refer to the number of individuals that reached the respective stage. Shaded areas represent standard errors around the means of four females, which were calculated across the 10 vials used per female. Data cover the time until the last Zoea V had moulted to the megalopa stage or died. Shaded areas represent the 95% confidence intervals of the fitted lines.

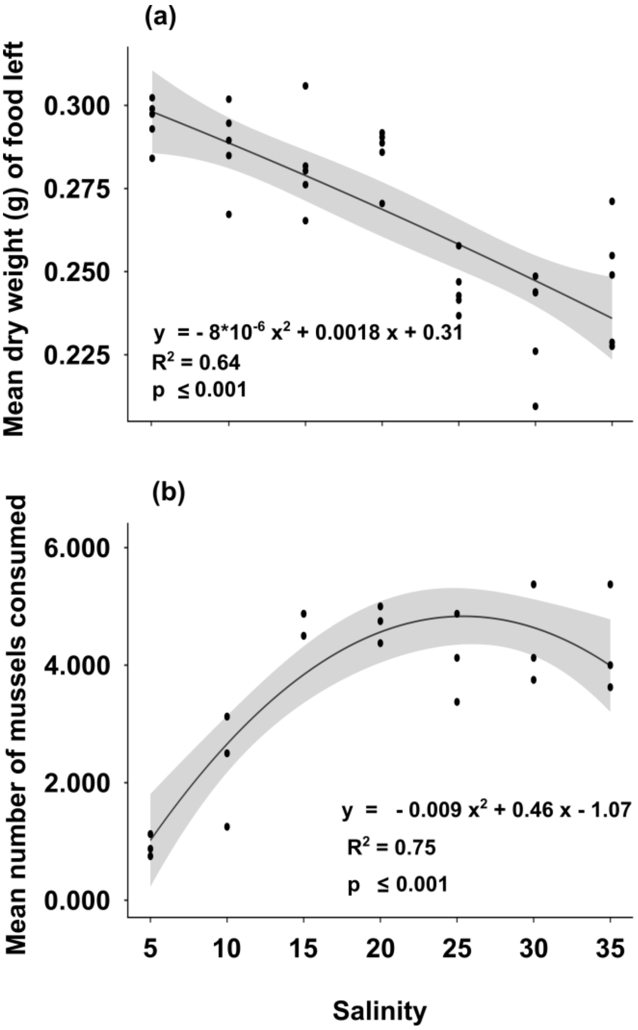

Figure 4. Influence of salinity (0 – 35) on feeding rates in (**a**) juveniles (n = 5), and (**b**) adults (n = 3) of *Hemigrapsus takanoi* from Kiel Fjord. Black dots represent the mean food uptake during 17 days. Data were evaluated using 2nd degree polynomial regressions. Grey areas represent the 95% confidence intervals of the fitted lines.

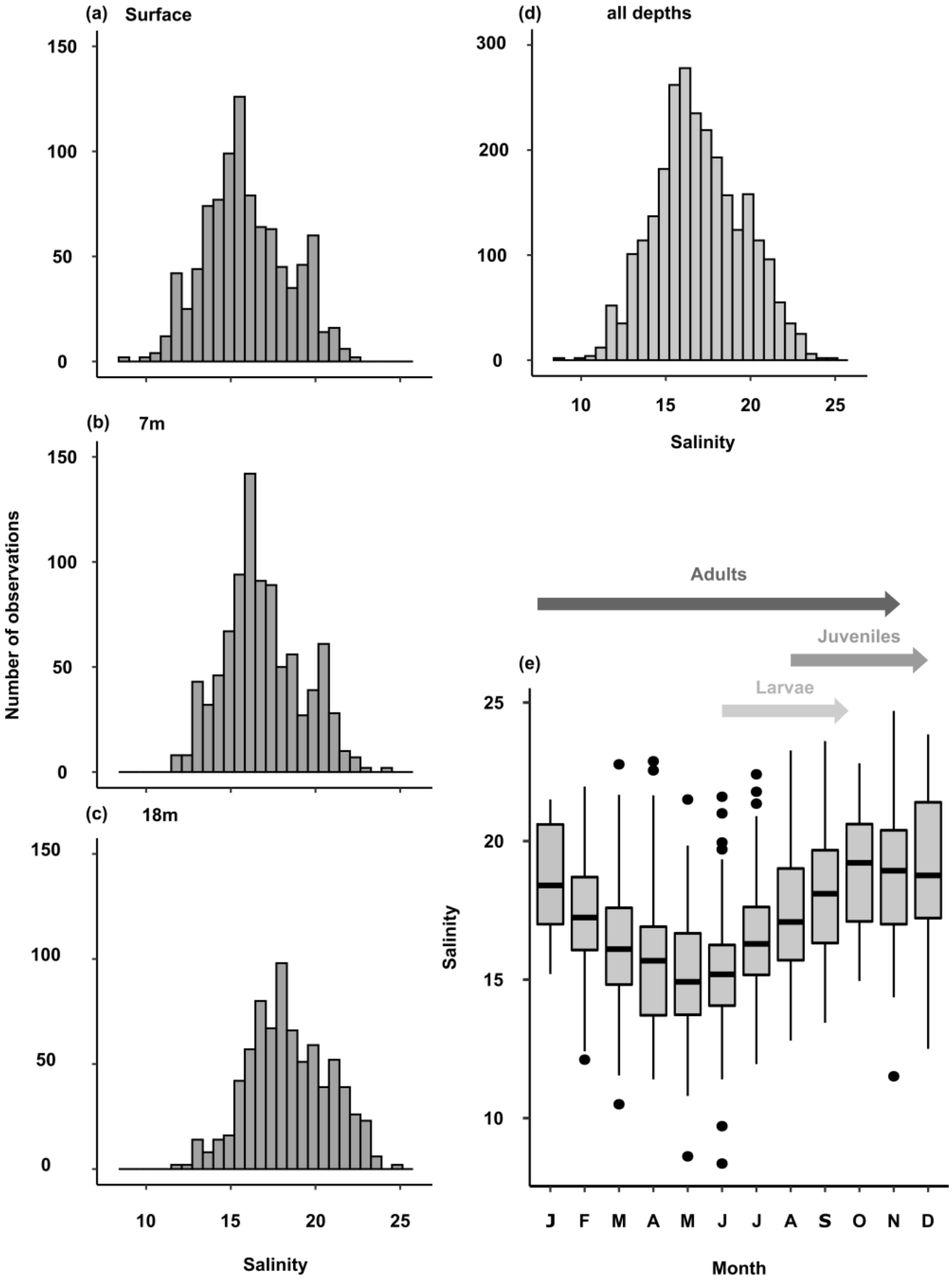

Figure 5. Salinity regimes of the inner Kiel Fjord based on a 13-years (from November 2005 to December 2018) dataset collected within the Kiel Fjord Zooplankton – fish larvae time series at GEOMAR. Data are presented using frequency plots (**a**, **b**, **c**, **d**) for the entire dataset for surface (surface to 1m; N=939; **a**), mid (7 m; N=902; **b**) and bottom (18 m; N=763; **c**) waters and for all water depths (**e**). Seasonal patterns among all years and depth layers (**e**) are presented as boxplots with medians, upper and lower quartiles, the non-outlier range and outliers. The approximate occurrence of larvae, juveniles and adults among the different seasons is indicated by arrows (upper right).

Supporting information for chapter II:

Supplementary figures

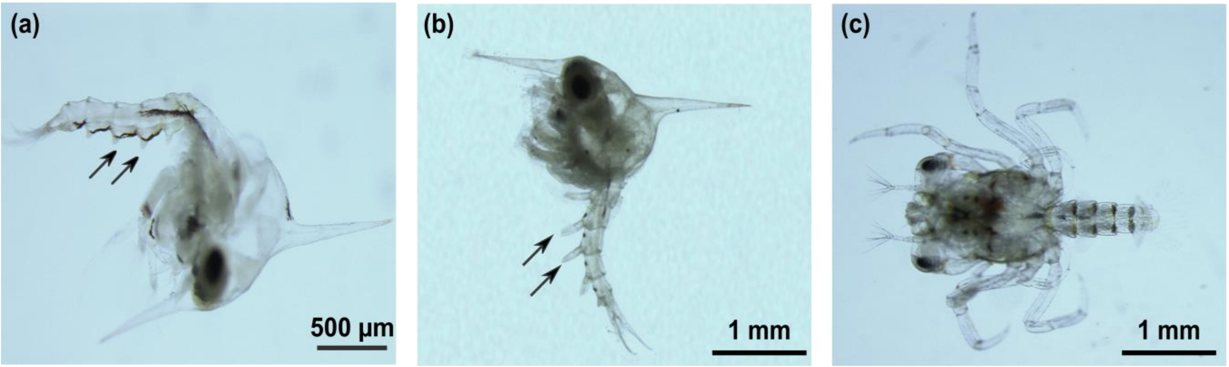

Figure S1. *Hemigrapsus takanoi* (**a**) Zoea IV with endopod buds (black arrows), (**b**) Zoea V with biramous pleopods (black arrows), and (**c**) a megalopa stage.

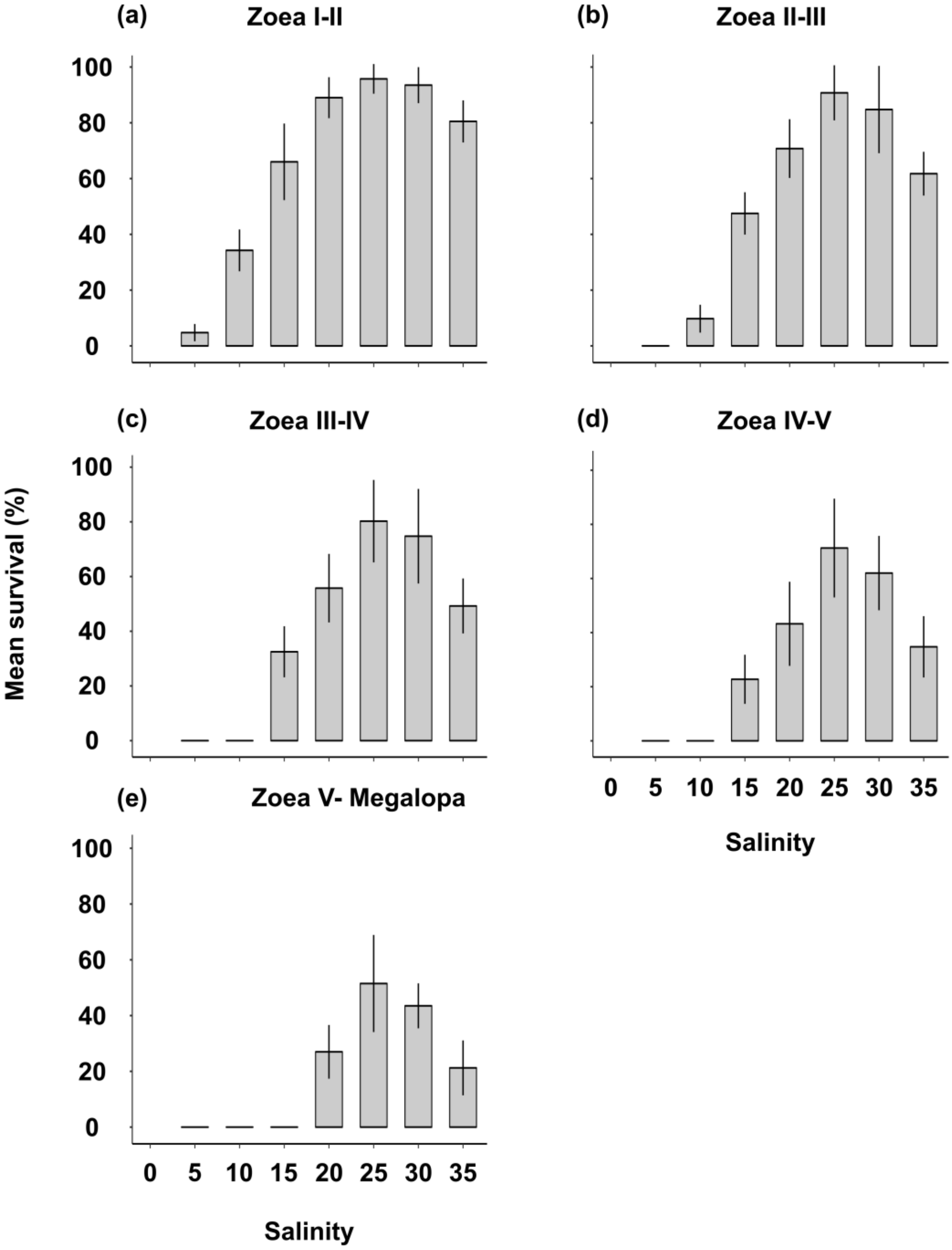

Figure S2. Influence of salinity (5 – 35) on survival of *Hemigrapsus takanoi* larvae (means ± SD). Percentage values of Zoea I – II (**a**), are calculated from initially 10 larvae reared in each of 10 vials per salinity level. Larvae came from four different females, while for Zoea II-megalopa stages (**b**–**e**), values are related to the values resulting from the moulting of the respective former stage.

Supplementary tables

Table S1: Results of pairwise Peto and Peto tests indicating significant differences in survival rates of *Hemigrapsus takanoi* larvae from Kiel Fjord between different salinity regimes. Significant results ($p \leq 0.05$) are highlighted in bold.

Salinity	0	5	10	15	20	25	30
5	**<0.001**	-	-	-	-	-	-
10	**<0.001**	**<0.001**	-	-	-	-	-
15	**<0.001**	**<0.001**	**<0.001**	-	-	-	-
20	**<0.001**	**<0.001**	**<0.001**	**<0.001**	-	-	-
25	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.76	-	-
30	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.29	0.28	-
35	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.24	0.21	0.051

Table S2: Percentage of individuals of *Hemigrapsus takanoi* from Kiel Fjord at different Zoea larval stages that survived to the megalopa stage under eight different salinity regimes (mean ± SD; n = 4 females).

Salinity Stage	0	5	10	15	20	25	30	35
Zoea I – II	0.0	4.7±3.0	34.2±7.5	66.0±13.7	89.0±7.3	95.7±5.3	93.5±6.4	80.5±7.5
Zoea II – III	0.0	0.0	9.7±4.9	47.5±7.5	70.7±10.5	90.7±9.8	84.7±15.6	61.7±7.8
Zoea III – IV	0.0	0.0	0.0	32.5±9.3	55.7±12.5	80.2±15	74.7±17.2	49.2±10.0
Zoea IV – V	0.0	0.0	0.0	22.7±9.0	43.2±15.5	71.2±18.2	62.0±13.7	34.7±11.3
Zoea V– megalopa	0.0	0.0	0.0	0.0	27.0±9.6	51.5±17.4	43.5±8.0	21.2±9.8

Chapter III

Salinity and temperature impacts on survival and feeding of *Hemigrapsus takanoi* larvae: restrictions for its dispersal (secondary spread) along the Baltic Sea

Ola Mohamed Nour [1, 2*], Martin Wahl [1], Christian Pansch [3†], Meike Stumpp [4†]

[1] GEOMAR Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

[2] Department of Biology and Geology, Faculty of Education, Alexandria University, 21526 Alexandria, Egypt

[3] Environmental and Marine Biology, Åbo Akademi University, 20520 Åbo, Finland

[4] Zoological Institute, Christian-Albrechts University, 24118 Kiel, Germany

*** Corresponding author:** e-mail: olasm.nour@gmail.com; GEOMAR, Düsternbrooker Weg 20, 24105 Kiel, Germany

[†] These authors contributed equally to this work

Key words: *Hemigrapsus takanoi*, Salinity, future warming, larval development, feeding, non-native species, Baltic Sea

Abstract

Knowledge on the potential of a species' dispersal is important when predicting its further spread into new habitats. Salinity is a common stressor restricting the distribution of various decapod crustaceans, and interactive effects with global climate change drivers are important to be considered. Particularly the larval stages are commonly determining a species tolerance and with this its potential to invade and successfully develop a sustaining population.

This laboratory study investigated the combined effect of 6 salinity (10–25) and 2 temperature (ambient 19 and 23°C) levels on larval survival, development to megalopa and feeding (in Zoea I, III, and V) in south-western Baltic Sea *Hemigrapsus takanoi*.

Larval survival to megalopa was generally favoured by increasing salinity. The percentage of larvae that reached the megalopa was higher in 19 compared to 23°C at all salinity levels. Higher temperatures accelerated the larval development to megalopa. Salinity and the interactions of salinity and temperature showed no significant effects on developmental speed. Noteworthy, however, no larva developed to megalopa in 23°C and a salinity of 16, while in 19°C some larvae could successfully develop under a salinity as low as 16. Feeding rates of the different zoea stages were affected by both, salinity and temperature, but not by their interaction. All larval stages fed generally more with increasing temperature and increasing salinity.

The results reveal that, *H. takanoi* population from Kiel Fjord (south-western Baltic Sea) is capable to complete its larval development under the current Kiel Fjord conditions. The geographical spread of this *H. takanoi* population into the wider Baltic Proper may, however, be restricted mainly due to the inability to establish and maintain a self-sustaining population under lower salinity conditions. Furthermore, projected desalination of the Baltic Sea together with rising temperatures due to global warming and heap peaks in summer may likely exert additional stress to this existing population, unless *H. takanoi* adapts at appropriate rates.

Introduction

Physiological characteristics, environmental factors, dispersal potential and ecological interactions, all determine the spatial distribution of marine organisms (Giménez and Anger 2001; De Grande et al. 2018). Crustacean decapods exhibit complex life cycles with development passing through a series of different phases, including embryonic, larval, juvenile and adult stages (Giménez and Anger 2003). Larval stages are crucial for gene flow, population connectivity and range shift by being the most dispersing life stage (Palumbi 2003, Cowen and Sponaugle 2009). Therefore, planktonic larvae are critical for the establishment and persistence of new populations (Anger 2006). The magnitude of larval dispersal capability is determined by few main key factors, as (i) their own behaviour, (ii) duration of development, and (iii) ocean currents (Weersing and Toonen 2009). On the other hand, larval survival and development are mainly affected by several abiotic and biotic factors (Simith et al. 2012; Jackson et al. 2014). Therefore, studying larval responses to changing environmental factors is key to understanding population dynamics, and the complex patterns of species connectivity (Selkoe et al. 2016).

Salinity is considered one of the crucial factors that strongly impact larval survival, feeding, metabolism, and larval development and moulting (Anger 2003). Larval development of marine species normally occurs within a well-defined range of salinity conditions characteristic to a species or population (Charmantier 1998). Reduction of survival occurs when water salinity is beyond the optimum range until a point beyond which no normal development and/or survival is possible (Sastry 1983). Adaptation to particular salinity regimes is important in each larval stage as a requirement for the establishment of a species in a given environment (Charmantier 1998). Previous studies on decapod larvae showed that tolerance to variable salinities, particularly hyper-regulation at low salinity, is due to an early appearance of osmoregulation abilities on this postembryonic stage (Charmantier and Anger 1999; Anger and Charmantier 2000). Larval ability to cope with salinities outside the optimum range are also strongly influenced by temperature (Crisp 1976; Anger 1991). In nature, environmental stress such as warming and desalination do usually not occur in isolation and can therefore interactively affect the organism (Hernández et al. 2012). Therefore, studying the combined effects of these two factors is important to assess the response of a specific population to abiotic changes (Epifanio 1998; Hernández et al. 2012).

The successful establishment of a species in a given habitat depends on its ability to adapt to the ambient environment (Charmantier 1998). Estuarine and coastal zones are ecosystems characterized by a high variability in environmental parameters such as salinity, and salinity is considered as an ecological key factor in those areas (Anger et al. 2008; Simith et al. 2012). Among these, the Baltic Sea is one of the largest brackish water bodies worldwide, characterized by strong salinity gradients from east to west (Janssen et al. 1999). The surface salinity ranges from 3–4 in the north-east, to 6–8 in the central Baltic, to 10–25 in the south-west of the central Baltic, and increases gradually to almost fully marine conditions through the Danish Straits and the Kattegat (Müller et al. 2016).

Biodiversity of marine organisms in the Baltic Sea is particularly affected by climate change (Holopanien et al. 2016), and at the same time, the Baltic Sea experiences some of the greatest changes in temperature and salinity (Reusch et al. 2018). By 2100, salinities of the surface waters are expected to decline by 1.5–2 units, while temperature will increase by 3–6 °C (Meier et al. 2012; Vuorinen et al. 2015). In addition, many euryhaline and eurythermal species are potential invaders to the Baltic Sea, and their invasion success is determined by their ability to survive and reproduce in the brackish environment (Paavola et al. 2005; Paiva et al. 2018). About 152 non-native and cryptogenic species have been recorded in the Baltic Sea (Casties et al. 2016; supplementary in Tsiamis et al. 2019). One of the recent invaders that reached the south-western Baltic in 2014 is the Asian brush clawed crab *Hemigrapsus takanoi* (Geburzi et al. 2015).

H. takanoi is a decapod crustacean originating from the north-west Pacific Ocean, and was brought to northern Europe more than 25 years ago (Gollasch 1999). In its native Asian range, this species is known to tolerate a wide range of salinities from 7 to 35, and from cold to warm temperature conditions (Mingkid et al. 2006a; Soors et al. 2010). In general, like most of members of the family Varunidae, *H. takanoi* larval development comprises five zoea and one single megalopa stage (Landeira et al. 2019). Although zoeae from its native range could moult to megalopa in salinities as low as 10, megalopae required higher (≥25) salinities to reach the juvenile (Mingkid et al. 2006b). Experimental studies on a Kiel Fjord (south-western Baltic Sea) population of *H. takanoi*, confirmed that juveniles and adults are euryhaline and can tolerate short-term exposure to salinities from 5 to 35 (Nour et al. in preparation). In contrary, larvae are considered to be stenohaline and required salinities ≥20 to reach the megalopa (Nour et al. in preparation). This suggests that salinity could restrict the expansion of this population to the south-western Baltic Sea, however, investigations on the interactive effects of salinity changes and future ocean warming are yet lacking.

In order to determine the salinity threshold and the interactive role of temperature for larval development in Kiel Fjord *H. takanoi*, we measured survival, development, and feeding rates of *H. takanoi* larvae from Zoea I to Megalopa in a salinity gradient from 10 to 25 (in steps of 3), at present-day summer conditions (19°C), and a future warming scenario (23°C). We hypothesized that the Kiel Fjord population is (a) already adapted to the current fjord conditions (salinity of 16 and a temperature of 19°C), and therefore able to complete the whole life cycle in these fjord conditions and that (b) ocean warming alters larval performance, and therefore limits their tolerance to low salinities, with direct consequences for the species future invasion potential into the Baltic proper.

Materials and Methods

Animal collection and maintenance

Ovigerous females of *H. takanoi* were collected from inner Kiel Fjord (54°32.9′N; 10°14.8′E) in early July 2018 by scrapping mussel aggregates at a depth of ~1.5 m. Animals were immediately brought to the laboratory facilities of GEOMAR, in buckets with seawater from the site of collection. Crabs were maintained individually in 2 L aquaria filled with well-aerated seawater at habitat conditions at the time of collection, i.e. a constant temperature of 19 ± 0.5°C and a salinity of 13, at a photoperiod of 12h:12h light:dark. Females were offered crushed mussels *ad libitum* as a food twice per week, and water was exchanged daily.

Experimental design

We assessed larval survival and developmental time from hatching to megalopa (Experiment 1), and feeding rate of Zoea I, III, and V (Experiment 2) at 12 combinations of temperature (19 and 23°C) and salinity (10, 13, 16, 19, 22, and 25) treatments, in a fully crossed experimental design. For both experiments ovigerous females with eggs close to hatching (following the criteria given by van den Brink et al. 2013) were selected. After hatching of their eggs, females were removed from the aquaria and larvae were immediately exposed and held at the respective experimental treatments.

Salinity levels were prepared by using filtered (0.2 µm Millipore) and well-aerated North Sea water (salinity ~33) diluted with deionized tap water (Epifanio 1998; Bas and Spivak 2000). Salinity was adjusted using a WTW Cond 3110 equipped with a Tetracon 325 electrode. Details for pH_{NBS} adjustment in Chapter II, materials and methods section. Experimental temperatures were achieved in constant temperature chambers at GEOMAR, Helmholtz-Centre for Ocean research Kiel. As a precaution against possible thermal shock, larvae were acclimated after hatching to the higher temperature treatment (i.e. 23°C) by gradually increasing the temperature at a rate of 1 °C per h (Hernández et al. 2012).

Experiment 1: Larval survival and development

Each treatment was carried out with three replicate 100 mL vials (technical replicates) filled with 80 mL seawater at the desired salinities. Each vial contained 10 vigorously swimming (i.e. apparently healthy) Zoea I larvae. In total, the experimental set up included 36 vials for each female separately (6 salinities x 2 temperatures x 3 vials). The offspring of five females (biological replicates) of a carapace width of 16.52 ± 0.69 (mean ± SD) were tested in total (5 females x 6 salinities x 2 temperatures with 3 vials each).

Water and food were exchanged daily. Freshly hatched *Artemia spp.* nauplii (<100 µm; Great Salt Lake Brand) were offered at a density of ~10 nauplii per mL (Anger et al. 2008). To avoid salinity changes in the experimental vials, nauplii were previously rinsed with filtered seawater as in the respective treatments before adding them to the culture vials (Lárez et al. 2000; Simith et al. 2012). Larvae were monitored daily until they died or moulted to megalopae. Survival was calculated as the sum of all larvae that reached the megalopa stage in each vial for each treatment. Development was estimated as the time (days) needed to reach the megalopae.

Experiment 2: Feeding rate of larvae

To determine feeding rates, 10 larvae from each zoea stage were held individually (technical replicates), in closed 25 mL glass vials containing 10 mL of seawater at the respective salinity and temperature treatment combination. After starvation for 24 h, 10 *Artemia spp.* nauplii per mL seawater were added to each vial. After 24 h, zoeae were carefully removed from the vials, death of individuals was recorded when existent, and the remaining individuals of *Artemia spp*. were counted. Feeding rate is presented as the number of individual *Artemia spp*. consumed in 24 h. The experiment was repeated for each of four ovigerous females (biological replicates) of a carapace width of 15.94 ± 1.11 (mean ± SD), for Zoea I, III and V larvae.

Zoeae I were obtained immediately after hatching. For Zoeae III and V, another part of the hatch was in parallel mass-reared in 2 L glass beakers filled with ~1.5 L seawater without aeration, with an initial number of ~2000 freshly hatched larvae per beaker for each treatment.

Water was changed daily, and food was offered as explained in Experiment 1. Larvae were checked daily, and dead larvae were removed. When larvae reached the required stage (i.e. Zoea III and V), and to obtain individuals with the same age, freshly moulted Zoea III and V were used to assess feeding rates. In total, for each female and each measurement stage, the experiment included 360 glass vials of 25 mL, each containing a single zoea larva (6 salinities x 2 temperatures x 10 vials x 3 zoea stages repeated with offspring of 4 females in total).

Data analyses

All analyses were performed using the statistical software package R (version 3.6.0, Development Core Team 2016). Graphical outputs were made using the ggplot2 package (Wickham 2016). For analyses, we used generalized linear mixed models (GLMMs) using the package lme4 (Bates et al. 2017). GLMMs were applied to: survival, developmental time (days), and feeding rate as response variables; salinity and temperature and their interactions were treated as fixed factors (explanatory variables) and female individuals were treated as random factor. As larvae reached the megalopa stage only in salinities from 16 to 25 in Experiment 1, and as larvae reached the Zoea V only in the same salinity range (Experiment 2), we only included these four salinities in the analyses of survival and developmental time to megalopae as well as of feeding, respectively.

Survival was analysed as proportions (i.e. alive or dead), with a binomial distribution. Developmental time and feeding rate were analysed as count data with a Poisson distribution. In Experiment 2 larvae were reared together in common 2 L glass bottles until they reached Zoeae III and V. Here, in order to avoid dependency, mean numbers of *Artemia spp.* consumed for the 10 zoeae for each female were calculated. Logarithmic link functions were used for all models. Assumptions for all models applied were confirmed using diagnostic plots of residuals (Zuur et al. 2010). The marginal R^2 (variance explained only by the fixed effect) and conditional R^2 (variance explained by fixed and random effects) of fitted GLMMs were calculated using the R package MuMln (Nakagawa and Schielzeth 2013).

In addition, when salinity significantly affected the examined response variable, we compared the optimum salinity of the response variable between the two different temperature conditions. we used a linear model (LM) by fitting a 2nd degree polynomial. We checked whether the assumptions of the LMs were met by detailed visual inspection of residual plots. From the general quadratic equation, the optimum salinity point (x-coordinate of the vertex) of the quadratic curve was calculated (Koram 2019) as following:

General quadratic equation:

$y = ax^2 + bx + c$ (Equation 1)

Where a and b are the slopes of x^2 and x; respectively, and c is the intercept of the regression line.

x-coordinate of the vertex (optimum point) = $-b/2a$ (Equation 2)

Results

Larval survival to megalopa

Larvae failed to reach the megalopa stage in the two lowest salinities 10 and 13, in both temperature treatments, and development ceased beyond the Zoea III stage. In salinity 16, although larvae reached the last zoea stage, none of larvae succeeded to moult to megalopa at the higher temperature (23°C; Fig. 1a). Larvae of only one female with median survival of 40% out of five succeeded to reach megalopa at ambient temperature (19°C; Fig. 1a).

The number of larvae that reached the megalopa stage in salinities ranging from 16 to 25 were significantly affected by both, salinity (GLMM: χ^2=460.41, df=1, p<0.001; Fig. 1a), and temperature (GLMM: χ^2=116.17, df=1, p<0.001; Fig. 1a). Salinity and temperature showed no significant interaction regarding survival success to megalopae (GLMM: χ^2=1.69, df=1, p=0.19; Fig. 1a), indicating that the sensitivity to salinity did not differ between temperatures. The obtained GLMM presented a marginal R^2 of 0.1 and a conditional R^2 of 0.22. In all salinity treatments, survival to megalopa was higher in 19°C than in 23°C. In both temperature treatments, salinity of 25 recorded the highest survival percentage (median survival of 30 and 20%, in 19 and 23°C, respectively). This percentage is decreased by 1.5 and 2 times at 19°C (median survival of 20%, salinities of 22 and 19) and 23°C (median survival of 10%, salinities of 22 and 19), respectively (Fig. 1a). Salinity of 22.5 was recorded as the salinity of best performance at 23°C, while there was a shift towards higher optimal salinity conditions (~24) at 19°C.

Developmental time (days) to megalopa

The time to reach the megalopa stage was not affected, neither by the salinity (GLMM: χ^2=0.55, df=1, p=0.46; Fig. 1b) nor by the interaction of salinity and temperature (GLMM: χ^2=0.003, df=1, p=0.95; Fig. 1b). The effect of temperature on developmental time was highly significant (GLMM: χ^2=221.97, df=1, p<0.001; Fig. 1b). The obtained GLMM presented a marginal R^2 of 0.75 and a conditional R^2 of 0.76. Increasing temperature accelerated larval development by ~1.6–1.8 times. At 23°C, development to the megalopa stage required 37–38 days (median time to megalopa, at salinities 19–25; Fig. 1b). At 19°C, 60–61 days were needed to complete the development to the megalopa stage (salinities 19–25), while at salinity 16 larvae required almost one week more (68 days; Fig. 1b).

Feeding rate of larval stages

In general, feeding rates were significantly affected by both salinity (GLMM: Zoea I: χ^2=12.3, df=1, p<0.001; Zoea III: χ^2=31.09, df=1, p<0.001; Zoea V: χ^2=21.83, df=1, p<0.001) and temperature (GLMM: Zoea I: χ^2=15.6, df=1, p<0.001; Zoea III: χ^2=32.12, df=1, p<0.001; Zoea V: χ^2=16.63, df=1, p<0.001; Fig. 2a–c). In all examined zoea stages, feeding rates increased with increasing salinity and were higher at higher temperature conditions (Fig. 2a–c). The differences between feeding rates at the different temperatures were ~1.2 (salinity 16), ~1.3 (salinity 19 and 25), and ~1.4 (salinity 22) times more in 23°C compared to 19°C, for Zoea I. For Zoea III, the differences were ~1.3 (salinity 16), 1.4 (salinity 19 and 22) and increased to 1.6 times more in 23°C compared to 19°C (salinity 25); and were ~1.3 times more in 23°C compared to 19°C in all salinity treatments for Zoea V.

The interactions between salinity and temperature had no significant effect on feeding rates (GLMM: Zoea I: χ^2=0.11, df=1, p=0.73; Zoea III: χ^2=0.74, df=1, p=0.38; Zoea V: χ^2=0.28, df=1, p=0.59; Fig. 2a–c). The obtained GLMMs presented a marginal R^2 of 0.47, 0.05, and 0.03 and a conditional R^2 of 0.42, 0.98, and 0.98, for Zoea I, III, and V; respectively. For Zoea III and V, the peak of feeding rate showed a preference for higher salinities at higher temperature. Peak performance shifted from salinity ~25 (at 19°C) to 31 (at 23°C) for Zoea III, and from 23.7 (at 19°C) to 25 (at 23°C) for Zoea V. In contrary, for Zoea I, in increased temperature, the peak of feeding was shifted to lower salinities, being at salinity 25.7 (at 19°C) and at salinity 23 (at 23°C).

In addition salinity (GLMM: 19°C: χ^2=19.52, df=1, p<0.001; 23°C: χ^2=44.92, df=1, p<0.001) and zoea stage (GLMM: 19°C: χ^2=41.32, df=1, p<0.001; 23°C: χ^2=42.87, df=1, p<0.001) significantly affected feeding rates, while their interactions had no effect (GLMM: 19°C: χ^2=0.28, df=2, p=0.86; 23°C: χ^2=1.25, df=2, p=0.53; Fig. S1a&b). The obtained GLMMs presented a marginal R^2 of 0.04 and 0.05 and a conditional R^2 of 0.97 and 0.98, for 19 and 23°C, respectively.

Discussion

The current study revealed that *H. takanoi* is likely able to complete its entire larval development under present-day environmental conditions in Kiel Fjord (south-western Baltic Sea). Salinity of 16 can be considered a lower threshold for *H. takanoi* larvae to complete development in the Baltic Sea, because larvae of only one female (out of five) were able moult to megalopa at this salinity (at 19°C). At conditions of the climate warming scenario (23°C), no larval succeeded to megalopa at a salinity of 16. Regardless of temperature, survival to megalopa decreased with decreasing salinity, with no effects on moulting time between all salinity levels. Elevated temperature on its own had a marked effect on larval development, with larvae developing quicker but showing poorer survival to megalopa among all salinity levels. Our results show that both, salinity and temperature can control *H. takanoi* larval development in the Baltic Sea, with no indication of significant interactions between these two factors.

The obtained results suggest that the Kiel Fjord *H. takanoi* population is self-sustaining, and larvae can complete their life cycle and moult to megalopa under the present-day conditions. Nonetheless, *H. takanoi* larvae successfully metamorphosed to megalopa in a narrow range of salinities (16–25) at ambient temperature. In addition, hatches of only one female (20% of examined females) reached the megalopa at salinity 16 and 19°C, indicating a low adaptation level of Kiel Fjord population.

Phenotypic plasticity (the acclimatization potential of physiological processes of a species to altered conditions; González-Ortegón and Giménez 2014) has been considered to be especially beneficial in habitats with strong variations in abiotic and biotic conditions making sure that some individuals are likely to survive suboptimal periods (Hughes and Stachowicz 2004; Roger et al. 2012). In many crustaceans, phenotypic plasticity of developmental stages showed an adaptative value and enabling organisms to buffer the variability in salinity that characterizes estuarian environments (e.g. *Rhithropanopeus harrisii*: Rosenberg and Costlow 1979; *Balanus amphitrite*: Qiu and Qian 1999; *Chasmagnathus granulate*: Charmantier et al. 2002). In several species of the family Grapsidae, a tendency towards variation in development from hatches of different females (Díaz and Bevilacqua 1987; Anger 1991) and among siblings from the same hatch (Montú et al. 1990) has been observed. Whether this intra-population variability in Kiel Fjord *H. takanoi* larval development in response to abiotic stress is related to plasticity or genetic diversity needs to be studied.

Developmental time to reach megalopa was not affected by the range of salinities tested, while it was inversely related to temperature, which is consistent with most other decapod species (Epifanio et al. 1998; Lárez et al. 2000). Our study showed that developmental time significantly differed between temperatures, i.e. 37–38 days at 23°C as compared to 60–68 days at 19°C. At higher temperature, within the physiologically tolerable salinity range of the species, a shortening of moulting intervals could be likely due to increasing metabolism, enzyme activities and hormone levels that are involved in the moulting process (Skinner1985; Lárez et al. 2000). A faster development is likely to increase recruitment success (Gonçalves et al. 1995), because the duration in the plankton is reduced, therefore decreasing the risk of predation (Morgan 1995). On the other hand, extension of larval development might increase a species’ dispersal, helping larvae to colonize new regions (Díaz and Bevilacqua 1986).

The results showed higher temperature to have bifold negative effects on *H. takanoi* larval development. Firstly, at all salinity levels the number of larvae that reached megalopa were lower at 23°C compared to 19°C. Secondly, higher temperature caused total failure to megalopa at salinity 16. This is surprising since *H. takanoi* adults can tolerate temperature up to 30°C (Berggren and Karlsoon 2017). A study from native regions showed successful larval development to megalopa at temperature of ~24°C and salinity range from 10 to 35 (Mingkind et al. 2006b). Larval response pattern to higher temperature as observed in this study might be an adaptation to the temperature conditions that prevail in north European water bodies where the examined population likely evolved. A similar response pattern was found in *Eriocheir sinensis* larvae from some invaded regions (Anger 1991). Our results also indicate that under a warming scenario, optimal larval survival shifted towards lower salinities (salinity of 22.5 at 23°C compared to 24 at 19°C). Nonetheless, if *H. takanoi* will not adapt to the warmer temperature, the Kiel Fjord population living at a salinity of around 16 (expected to decrease further) is expected to go extinct.

Both, increasing salinity and warming significantly increased the feeding for Zoea I, III, and V. Lower feeding rate in lower salinities could be related to osmoregulation requirements, presumably representing a metabolic dis-adjustment induced by osmotic stress (Anger 2003). This matches with observations on fewer survivors in low compared to higher salinities, indicating that larvae might need to save energy for osmoregulation in lower salinities (Anger 2003). Reduced rates of feeding, and consequently growth, have been observed in many studies when decapod larvae were exposed to osmotic stress (Johns 1982; Anger et al. 1998; Torres et al. 2007). Higher feeding rate in higher temperature on the other hand is most likely related to more energy required for metabolic processes (Paul and Nunes 1983), a pattern recorded for many other decapod larvae (Arnberg et al 2013 and reference therein). Reduced feeding under stress can alter an individual’s fitness, which in turn can affect population dynamics: Low feeding rates normally lead to low biomass and size of individuals (Giménez and Anger 2001; Giménez and Torres 2002), making them more vulnerable to predation (Torres et al. 2007) and cannibalism (Moksnes 2004). This reduction in body size and mass can also be transferred through several juvenile stages, affecting their fitness (Giménez et al. 2004; Giménez 2010).

Under present-day climate conditions, our data suggest that *H. takanoi* larvae could be a key factor limiting the spatial distribution of this species in the Baltic Sea. Larval sensitivity to low salinity may hinder the distribution boundaries of *H. takanoi* to the south-west. Whether *H. takanoi* possess a specific larval stage that can be further regarded as a bottleneck for osmoregulation ability is still unclear and should be considered in future studies. Osmoregulation capacities are known to be different among larval stages of a species (Charmantier 1998). For example, Zoea I and II stages of the red king crab *Paralithodes camtschaticus* are more tolerance to lower salinities than zoea IV (Parshin-Chudin et al. 2014). Zoea I of *Carcinus maenas* showed an osmoregulation capacity, while all other instars are considered osmo-conformers (Cieluch et al. 2004). Several studies considered the megalopa to be the bottleneck for successful development in response to environmental stress like ocean acidification (Walther et al. 2010; Schiffer et al. 2014). Whether *H. takanoi* posses a specific larval stage that can be regarded as a bottleneck for osmoregulation ability is still unclear and should be considered in future studies.

Conclusions

The present study shows that *H. takanoi* from Kiel Fjord (south-western Baltic Sea) has some (limited) potential to complete its larval development under the present-day Kiel Fjord condition. Nevertheless, our results demonstrate larval development to be challenged under the climate change scenarios imposed. Due to an increase in precipitation and river run-off, a decreasing salinity by 2 units in the Baltic Sea, in addition to elevated temperature are expected by the end of this century (Miere et al. 2012, Graewe et al. 2013), making it hard for *H. takanoi* to reproduce in the western Baltic Sea. Yet, it is unclear, how fast *H. takanoi* can adapt to ongoing environmental change considering that some adaptation must have taken place to enable a low percentage (20%) of the population to reproduce under present-day conditions. In future studies genetic diversity and adaptation potential of *H. takanoi* to future and more eastern Baltic Sea environmental conditions remains to be investigated.

Data availability

All data used in this study will be made publicly available on PANGAEA (https://www.pangaea.de) following acceptance as required by GEOMR Helmholtz Centre for Ocean Research Kiel regulations.

Ethical statement

This study was carried out and funded by GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, with no conflict of interest. All applicable international, national, and/or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed. Applying methods and protocols approved by the regulatory committee on the ethic of animal experiments, of Schleswig-Holstein Germany under the permit number: 1101, and in strict accordance to the relevant regulations and guidelines. All possible actions were taken to reduce animals suffering and stress during handling and sampling.

Acknowledgements

We thank Dr. Gabriela Torres for giving advice for larvae rearing techniques and Björn Buchholz for technical assistance. Dr. Mark Lenz for statistical advice. OMN acknowledges the financial support of the German Academic Exchange Service (DAAD) through the project German Egyptian Research Long-term Scholarship Programme (GERLS) 2015/16 (57147166).

References

Anger K (1991) Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). *Mar Ecol Prog Ser* 72: 103–110

Anger K (2003) Salinity as a key parameter in the larval biology of decapod crustaceans. *Inver Reprod Dev* 43(1): 29–45

Anger K (2006) Contributions of larval biology to crustacean research: a review. *Inver Reprod Dev* 49: 175–205

Anger K, Charmantier G (2000) Ontogeny of osmoregulation and salinity tolerance in a mangrove crab, *Sesarma curacaoense* (Decapods: Grapsidae). *J Exp Mar Biol Ecol* 251: 265–274

Anger K, Spivak E, Luppi T (1998) Effects of reduced salinities on development and bioenergetics of early larval shore crab, *Carcinus maenas*. *J Exp Mar Biol Ecol* 220: 287–304

Anger K, Spivak E, Luppi T, Bas C, Ismael D (2008) Larval salinity tolerance of the South American salt-marsh crab, *Neohelice* (*Chasmagnathus*) *granulata*: physiological constraints to estuarine retention, export and reimmigration. *Helgol Mar Res* 62: 93–102

Arnberg M, Calsoi P, Spicer JI, Tandberg AHS, Nilsen M, Wetsterlund S, Bechmann RK (2013) Elevated temperatures elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. *Mar Biol* 160: 2037–2048

Bas CC, Spivak ED (2000) Effect of salinity on embryos of two southwestern Atlantic estuarine grapsid crab species in vitro. *J Crustac Biol* 20(4): 647–656

Bates D, Maechler M, Bolker B, Walker S (2017). Linear Mixed-Effects Models using ‘Eigen’ and S4. R package version 1.1-13.http://cran.r-project.org/package=lme4

Berggren M, Karlsson R (2017) Invasiva, asiatiska krabbor längs Västkusten. *Fauna and Flora* 112: 22–26

Casties I, Seebens H, Briski E (2016) Importance of geographic origin for invasion success: A case study of the North and Baltic Seas versus the Great Lakes - St. Lawrence River region. *Ecol and Evol* 6: 8318–8329

Charmantier G (1998) Ontogeny of osmoregulation in crustaceans: a review. *Inver Reprod Dev* 33(2–3): 177–190

Charmantier G, Anger K (1999) Ontogeny of osmoregulation in the palaemonid shrimp

Palaemonetes argentinus (Crustacea: Decapoda). *Mar Ecol Prog Ser* 181: 125–129

Charmantier G, Giménez L, Charmantier-Daures M, Anger K (2002) Ontogeny of osmoregulation, physiological plasticity, and larval export strategy in grapsid crab *Chasmagnathus granulata* (Crustacea, Decaopda). *Mar Ecol Prog Ser* 229:185–194

Cieluch, U, Anger K, Aujoulat F, Buchholz F, Charmantier–Daures M, Charmantier G (2004) Ontogeny of osmoregulatory structures and functions in the green crab *Carcinus maenas* (Crustacea, Decapoda). *J Exp Biol* 207(2): 325–336

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Ann Rev Mar Sci* 1(1): 443–466

Crisp DJ (1976) Settlement responses in marine organisms. Pages 83-124 *in* R. C. Newell, ed. Adaptations to environment: essays on the physiology of marine animals. Butterworths, London.

De Grande FR, Granado P, Carretero FH, Costa TM (2018) Organic matter affects fiddler crab distribution? Results from field and laboratorial trials. *Estuar Coast Shelf Sci* 212: 138–145

Díaz H, Bevilacqua M (1986) Larval development of *Aratus pisonii* (Milne Edwards) (Brachyura, Grapsidae) from marine and estuarine environments reared under different salinity conditions. *J coast Res* 2: 43–49

Díaz H, Bevilacqua M (1987) Early developmental sequences of *Aratus pisonii* (H. Milne Edwards) (Brachyura, Grapsidae) under laboratory conditions. *Journal of Coastal Research* 3(1): 63–70

Epifanio CE, Dittel AI, Park S, Schwalm S, Fouts A (1998) Early life history of *Hemigrapsus sanguineus* a non-indigenous crab in the Middle Atlantic Bight (USA). *Mar Ecol Pro Ser* 170: 231–238

Geburzi JC, Graumann G, Köhnk S, Brandis D (2015) First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Rec* 4: 103–107

Giménez L (2010) Relationships between habitat conditions, larval traits, and juvenile performance in a marine invertebrate. *Ecology* 91: 1401−1413

Gimenez L, Anger K (2001) Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. *J Exp Mar Biol Ecol* 260: 241–257

Giménez L, Anger K (2003) Larval performance in an estuarine crab, *Chasmagnathus granulata*, is a consequence of both larval and embryonic experience. *Mar Ecol Prog Ser* 249: 251–264

Giménez L, Anger K, Torres G (2004) Linking life history traits in successive phases of a complex life cycle: effects of larval biomass on early juvenile development in an estuarine crab, *Chasmagnathus granulata. Oikos* 104: 570−580

Giménez L, Torres G (2002) Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experienced during embryonic development, and the initial larval biomass. *Mar Biol* 141(5): 877–885

Gollasch S (1999) The Asian decapod *Hemigrapsus penicillatus* (de Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective. *Helgol Meeres* 52: 359–366

Gonçalves F, Ribeiro R, Soares A (1995) Laboratory study of effects of temperature and salinity on survival and larval development of a population of *Rhithropanopeus harrisii* from the Mondego River estuary, Portugal. *Mar Biol* 121: 639–645

González-Ortegón E, Giménez L (2014) Environmentally mediated phenotypic links and performance in larvae of a marine invertebrate. *Mar Ecol Pro Ser* 502: 185–195

Gräwe U, Friedland R, Burchard H (2013) The future of the western Baltic Sea: two possible scenarios. *Ocean Dyn* 63: 901–921

Hernández JE, Palazón-Fernández JL, Hernández G, Bolaños J (2012) The effect of temperature and salinity on the larval developemnt of *Stenorhynchus seticornis* (Brachyura: Inachidae) reared in the laboratory. *J Mar Biol Assoc UK* 92(3): 505–513

Holopanien R, Lehtiniemi M, Miere HEM, Albertsson J, Gorokhova E, Kotta J, Viitasalo M (2016) Impacts of changing climate on the non-indigenous invertebrates in the northern Baltic Sea by the end of the twenty-first century. *Biol Invasions* 18: 3015–3032

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *PNAS*: 8998–9002

Koram SS (2019) Key concept of quadratic functions and Inequalities-First Edition: *Springer Series in Geomechanics and Geoengineering* 13(1): 9– 22

Jackson TD, Torres G, Giménez (2014) Survival and development of larvae of two decapod crustaceans under limited access to prey across a thermal range. *J Plankton Res* 36(6): 1476–1487

Janssen F, Schrum C, Backhaus JO (1999) A climatological data set of temperature and salinity for the Baltic Sea and the North Sea. *De Hy Z* 51: 5–245

Johns DM (1982) Physiological studies on *Cancer irroratus* larvae. III. Effects of temperature and salinity on the partitioning of energy resources during development. *Mar Ecol Prog Ser* 8:75–85

Landeira JS, Cuesta JA, Tanaka Y (2019) Larval development of the brush-clawed shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae). *J Mar Biol Assoc UK* 99(5): 1153–1164

Lárez MB, Palazón-Fernández JL, Bolaños CJ (2000) The effect of salinity and temperature on the larval development of *Mithrax caribbaeus* Rathbun, 1920 (Brachyura: Majidae) reared in the laboratory. *J Palnkton Res* 22:1855–1869

Meier HEM, Hordoir R, Andersson HC, Dieterich C, Eilola K, Gustafsson BG, Höglund A, Schimanke S (2012) Modeling the combined impact of changing climate and changing nutrient loads on the Baltic Sea environment in an ensemble of transient simulations for 1961 – 2099. *Clim Dyn* 39: 2421–2441

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

Mingkid WM, Masashi Yokota M, Watanabe S (2006b) Salinity tolerance of larvae in the penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). *La mer* 44: 17–21

Moksnes P-O (2004) Interference competition for space in nursery habitats: density-dependent effects on growth and dispersal in juvenile shore crabs *Carcinus maenas*. *Mar Ecol Prog Ser* 281: 181–191

Montú IM, Anger, K, de Bakker C (1990) Variability in the larval development of *Metasesarma rubripes* (Decapoda, Grapsidae) reared in the laboratory. Neritica, Pontal do Sul. PR (Brazil) 5: 113–128

Morgan SG (1995) Life and death in the plankton: larval mortality and adaptation. In McEdward L. (ed.) Ecology of marine invertebrate larvae. Boca Raton, FL: CRC Press, pp. 279–321

Müller JD, Schneider B, Rehder G (2016) Long-term alkalinity trends in the Baltic Sea and their implictaions for CO2-induced acidification. *Limnol Oceanogr* 61: 1984–2002

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol* 4: 133–142

Nour OM, Pansch C, Lenz M, Wahl M, Clemmesen C, Stumpp M (2020) To invade or not to invade: Impaired larval development at low salinities could limits the spread of the non-native crab *Hemigrapsus takanoi* in the Baltic Sea. *submitted to Marine Biology*

Paavola M, Olenie S, Leppäkoski E (2005) Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuar Coast Shelf Sci* 64(4): 738 –750

Paiva F, Barco A, Chen Y, Mirzajani A, Chan FT, Lauringson V, Baltazar-Soares M et al (2018) Is salinity an obstacle for biological invasions? *Glob Change Biol* 24(6):2708–2720

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecol Appl* 13(1): 146–158

Parshin–Chudin AV, Borisov RR, Kovacheva NP, Lebedev RO, Nazartseva MY (2014) Effect of salinity on the survival of the red king crab; *Paralithodes camtschatic*us (Tilesius, 1815), at early stages of ontogeny. *Russ J Ecol* 45(2): 150–152

Paul AJ, Nunes P (1983) Temperature modification of respiratory metabolism and caloric intake of *Pandalus borealis* (Krøyer) first zoeae. *J Exp Mar Biol Ecol* 66: 163–168

Qiu J, Qian P (1999) Tolerance of the barnacle *Balanus amphitrite* to salinity and temperature stress: effects of previous experience. *Mar Ecol Prog Ser* 188:123–132

R Core Team (2016) R: a language and environment for statistical computing. https://www.Rproject.org

Reusch TBH, Dierking J, Andersson HC, Bonsdorff E, Carstensen J, Casini M et al (2018) The Baltic Sea as a time machine for the future coastal ocean. *Sci Adv* 4: eaar8195.doi: 10.1126/sciadv.aar 8195

Roger F, Godhe A, Gamfeldt L (2012) Genetic diversity and ecosystem functioning in the face of multiple stressor. *PloS One* 7(9): e45007

Rosenberg R, Costlow J (1979) Delayed response to irreversible non-genetic adaptation to salinity in early development of the brachyuran crab *Rhithropanopeus harrisii*, and some notes on adaptation to temperature. *Ophelia* 18: 97–112

Sastry AN (1983) Ecological aspects of reproduction. Pp. 179–270 *in* F. J. Vernberg and W. B. Vernberg, eds. The Biology of Crustacea. Vol. 8. Environmental Adaptations. Academic Press, New York

Schiffer M, Harms L, Lucassen M, Mark FC, Pörtner H-O, Storch D (2014) Temperature tolerance to different larval stages of the spider crab *Hyas araneus* exposed to elevated seawater PCO_2. *Front Zool* 11: 87

Selkoe KA, D'Aloia CC, Crandall ED, Iacchei M, Liggins L, Puritz JB, Von Der Heyden S, Toonen RJ (2016) A decade of seascape genetics: contributions to basic and applied marine connectivity. *Mar Ecol Prog Ser* 554: 1–19

Simith DJB, de Souza AS, Maciel CR, Fernando M, Abrunhosa FA, Diele K (2012) Influence of salinity on the larval development of the fiddler crab *Uca vocator* (Ocypodidae) as an indicator of ontogenetic migration towards off shore waters. *Helgol Mar Res* 66: 77–85

Skinner DM (1985) Molting and regeneration. In Bliss D. and Mantel L.H. (eds) The biology of Crustacea, Volume 9. New York: Academic Press, pp. 43–146

Soors J, Faasse M, Stevens M, Verbessem I, de Regge N, Van den Bergh E (2010) New crustacean invaders in the Schelde estuary (Belgium). *Belg J Zool* 140: 3–10

Torres G, Giménez L, Anger A (2007) Effects of osmotic stress on crustacean larval growth and protein and lipid levels are related to life-histories: The genus *Aramses* as a model. *Comp Biochem Phys B* 148: 209–224

Tsiamis K, Palialexis A, Stefanova K, Gladan ZN, Skejić S, Despalatović M, Cvitović I, Dragidčecvić B, Dulčić J, Vidjak O, Bojanić N, Žuljević A et al. (2019) Non-indigenous species refined national baseline inventories: a synthesis in the context of the European Union's Marine Strategy Framework Directive. *Mar Pollut Bull* 145: 429–435

van den Brink A, Godschalk M, Smaal A, Lindeboom H, McLay C (2013) Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *J Mar Biol Associ UK* 93:189–196

Vuorinen I, Hännine J, Rajasilta M, Laine P, Eklund J, Montesino-Pouzols, Corona F, Junker K, Meier HEM, Dippner JW (2015) Scenario simulations of future salinity and ecological consequence in the Baltic Sea and adjacent North Sea areas-implications of environmental monitoring. *Ecol indic* 50: 196 –205

Walther K, Anger K, Pörtner HO (2010) Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79°N). *Mar Ecol Prog Ser* 417:159–170

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York

Weersing K, Toonen RJ (2009) Population genetics, larval dispersal, and connectivity in marine systems. *Mar Ecol Prog Ser* 393: 1–12

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1: 3–14

Figures

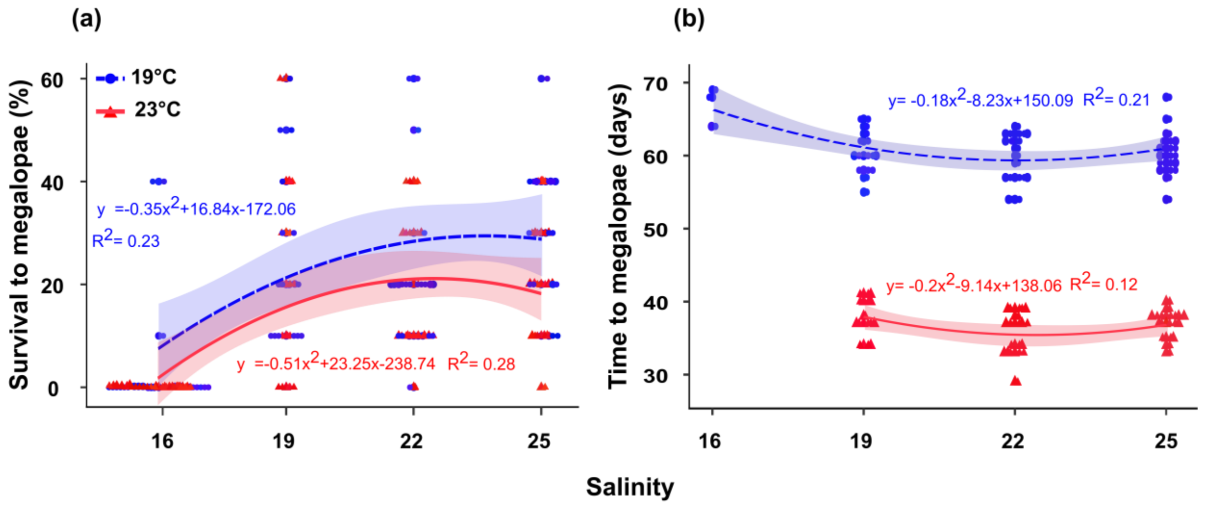

Figure 1. Influence of salinity (10–25) and temperature (19°C “blue circles” and 23°C “red triangles”) on (**a**) the percentage of larvae that reached the megalopa stage, and (**b**) the time needed to reach this stage in *Hemigrapsus takanoi* from Kiel Fjord. Salinity levels at which larvae did not reach the megalopa stage (i.e. salinities 10 and 13) were removed from the analysis, while for modelling the time needed to reach the megalopa stage, the salinity of 16 is also excluded for temperature of 23°C. Each data point represents the proportion of survivors in a single vial with 10 larvae or the mean time needed to reach the megalopa stage in a single vial. 2nd degree polynomials were fitted to the data. Coloured shaded areas represent the 95% confidence intervals of the fitted lines.

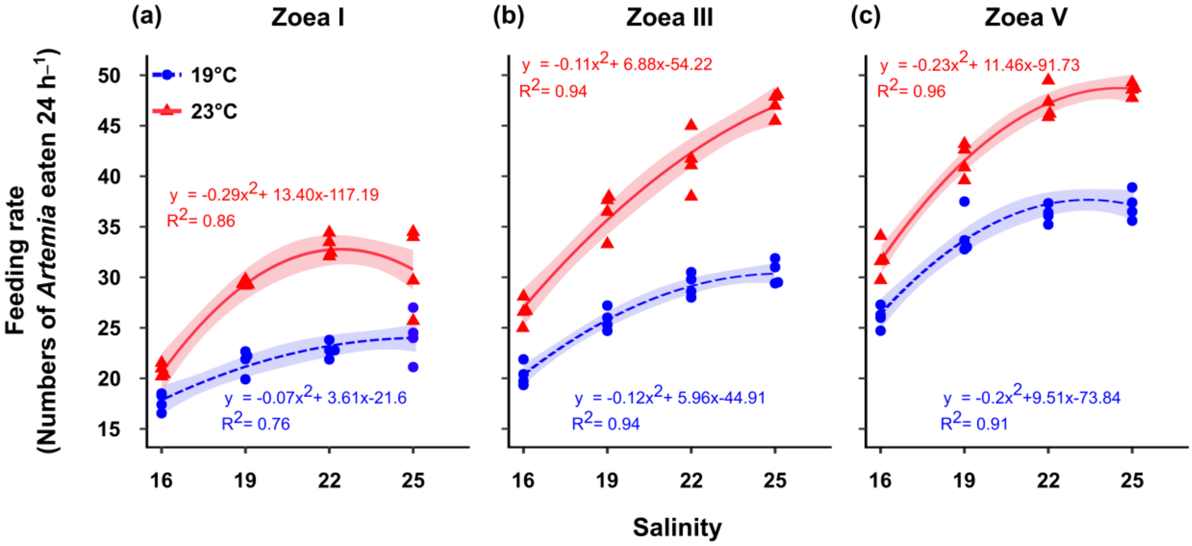

Figure 2. Influence of salinity (10–25) and temperature (19°C "blue circles" and 23°C "red triangles") on the feeding rate of **(a)** Zoea I, **(b)** Zoea III, and **(c)** Zoea V stages of *Hemigrapsus takanoi* from Kiel Fjord. Salinity levels at which larvae did not reach complete the zoea development (i.e. salinities 10 and 13) were removed from the analysis. Each data point represents the mean feeding rate of 10 separately zoea stage from a single female. Feeding rate was estimated as the number of *Artemia sp*. consumed in 24 h. 2nd degree polynomials were fitted to the data. Coloured shaded areas represent the 95% confidence intervals of the fitted lines.

Supporting information for chapter III:

Supplementary figure

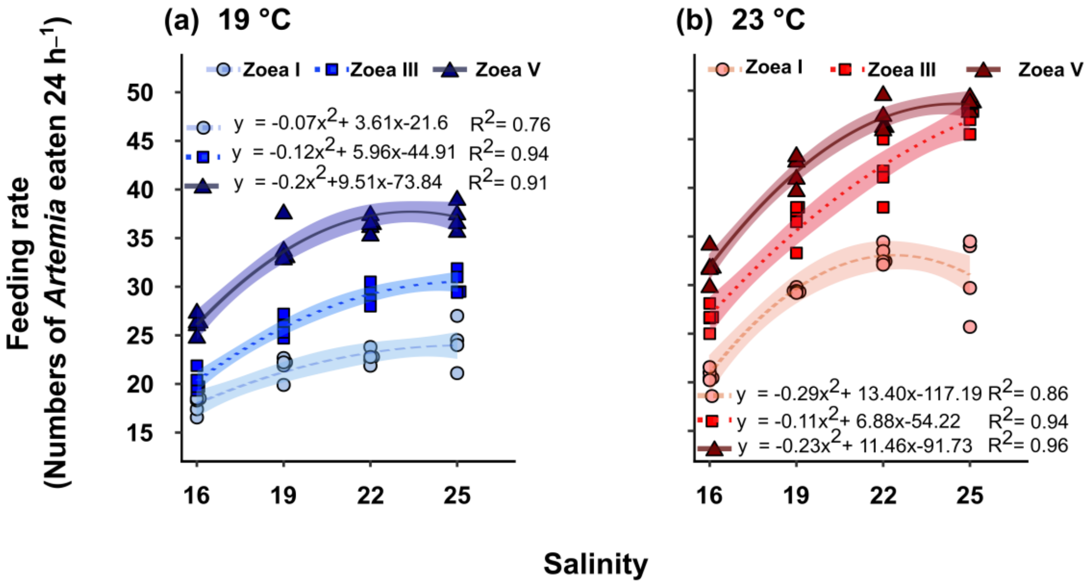

Figure S1. Influence of salinity (10–25) and different zoea stages of *Hemigrapsus takanoi* from Kiel Fjord (Zoea I “orange circles”, Zoea III “green squares”, and Zoea V “grey triangles”) on feeding rates at **(a)** 19°C, and **(b)** 23°C. Salinity levels at which larvae did not reach complete the zoea development (i.e. salinities 10 and 13) were removed from the analysis. Each data point represents the mean feeding rate of 10 separately zoea stage from a single female. Feeding rate was estimated as the number of *Artemia spp*. consumed in 24 h. 2nd degree polynomials were fitted to the data. Coloured shaded areas represent the 95% confidence intervals of the fitted lines.

4. General discussion

This thesis presents on the structure of a *H. takanoi* population from south-western Baltic Sea, and its sensitivity to low-salinity regimes as a barrier for establishment and dispersal under current and future warming scenarios.

Chapter I of this thesis evaluated *H. takanoi* population density and characteristics from inner Kiel Fjord. *H. takanoi* was monitored bi-weekly over an entire year (Chapter I). Knowledge on population body-size parameters, like size distribution, and carapace width-body weight relationship, as well as size at the onset of sexual maturity, are fundamental measures for understanding the species potential effects and ecological performances (Sharifian et al. 2017). *H. takanoi* is known as a predator on mussels from both, native (black pigmy mussel *Xenostrobus securis;* Doi et al. 2009) and non-native regions (blue mussels *Mytilus edulis*; Bouwmeester et al. 2019). In this study, I have shown that *H. takanoi* from the inner Kiel Fjord is able to open a wide size range of mussels (*M. edulis*) from 3 up to 21 mm. However, consumption rate was mainly affected by sex and season (Chapter I). In chapter II, I confirmed that salinity will be a key factor determining the establishment of the *H. takanoi* population in south-western Baltic Sea, and can be considered a barrier for further spread of the population towards the central Baltic Sea (Chapter II). Even though, this study showed the tolerance of juvenile and adult stages to survive at salinities as low as 5 for a period of 17 days, their fitness parameters (i.e. consumption rate) were yet affected by these low salinities. Furthermore, larval developmental ceased beyond the Zoea V stage in salinities at 15 and below. Survival to megalopa was with 50% survival highest at a salinity of 25. Developmental duration was not affected by salinity (Chapter II).

Thus, the complete reproductive cycle of *H. takanoi* likely takes place in the inner Kiel Fjord. This is supported by data presented in this thesis, including (1) presence of ovigerous females during the reproductive season, (2) larval development under laboratory experiments similar to Kiel Fjord prevalent conditions, (3) the presence of small juveniles after the reproductive season, and (4) presence of different larval stages in planktonic samples from inner Kiel Fjord during May–August 2016 (Geburzi et al. under preparation). However, Kiel Fjord with an average surface water salinity of 15.9 (Chapter II) might represent the edge of distribution where *H. takanoi* populations are able to complete their full life cycle. In chapter III, I systematically identified the salinity limits to be right at Kiel Fjord conditions between 16 and 19 for larval development. I was able to pinpoint a salinity of 16 as a most likely threshold below which larval development of the *H. takanoi* inner Kiel Fjord population does not complete the full life cycle (Chapter III).

Finally, in chapter III, I show evidence that climate-driven environmental variables, more specifically low salinity and increased seawater temperature as expected to be found in the Baltic Sea in the near future (Müller et al. 2016; Reusch et al. 2018), will dramatically affect *H. takanoi's* early life history stages. Elevated temperatures of 4°C above the ambient 19°C will not ameliorate the negative effect of low salinity on larval development. In contrary, this applied warming caused failure of larvae to develop in salinities of 16 and below. Moreover, a decline of larval moulting to megalopa was observed under the warming scenario at salinity levels of 19, 22 and 25, supporting this finding (Chapter III).

4.1. *Hemigrapsus takanoi* abundance in Kiel Fjord

The muddy harbours of Kiel Fjord, with plenty of shelter opportunities, provides a suitable environment for *H. takanoi* to establish. Since the one-year monitoring was conducted from only one location, the results of this study provide a general insights of *H. takanoi* population characteristics in Kiel Fjord. *H. takanoi* showed a clear pattern in monthly abundance: Numbers increased in spring and summer and declined during autumn and winter. This low abundance during the colder seasons might be due to movement of crabs into more deeper waters to avoid harsh weather, a behaviour that was also observed for the congener *H. sanguineus* during winter months (McDermott 1998). I also found clear patterns for size and biomass of this species, and its sex ratio: Males were larger and heavier than females, with slightly more females being caught by the methods applied.

The two sampling methods scraping and traps that I applied in this study, from my point of view, were sufficient to give insights on the current population status. However, my sampling methods were not effective in collecting small juveniles (≤ 5mm), and accurate monitoring of newly settled individuals could possibly have shown pulses in abundance related to recruitment processes. A total of 596 adult crabs, collected from one site over an entire year, is an obvious evidence for high abundances, however, the investigation was certainly not including all individuals available at the time. Therefore, in addition, population density was assessed in July 2019, at peak-season (as recorded in summer 2017; **Box. 4.1**). Results from this are a snapshot, however, offer a relatively accurate representation of proportional density for *H. takanoi,* and also confirmed females' dominance over males, with the same sex ratio (1.4:1) as was recorded during the 2017 long-term trap and scraping sampling (Chapter I). In addition, the data revealed that the Kiel Fjord ecosystem is capable to sustain such highly densities of *H. takanoi*, when comparing this habitat to other non-native European regions. For example, a maximum number of 60 and 18 adult *H. takanoi* individuals per m^2 were recorded along the Opal coast of France (Dauvin et al. 2009), and the Atlantic Wadden Sea (Landschoff et al. 2013), respectively, compared to a maximum of 432 individuals per m^2 recorded from inner Kiel Fjord (O Nour, unpublished data, **Box 4.1**).

Box 4.1 *Hemigrapsus takanoi* density in inner Kiel Fjord in July 2019

Aim: Snapshot identification of the total number of *H. takanoi* individuals per m^2 in the inner Kiel Fjord.

Materials and methods: A cylinder with a diameter of 237 mm, and a total area of 0.044 m^2 was deployed in the sediment in 9 different spots of the inner most part of Kiel Fjord (54°32.9′N; 10°14.8′E) on July 24th, 2019. All *H. takanoi* inside this cylinder were counted and the average number of individuals per m^2 was calculated. In addition, in order to relate this sampling effort to the methods used during the monitoring study in 2017, the 2 sample methods used in Chapter I were also applied on the same day of the snapshot sampling. A scraper covering a total area of ~ 0.28 m^2, was used for ~20 minutes (~35 scrapes), and 2 traps filled with crushed mussels were deployed for 24h.

Results*:* An average of 172 ± 139 (SD) individuals per m^2 were collected by the snapshot cylinder sampling (**Figure 4.1**), with 68 and 432 as the minimum and maximum values recorded during the sampling, respectively. A sex ratio of 1.4:1 was recorded with a dominance of females. 110 individuals per m^2 were collected by scraping, and a mean of 6.1 ± 0.7 were caught by the traps.

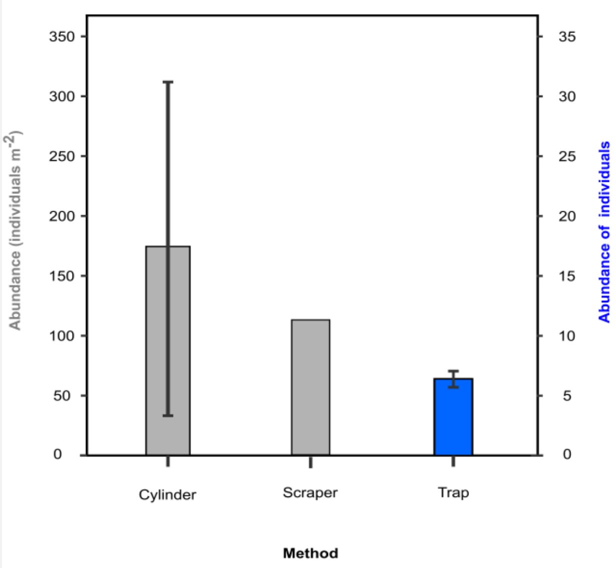

Figure 4.1 Abundance of adult individuals of *Hemigrapsus takanoi* per m^2 in the inner Kiel Fjord during a snapshot sampling in July 2019, using a cylinder with a diameter of 237 mm and scraping for ~ 20 minutes (left side y-axis), as well as the abundance of individuals found in the 2 traps after 24 h (right side y-axis). Data collected by cylinder is the mean of the 9 spots, and by the traps is the mean of the 2 traps. Error bars are standard deviations.

4.2. The current status of the Kiel Fjord *H. takanoi* population

As mentioned in the introduction (see also **Box 1.1**), invasive species gain their names after (i) establishing a population outside their native region, plus (ii) if they cause a harmful effect in their recipient regions. Whether or not *H. takanoi* dramatically alters Kiel Fjord (south-western Baltic Sea) ecosystems is discussed further in the coming sections.

From the data collected during the bi-weekly monitoring in 2017 (Chapter I), and by observing the population dynamics in July 2017, 2018, and 2019 (**Figure 4.2.1**), I suggest that there is a strong indication that *H. takanoi* remains present and abundant at the site of its original discovery in the south-western Baltic Sea (Geburzi et al. 2015). I also suggest that this population is either still in an establishment phase or at the beginning of an expansion phase (**Figure 4.2.2**). Although a large decline in abundance was recorded during summer 2018 (**Figure 4.2.1**), even with absolutely no crab collected during some days (unpublished data), an immense occurrence of juvenile numbers during autumn 2018 was seen (personal observations, ≥ 300 juveniles collected in one week by comparable sampling methods). Additionally, results from 2019 (**Figure 4.2.1**), may support the recovery of the population.

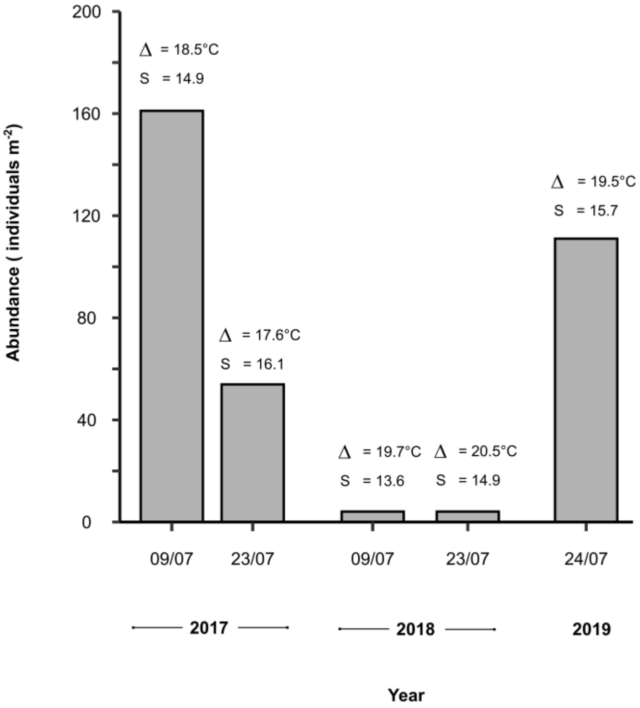

Figure 4.2.1: Abundance of adult individuals of *Hemigrapsus takanoi* per m^2 in the inner Kiel Fjord during July 2017 (Chapter I), July 2018 and July 2019 (unpublished data), collected by scraping for ~ 20 minutes. The applied scraping covered a total area of ~ 0.28 m^2. Values of temperature (Δ) and salinity (S) for each sampling date are given above the column.

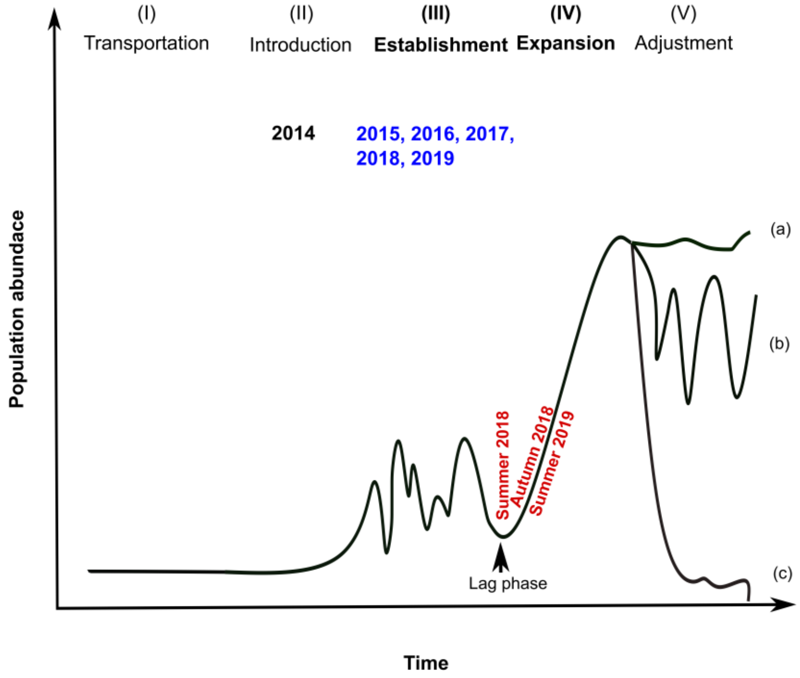

Figure 4.2.2: A hypothetical illustration of the potential invasion process (modified from Boudouresque and Verlaque 2012) of the introduced *Hemigrapsus takanoi* population in Kiel Fjord. The phases of invasion are illustrated by (I)–(V). Transportation and introduction of the species likely occurred during 2014 or earlier (Geburzi et al. 2015). The presumably current phases are highlighted in bold ((III)-Establishment or (IV)-Expansion). Tentative years and seasons are highlighted in blue and red for the presumably establishment and expansion phases, respectively. An expansion and growth of the population potentially started in autumn 2018 to summer 2019, after a breakdown with a short lag phase during a strong heatwave in summer 2018. Long-term monitoring of the current population is needed to determine the state of the population in the future and whether the population will undergo a static- (a), fluctuations- (b), or decline- (c) pattern when reaching the adjustment phase.

After their introduction, and before the phase of exponential expansion, many species undergo a lag phase in which small population sizes with very little or no population growth prevail (Crooks 2005, Lejeusne et al. 2014). The small number of crabs caught during summer 2018, which is striking considering summer is assumed to be the peak of abundance (Chapter I), may consider a very short lag time for *H. takanoi* in Kiel Fjord. An extreme event (marine heatwave) occurred in Baltic Sea during summer 2018 (Wolf et al. pers. commun.), which may have contributed to these low abundances. However, adult *H. takanoi* from Kiel Fjord population can tolerate future warming scenario (24°C) even with some peaks up to 27°C (Lugo et al. 2020). Thus, this low abundance might also be due to the movement of *H. takanoi* deeper into colder water during such extreme marine heat wave event.

In general, the pattern of *H. takanoi* population dynamics recorded in the Kiel Fjord is similar to that of Wadden Sea populations. *H. takanoi* from the Wadden Sea, after a very short lag phase (if any at all; Landschoff et al. 2013), appeared to be continuously growing and spreading (Jonas Geburzi, unpublished data). Continued work observing the populations of *H. takanoi* in

Kiel Fjord is needed to determine if the current population is going to (i) reach a relatively plateau stage, (ii) undergo strong oscillations, or (iii) sharply decline over time in the future.

4.3 Potential effects on the Baltic Sea ecosystem: competition with native crab, predation on mussel populations, and impacts on the food web

H. takanoi from non-native European waters was showed to have potentially negative effects on native juvenile crabs of the species *C. maenas,* by reducing their recruitment success (Geburzi et al. 2018), and by competing and expelling them from their shelters (van den Brink et al. 2012). In addition, the mussels' prey size range of both species overlaps, which is likely to result in resource competition between similarly sized specimens of the invasive and the native crab. However, the two different crab species may also compete over other available prey such as macroalgae or other invertebrate prey species as *Littorina* spp. or barnacles (Griffen et al. 2008; 2012).

In the western Baltic Sea, *M. edulis* is among the most abundant organisms, an important habitat builder, with about 90 % contribution to local hard bottom biomass (Enderlein and Wahl 2004; Johannesson et al. 2011), and with vital ecological roles in maintaining biodiversity (Kautsky and Evans 1987; Norling and Kautsky 2008). The present study showed that *H. takanoi* prey size preference on blue mussel *M. edulis* appeared to increase steeply with predator size. Males showed an ability to open mussels up to 21 mm, while females could open mussels up to 15 mm only. The two main factors that determine the maximum size of shelled prey that can be eaten by crab predators are 1) the size and 2) the biomechanics of the predator's claws (mainly the chela; Huges and Elner 1979). Claw size was observed to be relatively larger in males. Claw height generally increased with body size, however, this increase was three times stronger in males compared to similar-sized females, which might give males a mechanical advantage.

The results show increasing consumption rates with increasing temperature, which is likely mainly due to an increase in metabolic rates at warmer body temperatures (Wyban et al. 1995; Thomas et al. 2000). Hence, under global warming, *H. takanoi* might increase feeding pressure on *M. edulis* populations (Lugo et al. 2020). Besides, male crabs may impose more per-individual pressure on Baltic Sea mussels than females, as they consume twice as much biomass of mussels than females during spring and autumn, even though females appeared to be generally more abundant.

M. edulis coverage from the bottom of Kiel Bight showed a sharp decline since 2006 likely due to a combination of changes in different environmental factors (temperature, speed of water current, and concentration of chlorophyll-a; Franz et al. 2019a). Species diversity in the Kiel Bight was shown to generally increase in the presence of the predators *C. maenas* and *A. rubens,* mostly by controlling blue mussel dominance (Enderlein and Wahl 2004). Moreover, reduction of *M. edulis* growth was observed in the presence of *C. maenas* and *A. rubens* (Dorestov and Wahl 2001). To this point, no data are available on the impacts of *H. takanoi* on mussel growth and dominance, making it difficult to make further predictions. However, this overlap in prey size classes of mussels between native and non-native crabs of similar sizes (Bouwmeester et al. 2019), in addition to any further competitive interactions between *H. takanoi* and the two main predators (*C. maenas* and *A. rubens*) on mussels, will probably add an exert predation pressure on Baltic Sea *M. edulis* populations.

Whether *H. takanoi* will be a threat to Baltic Sea biodiversity or not is up to now not clear. Nevertheless, the trophic position of *H. takanoi* populations existing in the south-western Baltic Sea, as well as its role in the food web, will need to be investigated in the future.

H. takanoi early larval stages might play a crucial role in western Baltic Sea food webs due to predation on microzooplankton, and at the same time, as prey for planktivorous organisms such as fish larvae (Löder et al. 2011). *H. takanoi* is known to be an omnivore (Doi et al. 2009). Potentially, females and small juveniles, and as also known for the congener *H. sanguineus,* with their weak claws, may rely on others prey rather than bivalves with relatively hard shells (Brousseau and Baglivo 2006). Thus, might likely feed mainly on detritus and other plant materials and partly on other organic materials (Brousseau and Baglivo 2006; Doi et al. 2009). Furthermore, the burrowing behaviour in the mud, mainly by females of *H. takanoi,* observed during the sampling in 2017, may add some benefits to the sediments through bioturbation (Kristensen et al. 2012). In addition, *H. takanoi* from some non-native regions showed to be infected by the parasite, the acanthocephalans *Profilicollis botulus* (Goedknegt et al. 2017). *P. botulus* is native to Europe and occurs in many native European birds that are considered the definitive host of this parasite. Evidence of high mortality of eider ducks (*Somateria mollissima*) by *P. botulus* is known from European and US regions (Garden et al. 1964). *H. takanoi* can be considered an intermediate host for this parasite, and increasing numbers of intermediate hosts will, therefore, potentially lead to increasing population size of *P. botulus* in crabs, ultimately amplifying the birds' infection potential, that also rely on crabs in their diet. *H. takanoi* could therefore also negatively impact higher trophic levels in a process known as parasite ‘spillback’, meaning that when newly introduced species act as a new host for a native parasite, from which an infection could spillback to native fauna (Daszak et al. 2000, Tompkins and Poulin 2006).

4.4 Salinity effects on *H. takanoi* and different response of the different life stages

In habitats like estuarian and coastal zones, salinity is considered one of the key ecological factors, because those habitats are normally characterized by a high variability in salinity (Anger 2003). Juvenile and adult estuarian crabs are well adapted to this often-wide range of salinities in their habitats, while their pelagic larvae are often sensitive toward varying and extreme salinity conditions (Charmantier 1998; Charmantier et al. 2002).

Marine organisms are classified into two categories according to their osmoregulation capacity: (i) *Osmo-conformers* are iso-osmotic in their haemolymph compared to an external medium due to a lack of compensatory mechanisms; (ii) *Osmo-regulators*, regardless of the external medium, have the ability to regulate their internal extracellular fluids (e.g. haemolymph in crustaceans). Organisms are further classified into their salinity tolerance range into: (i) *Stenohaline* species, which have a narrow tolerance range and cannot tolerate wide fluctuations in habitat salinity, or (ii) *Euryhaline* species, which are tolerant to a wide range of salinities (for details see: Rivera-Ingraham and Lignot 2017). *H. takanoi* is known to be an osmoregulatory species (Shinji et al. 2009). In the present study, juveniles and adults of the *H. takanoi* population from Kiel Fjord showed to be rather euryhaline, with 100 % survival. The specimens tested tolerated a broad range of salinities from 5 to 35 for an experimental period of 17 days. Their feeding rates were significantly increasing with increasing salinity. These findings demonstrated a salinity preference towards higher salinities, but yet a wide tolerance to salinity variability. However, the larval response to salinity revealed that they are probably more stenohaline marine species (their lower level of salinity tolerance ~30: Remane 1934; ~24: Bulger et al. 1993). Populations of non-native species are known to be often more resistant to stressful fluctuations in their newly introduced habitats, when compared to their regions of origin (Richards et al. 2006; Lenz et al. 2011). An experimental study from the native habitat confirmed that *H. takanoi* larvae are able to reach megalopa in salinities between 10 to 35 (Mingkid et al. 2006b). From the presented data, however, *H. takanoi* larvae from Kiel Fjord occupy a narrower salinity-range niche when compared to their native region, and larvae did not develop beyond the Zoea III stage at salinities of 10 and below (Chapter II). A preference for higher salinities was indicated by the highest number of megalopae occurring at salinity 25 (Chapters II and III). Furthermore, a salinity of 16 seems to be the lower threshold salinity to complete larval development of the current *H. takanoi* Kiel Fjord population (Chapter III).

Both, a successful reproductive cycle and the settlement process play a crucial role in the establishment of a sustainable population of marine invertebrates (McFarland and Hare 2018). I can at this point only speculate that larvae are the bottleneck for the successful establishment of *H. takanoi* in the inner Kiel Fjord. An expected desalination of seawater within the Baltic Sea due to climate changes (Meier et al. 2012; Müller et al. 2016) might, however, further add pressure on *H. takanoi* larval development in the near future.

4.5 The larval salinity performance window under climate changes

Tolerance windows of a species and its optimized performance towards environmental factors are basic characters defining species fitness and survival. In addition, establishment success and geographical ranges can be determined from these measures (Pörtner 2008; Pörtner and Farrell 2008). Numerous studies revealed that combined or interactive effects of environmental factors (like pH, oxygen, temperature and salinity), might shift the performance and alter the tolerance window response of an organism compared to its response toward a single environmental parameter alone (Pörtner 2012; Madeira et al. 2014). For example, in many invertebrate taxa, thermal tolerance windows are narrowed under the effects of enhanced CO_2 and hypoxia levels (Pörtner et al. 2000 2005). Similarly, a narrowing of the thermal tolerance window of organisms exposed to high CO_2 levels are shown for bivalves and brachyuran crabs under the interactive effects of elevated seawater pCO_2 and temperature extremes (Pörtner et al. 2005; Walther et al. 2010; Schiffer et al. 2014). Due to climate change, marine species are exposed to changes in several environmental parameters, and these environmental fluctuations occur simultaneously. Thus, in the light of climate change, it is important include these simultaneous multi-stressor effects (Wahl et al. 2016). Sensitivities of different life stages to such multi-stressor interactions also require consideration (Pörtner 2012).

The present study focussed on larvae as their tolerance windows are often narrower compared to juveniles and adults (Charmantier 1998; Mingkid et al. 2006b). Two temperature levels were used corresponding to the current summer temperature conditions of 19 °C in the Baltic Sea, at the season when larval hatching and development take place. The higher temperature of 23°C was chosen based on projected warming of surface seawater within the Baltic Sea (Meier et al. 2012). These two temperatures were tested in combination with six salinity levels (10–25). Although the study showed no interaction between salinity and temperature on *H. takanoi* larval survival, higher temperature enhanced larval mortality at each salinity level. Considering a salinity of 25, in which the highest survival to megalopa was recorded, a decrease of survival to megalopa by 1.5 times was seen in 23°C compared to 19°C. In addition, the warming scenario caused a developmental failure to megalopa at salinity of 16. Moreover, potential global warming induced shifts in larval survival towards lower salinities. The best performance salinity for *H. takanoi* larval survival at 19°C was ~ 24, while at 23°C shifted to lower optimal salinity conditions ~ 22.5. In summary, warming narrows the salinity tolerance window of *H. takanoi* larval survival.

4.6 Other potential drivers of population dynamics in the Kiel Fjord

This present study highlights the magnitude of intra-population variability in responses to different environmental stressors, as only one brood out of 5 females could succeeded to reach megalopa at salinity 16 (Chapter III). Hence, the potential source of this variability may be likely due to some adaptation or a phenotypic plasticity. Developmental plasticity is a common event for early life history stages in response to environmental stress and is known for many brachyuran crabs (Giménez and Torres 2002, Storch et al. 2009). As an adaptive response toward unfavourable conditions, many crab larvae undergo development along long pathways, by adding an additional larval stage through their development (i.e. development through additional instars; Giménez 2003; González-Ortegón and Giménez 2014). However, this was not observed in the present study of *H. takanoi*, were development occurred with normal numbers of larval stages (5 Zoaea and 1 megalopa stage; Landeira et al. 2019). Intra-population genetic variations might also play a potential role in offspring variability. In general, *H. takanoi* from European coasts showed a high genetic similarity among populations (1.1% diversity among populations) and high genetic variations within population (96%; Geburzi et al. in preparation). Whatever the main driving force for this intraspecific variability in larval survival at a salinity of 16 is (Chapter III), this should increase the likelihood of successful larval recruitment and encourage population persistence (Hadfield and Strathmann 1996).

Results derived from experiments that were conducted in the same way confirmed the important interannual differences within *H. takanoi* populations. Larvae hatched from females collected in 2017 (20°C; Chapter II), at salinity 25 (recorded the highest number of survivors to the megalopa stage), showed generally higher survival (50%), and faster development (~34 days) compared to offspring of the 2018 (19°C; Chapter III) batch (survival ~30%, developmental time ~60 days), which is likely not explained by the minor temperature difference of 1 °C. Similarity, such intraspecific variation in response to thermal mitigation of low-salinity stress varied among larvae of *C. maenas* from North Sea produced by different females during different years (Spitzner et al. 2019).

In Kiel Fjord at the sampling location of *H. takanoi*, besides common natural fluctuations in salinity, two extreme events were recorded in the past three years. These can be considered harsh and extreme conditions since the potential arrival of *H. takanoi* to the western Baltic Sea, which might have affected the current population, and which may explain these interannual differences within the same population (**Figure 4.2.1**). An extreme upwelling event was recorded in September 2017 (GEOMAR website), and a heat wave during summer (June and August) 2018 (Wolf et al. pers. commun.). For the experiment conducted during 2017, ovigerous crabs were collected (mid-July to early-August 2017) before the occurrence of the hypoxic upwelling event (September 2017), which could not have affected larval developmental under laboratory conditions indicating the relatively higher larval survival rate (50%). However, the occurrence of this extreme upwelling event in the field was after the spawning of *H. takanoi* and during larval development, which might have caused a negative effects on the newly settled juveniles in the field, and consequently on the whole population (as recorded from the low abundance sampling during July 2018). This could also exert some influences on the offspring of the following year (Giménez and Torres 2002; Giménez and Anger 2003). Besides the heatwave in summer (June) 2018, as ovigerous crabs were collected in early July, all of this could be the one of the reasons for the low survival rate during the experiment in 2018. In summary, a clear correlation of these events with *H. takanoi's* performance lacks evidence and is barely a speculation, for which experimental assessments might provide proof in the future.

4.7 Factors of *H. takanoi*'s success and its potential distribution limits along the Baltic Sea

Apparently, the success of *H. takanoi* establishing in the south-western Baltic Sea, and generally in Europe, is based on a combination of factors, which were mostly analysed in this study, and which can be combined with knowledge from earlier literature. Their high fecundity and the r-selected reproductive strategy are likely the two main factors for *H. takanoi* establishment success in European water bodies (van den Brink et al. 2012, Gothland et al. 2014). Moreover, temporal shifting of recruitment periods between *H. takanoi* and native *C. maenas* is considered a main ecological factor for the success of *H. takanoi* in the intertidal Wadden Sea: The slightly different spawning seasons enhance recruitment success and ultimately population growth by reducing interspecific competition between the two species (Geburzi et al. 2018). This could also be a reason for the success in the south-western Baltic Sea, as the spawning of *C. maenas* started early in April in 2017 (personal observation) while *H. takanoi* started spawning in June in 2017 (Nour et al. 2020). Therefore, recruitment of juveniles of *C. maenas* most likely occurred at different times when compared to those of *H. takanoi*. An additional factor that could increase their success in invading European coasts is that *H. takanoi* is less infected by parasites outside their native regions and also less infected compared to the native *C. maenas* (Goedknegt et al. 2017 and references therein). Whether or not *H. takanoi* benefits from lower infection levels than the native species is, however, still unclear (Goedknegt et al. 2017).

Currently, Kiel Fjord represents the south-western most Baltic Sea area where *H. takanoi* seems able to establish a self-sustaining population (Chapters II and III). The potential of *H. takanoi* to inhabit extreme brackish environments like the Baltic Sea Proper is also known from their native regions, where adults are regularly found in estuaries with salinities as low as 7 (Mingkid et al. 2006a). However, the fact that their larvae rely on higher salinities to undergo successful development (Chapter II and III; Mingkid et al. 2006b), give a clear evidence that *H. takanoi* will probably not be able to further spread into the Baltic Proper, without appropriate acclimation and adaptation.

5. Conclusions and future research perspectives

The present study provides information about population dynamics, size and sex ratio of *H. takanoi* form south-western Baltic Sea. The study provide evidence that *H. takanoi* established a population in the Kiel Fjord (Research question 1). However, if the current population has entered its expansion phase or not, needs to be confirmed in the coming years. Furthermore, this study suggests that feeding pressure on Baltic Sea *M. edulis* likely changes in the future, mainly due to the additional pressure caused by the introduced *H. takanoi* (Research question 2, **Figure 5.1 A**). Consumption rates of *H. takanoi* increased in warmer seasons, and males consumed more food than females. The thesis also highlights direct effects of salinity on the performance of different life history stages of *H. takanoi* (Research question 3). Juveniles and adults demonstrated to be euryhaline and to be able to tolerate a wide range of salinities, while larvae were sensitive to salinities below 16. High mortality rates of larvae in response to low salinity confirmed that planktonic larval stages are ontogenetic bottleneck during the life cycle of *H. takanoi* (**Figure 5.1 B**). Furthermore, this thesis emphasizes the relevance of salinity as a main abiotic factor driving the distribution of this species in the Baltic Proper (Research question 4). Finally, larval stages are sensitive to future climate change conditions, and expected warming and desalinization might drive the distribution of *H. takanoi* back towards the Kattegat, if acclimation and adaptation capacity of this species is limited (**Figure 5.1**).

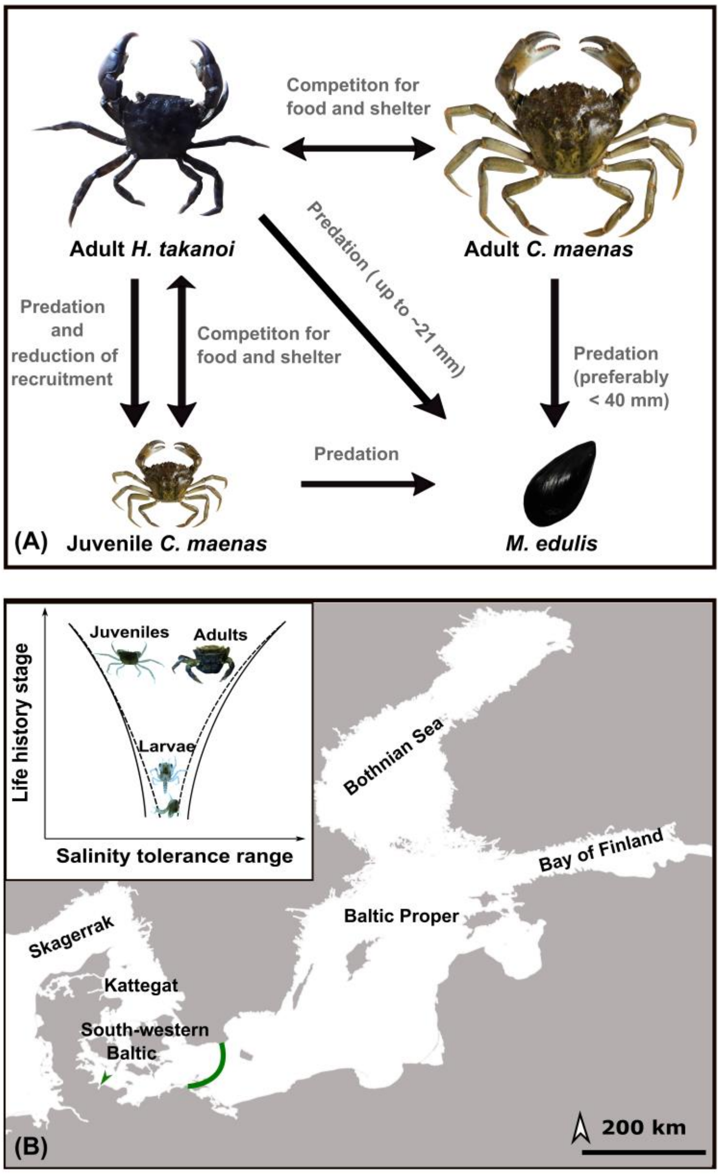

Figure 5.1 Summary figure of the thesis (**A**) Simplified illustration of the potential interactions between the non-native adult *H. takanoi* and native juvenile and adult *Carcinus maenas*, and predation competition on the common blue mussel *Mytilus edulis*. Competition for shelters and food between native *C. maenas* and non-native *H. takanoi*, and decreasing in recruitment of juveniles *C. maenas* in the presence of adults *H. takanoi* are known from previous studies (van den Brink 2012; Geburzi et al. 2018). Adults *H. takanoi* from the Baltic Sea can open mussels up to 21 mm in size (Chapter I). Similar sizes of *C. maenas* showed an overlap in their prey size classes of mussels (Bouwmeester et al. 2019). In general, *C. maenas* adults from Baltic Sea prefer mussel sizes less than 40 mm. Pictures for *C. maenas* were obtained from: www.iucngisd .org.
(**B**) Suggested restriction of *Hemigrapsus takanoi's* dispersal to the south-western Baltic Sea (green line), and its likely limitation to further invade the Baltic Proper due to limitations in low-salinity tolerance. Salinity of 16 was identified to be lower threshold salinity for larval development and to complete the life cycle in this study (Chapter III). The green arrow shows the Kiel Fjord, where *H. takanoi* was originally recorded in 2014 (Geburzi et al. 2015), and in which the species established a population (Chapter 1 and Box 4.1 in the Discussion).
An upper left insert: Schematic model of the salinity tolerance of ontogenetic stages of *H. takanoi* from the south-western Baltic Sea (modified from Walther et al. 2010 and Schiffer et al. 2014; and with regard to data collected in Chapter II and III). Increasing seawater temperature (dashed line) in the course of global warming or during heatwaves in summer may narrow the salinity tolerance of larval stages (Chapter III). Salinity tolerance of juvenile and adult stages under higher temperature were not tested.

The results of this thesis point towards two major open research questions considering several levels of biological organization:

i) *H. takanoi* population dynamics in Kiel Fjord and in response to future climate change in the Baltic Sea

As revealed by the thesis results, *H. takanoi* succeeded to establish a self-sustaining population in the inner Kiel Fjord (south-western Baltic Sea). However, whether this population will be able to adapt to the future environmental fluctuations, and as such to its extreme events, is still not clear. Long-term monitoring is a necessary measure to study the stability of the current population.

The present study focused on the effect of predicted future warming and freshening on early life history stages of *H. takanoi*, and the results showed that larvae are strongly affected by the predicted climate change scenario limiting their survival. However, climate change effects on species performance remains uncertain as long as no information about the interactive effects of all the different environmental stressors are tested. The ability of the species to adapt to these shifts, in addition, the eco-developmental potential of this species must be taken into account as well, considering all its ontogenetic stages. The presented results enable new perspectives for intra-population variability in response to salinity stress, were larvae of only one out of five females could develop to megalopa in salinity of 16 (Chapter III). This raises the question, whether this intra-population variability will allow *H. takanoi* to adapt to lower salinity in the near future, and hence, the population to spread towards the Baltic Proper. Therefore, it would be interesting to consider phenotypic plasticity and genetic variability within population under future experimental studies.

ii) Osmoregulatory capacity of ontogenetic stages of *H. takanoi*:

Different ontogenetic stages of the same species differ in their tolerance to low salinities (Charmantier 1998; Anger et al. 2008). As requirement for species establishment in a given environment, adaptation to particular salinity regimes is necessary (Charmantier 1998). The current study investigated tolerance of juveniles and adults to salinity stress for a period of 17 days as well as larval development from hatching to settlement. The results indicate that *H. takanoi* larvae are less robust towards salinity stress than juvenile and adult stages, and that larvae can be considered a bottleneck of the *H. takanoi* population from south-western Baltic Sea. Information on osmoregulatory capacity of larvae, juveniles and adults may explain the patterns observed in this study. For a complete picture of salinity tolerance of juveniles and adults, however, longer incubation times in combination with characterization of osmoregulatory capacity and performance are necessary. Several studies considered megalopa to be the bottleneck stage for successful survival and availability of a species on response to ocean acidification (Walther et al. 2010; Schiffer et al. 2014). For osmotic stress, zoaea could be sensitive to such osmoregulation process, since transportation of gases and ions between the external medium and internal body fluids takes place in the integument at all zoaea stages (Talbot et al. 1972; Felder et al. 1986). Whether *H. takanoi* possess a specific zoea stage that can be regarded as a bottleneck for osmoregulation ability, or if eggs production and hatching success will be the limiting factor under lower salinity conditions, are still unclear and should be considered in future studies.

Summarizing, despite *H. takanois* tolerance to low salinity in juvenile and adult stage, *H. takanoi* might encounter potential disadvantages during ongoing climate change, because the larval stages responded sensitive to conditions that can be expected in the future. Additional environmental variables like $p\mathrm{CO}_2$, pH, UV, nutrients, and many more, and their interactions with temperature and salinity may further affect larval development (as well as adults) and should be considered in future studies.

6. References

Alpert P (2006) The advantages and disadvantages of being introduced. *Biological Invasions* 8: 1523–1534

Anger K (2003) Salinity as a key parameter in the larval biology of decapod crustaceans. *Invertebrate Reproduction and Development* 43(1): 29–45

Anger K, Spivak E, Luppi T, Bas C, Ismael D (2008) Larval salinity tolerance of the South American salt-marsh crab, *Neohelice* (*Chasmagnathus*) *granulata*: physiological constraints to estuarine retention, export and reimmigration. *Helgoland Marine Research* 62: 93–102

Armonies W (2001) What an introduced species can tell us about the spatial extension of benthic populations. *Marine Ecology Progress Series* 209: 289–294

Asakura A, Watanabae S (2005) *Hemigrapsus takanoi*, new species, a sibling species of the common Japanese intertidal crab *H. penicillatus* (Decapoda: Brachyura: Grapsoidea). *Journal of Crustacean Biology* 25: 279–292

Ashelby CW, Sewell J, Rostron J, Shrubsole R, Child T, Clark PF (2017) Evidence for the invasion and successful establishment of *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Varunidae) in Great Britain. *Crustaceana* 90: 695–708

Azzurro E, Tuset VM, Lombarte A et al (2014a) External morphology explains the success of biological invasions. *Ecology Letters* 17:1455–1463

Azzurro E, Goren M, Diamant A, Galil B, Bernardi G (2014b) Establishing the identity and assessing the dynamics of invasion in the Mediterranean Sea by the dusky sweeper, *Pempheris rhomboidea* Kossmann & Räuber, 1877 (Pempheridae, Perciformes). *Biological Invasions* doi:10.1007/s10530-014-0836-5

Bax NJ (1999) Eradicating a dreissenid from Australia. *Dreissena*!10: 1–5

Beisel JN, Leveque C (2010) Introductions d'especes dans les milieux aquatiques: Faut-il avoir peur des invasions biologiques? Editions Quae, 232 pp

Berggren M, Karlsson R (2017) Invasiva, asiatiska krabbor längs Västkusten. *Fauna and Flora* 112: 22–26

Blackburn TM, Pyšek P, Bacher S et al (2011) A proposed unified framework for biological invasions. *Trends in Ecology and Evolution* 26: 333–339

Blasi JC, O'Connor NJ (2016) Amphipods as potential prey of the Asian shore crab *Hemigrapsus sanguineus*: laboratory and field experiments. *Journal of Experimental Marine Biology and Ecology* 474: 18–22

Boudouresque CF, Verlaque M (2002) Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Marine Pollution Bulletin* 44: 32–38

Boudouresque CF, Verlaque M (2012) An overview of species introduction and invasion processes in marine and coastal lagoon habitats. *Cahiers de Biologie Marine* 53: 309– 317

Bouwmeester MM, Waser AM, van der Meer J, Thieltges DW (2019) Prey size selection in invasive (*Hemigrapsus sanguineus* and *H. takanoi*) compared with native (*Carcinus maenas*) marine crabs. *Journal of the Marine Biological Association of the United Kingdom* 100(1): 73–77

Branch G M, Steffani CN (2004) Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). *Journal of Experimental Marine Biology and Ecology* 300:189–215

Breton G, Faasse M, Noël PY, Vincent T (2002) A new alien crab in Europe: *Hemigrapsus sanguineus* (Decapoda: Brachyura: Grapsidae). *Journal of Crustacean Biology* 22 (1): 184–189

Broecker WS (2003) The oceanic CaCO3 cycle. In the Oceans and Marine Geochemistry, Treatise on Geochemistry, ed. H Elderfield. London: Elsevier, pp. 529-49

Brousseau DJ, Baglivo JA (2005) Laboratory investigations of food selection by the Asian shore crab, *Hemigrapsus sanguineus*: algal versus animal preference. *Journal of Crustacean Biology* 25(1): 130–134

Bulger AJ, Hayden BP, Monaco ME, Nelson DM, McCormick-Ray MG (1993) Biologically-based estuarine salinity zones derived from a multivariate analysis. *Estuaries* 16: 311–322

Charmantier G (1998) Ontogeny of osmoregulation in crustaceans: a review. *Invertebrate Reproduction and Development* 33(2–3): 177–190

Charmantier G, Giménez L, Charmantier-Daures M, Anger K (2002) Ontogeny of osmoregulation, physiological plasticity, and larval export strategy in grapsid crab *Chasmagnathus granulata* (Crustacea, Decaopda). *Marine Ecology Progress Series* 229:185–194

Carlton JT (1996) Biological Invasions and Cryptogenic Species. *Ecology* 77: 1653–1655

Casties I, Seebens H, Briski E (2016) Importance of geographic origin for invasion success : A case study of the North and Baltic Seas versus the Great Lakes –St. Lawrence River region. *Ecology and Evolution* 6: 8318–8329

Caut S, Angulo E, Courchamp F (2009) Avoiding surprise effects on Surprise Island: alien species control in a multi-trophic level perspective. *Biological Invasions* 11: 1689–1703

Chown S, Sinclair BJ, Van Vuuren BJ (2008) DNA barcoding and the documentation of alien species establishment on sub-Antarctic Marion Island. *Polar Biology* 31: 651–655

Clarke Murray C, Pakhomov EA, Therriault TW (2011) Recreational boating: A large unregulated vector transporting marine invasive species. *Diversity and Distribution* 17:1161–1172

Colautti RI, Bailey SA, van Overdijk CDA, Amundsen K, MacIsaac HJ (2006) Characterised and projected costs of nonindigenous species in Canada. *Biological Invasions* 8: 45–59

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define "invasive" species. *Diversity and Distributions* 10: 135–141

Convention on Biological Diversity (2000) CBD, Nairobi, Kenya. Alien species that threaten ecosystems, habitats or species, COP 5 Decision V/8. https://www.cbd.int/decision/cop/default.shtml?id=7150 (accessed 10 December 2018)

Crooks JA (2005) Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Ecoscience* 12(3): 316–329

Crooks JA, Soule´ ME (1999) Lag times in population explosions of invasive species: causes and implications. In: Sandlund OT, Schei PJ, Viken A (eds) Invasive species and biodiversity management. Kluwer Academic Press, Dordrecht, pp 103–125

Crowl TA, Crist TO, Parmenter RR, Lugo AE (2008) The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment* 6: 238–246

Culver CS, Kuris AM (2000) The apparent eradication of a locally established introduced marine pest. *Biological Invasi*ons 2: 245–253

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

Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Scienc*e 287: 443–449

Dauvin JC, Delhay JB (2010) First record of *Hemigrapsus takanoi* (Crustacea: Decapoda: Grapsidae) on the western coast of northern cotentin, Normandy, western English channel. *Marine Biodiversity Records* 3: e101, doi:10.1017/S1755267210000928

Dauvin JC, Rius AT, Ruellet T (2009) Recent expansion of two invasive crabs species *Hemigrapsus sanguineus* (de Haan 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal coast, France. *Aquatic Invasions* 4: 451–465

Dawson M (2002) Plant quarantine: a tool for preventing the introduction and spread of alien species harmful to plants. In: Claudi R, Nantel P and Muckle-Jeffs E (eds) Alien Invaders in Canada's Waters, Wetlands and Forests. Canadian Forest Service, Ottawa, Canada, pp. 243–251

De Haan W (1835).Crustacea. In: Fauna Japonica sive Descriptio Animalium, Quae in Itinere per Japoniam, Jussu et Auspiciis Superiorum, qui Summum in India Batava Imperium Tenent, Suscepto, Annis 1823-1830 Collegit, Notis, Observationibus et Adumbrationibus Illustravit (Ed., von Siebold PF). Lugduni-Batavorum, Leiden, pp. 1-243

Dobretsov S, Wahl M (2001) Recruitment preferences of blue mussel spat (*Mytilus edulis*) for different substrata and microhabitats in the White Sea (Russia). *Hydrobiologia* 445: 27–35

Doi W, Iinuma Y, Yokota M, Watanabe S (2009) Comparative feeding behavior of invasive (*Carcinus aestuarii*) and native crabs (*Hemigrapsus takanoi*). *Crustacean Reserach* 38: 1–11

Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? *Trends in Ecology and Evolution* 14: 135–139

Enderlein P, Wahl M (2004) Dominance of blue mussels versus consumer-mediated enhancement of benthic diversity. *Journal of Sea Research* 51: 145–155

Falk-Petersen J, Bøhn T, Sandlund OT (2006) On numerous concepts in invasion biology. *Biological Invasions* 8: 1409–1424

Felder JM, Felder DL, Hand SC (1986) Ontogeny of osmoregulation in the estuarine ghost shrimp *Callianassa jamaicense* var. *louisianensis* Schmitt (Decapoda, Thalassinidea). *Journal of Experimental Marine Biology and Ecology* 99: 91–105

Franz M, Barboza FR, Hinrichsen H-H, Andreas Lehmann A, Scotti M, Hiebenthal C, Molis M, Schütt R, Wahl M (2019a) Long-term records of hard-bottom communities in the southwestern Baltic Sea reveal the decline of a foundation species. *Estuarine, Coastal and*

Shelf Science 219: 242–251

Franz M, Lieberum C, Bock G, Karez R (2019b) Environmental parameters of shallow water habitats in the SW Baltic Sea. *Earth System Science Data* 11: 947–957

Galil BS (2007) Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. *Marine Pollution Bulletin* 55: 314–322

Galil B, Marchini A, Occhipinti-Ambrogi A, Ojaveer H (2017) The enlargement of the Suez Canal-erythraean introductions and management challenges. *Management of Biological Invasions* 8(2): 141–152

García-Guerrero M, Villarreal H, Racotta IS (2003) Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 135(1): 147–154

Garden EA, Rayski C, Thom VM (1964) A parasitic disease in Eider Ducks. *Bird Study* 11: 280–287

Garside CJ, Glasby TM, Stone LJ et al (2015) The timing of *Carcinus maenas* recruitment to a south-east Australian estuary differs to that of native crabs. *Hydrobiologia* 762: 41–53

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

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

GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel. Retrieved from www.geomar.de/service/wetter

Giménez L (2003) Potential effects of physiological plastic responses to salinity on population networks of the estuarine crab *Chasmagnathus granulata*. *Helgoland Marine Research* 56: 265–273

Giménez L, Anger K (2003) Larval performance in an estuarine crab, *Chasmagnathus granulata,* is a consequence of both larval and embryonic experience. *Marine Ecology Progress Series* 249: 251–264

Giménez L, Torres G (2002) Larval growth in the estuarine crab *Chasmagnathus granulata*: the importance of salinity experienced during embryonic development, and the initial larval biomass. *Marine Biology* 141(5): 877–885

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

Goedknegt MA; Havermans J, Waser AW, Luttikhuizen PC, Velilla E, Camphuysen KCJ, van den Meer J, Thieltges DW (2017) Cross-species comparison of paraites richness, prevalence, and intensity in a native comapred to two invasive brachyuran crabs. *Aquatic Invasions* 12(2): 201–212

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

González-Ortegón E, Giménez L (2014) Environmentally mediated phenotypic links and performance in larvae of a marine invertebrate. *Marine Ecology Progress Series* 502: 185–195

Gothland M, Dauvin JC, Denis L, Dufossé F, Jobert S, Ovaert J, Pezy JP, Tous Rius A, Spilmont N (2014) Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science* 142: 41–49

Gouillieux B, Cécile Massé (2019) First record of *Monocorophium uenoi* (Stephenson, 1932) (Crustacea: Amphipoda: Corophiidae) in the Bay of Biscay, French Atlantic coast. *BioInvasions Records* 8 (1): 87–95

Gräwe U, Friedland R, Burchard H (2013) The future of the western Baltic Sea: two possible scenarios. *Ocean Dynamics* 63: 901–921

Griffen BD, Altman I, Bess BM, Hurley J, Penfield A (2012) The role of foraging in the success of invasive Asian shore crabs in New England. Biological Invasions 14: 2545–2558

Griffen BD, Altman I, Hurley J et al (2011) Reduced fecundity by one invader in the presence of another: a potential mechanism leading to species replacement. *Journal of Experimental Marine Biology and Ecology* 406: 6–13

Griffen BD, Guy T, Buck JC (2008) Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology* 77: 32–40

Grosholz E, Lovell S, Besedin E, Katz M (2011) Modelling the impacts of the European green crab on commercial shellfisheries. *Ecological Application* 21: 915–924

Guy-Haim T, Lyons DA, Kotta J, Ojaveer H et al (2018) Diverse effects of invasive ecosystem engineers on marine biodiversity and ecosystem functions: a global review and meta-analysis. *Global Change Biology* 24: 906–924

Hadfield M, Strathmann MF (1996) Variability, flexibility and plasticity in life histories of marine invertebrates. *Oceanologica Acta* 19(3–4): 323–334

Hammann M, Wang G, Boo SM, Aguilar-Rosas LE, Weinberger F (2016) Selection of heat shock resistance traits during the invasion of the seaweed *Gracilaria vermiculophylla*. *Marine Biology* 163: 104. doi:10.1007/s00227-016-2881-3

Hansen HP, Giesenhagen HC, Behrends G (1999) Seasonal and long-term control of bottom-water oxygen deficiency in a stratified shallow-water coastal system. *ICES Journal of Marine Science* 56: 65–71

Harris S, Timmins SM (2009) Estimating the Benefit of Early Control of all Newly Naturalised Plants (Science for Conservation: Vol. 292), New Zealand Department of Conservation

Hoegh-Guldberg O, Pearse JS (1995) Temperature, food availability, and the development of marine invertebrate larvae. *Integrative and Comparative Biology* 35(4): 415–425

Holopanien R, Lehtiniemi M, Miere HEM, Albertsson J, Gorokhova E, Kotta J, Viitasalo M (2016) Impacts of changing climate on the non-indigenous invertebrates in the northern Baltic Sea by the end of the twenty-first century. *Biological Invasions* 18: 3015–3032

Hughes, RN, Elner, RW (1979) Tactics of a predator, *Carcinus maenas* and morphological responses of the prey, *Nucella lapillus. Journal of Animal Ecology* 48: 65–78

IPCC (2007) Topic 1: Observed Changes and their Causes. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, Miller HL et al (Eds.), Climate change 2007. The physical science basis: Working group I contribution to the fourth assessment report of the IPCC. Cambridge, UK: Cambridge University Press

Islam QT, Razzak MA, Islam MA, Bari MI, Basher A, Chowdhury FR, Sayeduzzaman AB, Ahasan HA, Faiz MA, Arakawa O, Yotsu-Yamashita M, Kuch U, Mebs D (2011) Puffer fish poisoning in Bangladesh: clinical and toxicological results from large outbreaks in 2008. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 105(2): 74–80

Jackson DA, Mandrak NE (2002) Changing fish biodiversity: predicting the loss of cyprinid biodiversity due to global climate change. Pages 89–98 in N. A. McGinn, editor. Fisheries in a changing climate. Symposium 32. American Fisheries Society, Bethesda, Maryland

Johannesson K, Smolarz K, Grahn M, Andre C (2011) The future of Baltic Sea populations: local extinction or evolutionary rescue? *AMBIO* 40: 179–190

Karlsson R, Obst M, Berggren M (2019) Analysis of potential distribution and impacts for two species of alien crabs in Northern Europe. *Biological Invasions* 21: 3109–3119

Katsanevakis S, Zenetos A, Belchior C et al. (2013) Invading European Seas: assessing pathways of introduction of marine aliens. *Ocean and Coastal Management* 76: 64–74

Kautsky N, Evans S (1987) Role of biodeposition by Mytilus edulis in the circulation of matter and nutrients in a Baltic coastal ecosystem. *Marine Ecology Progress Series* 38: 201–212

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

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199–204

Kettunen M, Genovesi P, Gollasch S, Pagad S, Starfinger, U. ten Brink, P. & Shine, C. 2009. Technical support to EU strategy on invasive species (IAS) - Assessment of the impacts of IAS in Europe and the EU (Final module report for the European Commission). Institute for European Environmental Policy (IEEP), Brussels, Belgium. 43 pp. + Annexes (draft version, to be finalised June 2009)

Kristensen E, Penha-Lopes G, Delefosse M, Valdemarsen T, Quintana CO, Banta GT (2012) What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Marine Ecology Progress Series* 446: 285–302

Lafferty KD, Kuris AM (1996): Biological Control of Marine Pests. *Ecology* 77 (7): 1989–2000

Landeira JS, Cuesta JA, Tanaka Y (2019) Larval development of the brush-clawed shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae). *Journal of the Marine Biological Association of Uinted Kingdom* 99(5): 1153–1164

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

Lee S, Lee S-K, Rho HS, Kim W (2013) New report of the Varunid Crabs, *Hemigrapsus takanoi* and *Sestrostoma toriumii* (Crustacea: Decapoda: Varunidae) from Korea. *Animal Systematics, Evolution and Diversity* 29 (2): 152–159

Lehtiniemi M, Lehmann A, Javidpour J, Myrberg K (2011) Spreading and physico-biological reproduction limitations of the invasive American comb jelly *Mnemiopsis leidyi* in the Baltic Sea. Biological Invasions 14:341–354

Lejeusne C, Latchere O, Petit N, Rico C, Green AJ (2014) Do invaders always perform better ? comparing the response of native and invasive shrimps to temperature and salinity gradients in South-West Spain. *Estuarine, Coastal and Shelf Science* 136: 102–111

Lenz M, da Gama BA, Gerner NV, Gobin J, Frederike G, Harry A, Jenkins SR et al (2011) Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: results from a globally replicated study. *Environmental Research* 111: 943–952

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

Leppäranta M, Myrberg K (2009) Physical oceanography of the Baltic Sea. Springer-Verlag, Berlin

Lockwood JL, Cassey P, Blackburn TM (2005): The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20 (5): 223–228

Löder MGJ, Meunier C, Wiltshire KH, Boersma M, Aberle N (2011) The role of cilites, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. *Marine Biology* 158(7): 1551–1580

Lugo SCM, Baumeister M, Nour OM, Wolf F, Stumpp M, Pansch C (2020) Warming and temperature variability determine the performance of two invertebrate predators. *Scientific Reports* 10:6780, https://doi.org/10.1038/s41598-020-63679-0

Makino W, Miura O, Kaiser F, Geffray M, KatsubeT, Urabe J (2018) Evidence of multiple introductions and genetic admixture of the Asian brush-clawed shore crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae) along the Northern European coast. *Biological Invasions* 20: 825–842

Madeira D, Narciso L, Diniz MS, Vinagre C (2014) Synergy of environmental variables alters the thermal window and heat shock response: An experimental test with the crab *Pachygrapsus marmoratus. Marine Environmental Research* 98: 21–28

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

Markert A, Raupach MJ, Segelken-Voigt A, Wehrmann A (2014) Molecular identification and morphological characteristics of native and invasive Asian brush-clawed crabs (Crustacea: Brachyura) from Japanese and German coasts: *Hemigrapsus penicillatu*s (De Haan, 1835) versus *Hemigrapsus takano*i Asakura & Watanabe 2005. *Diversity and Evolution* 14: 369–382

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

McFarland K, Hare MP (2018) Restoring oysters to urban estuaries: Redefining habitat quality for eastern oyster performance near New York City. *PLoS ONE* 13 (11): e0207368

McLay CL, López Greco LS (2011) A hypothesis about the origin of sperm storage in the Eubrachyura, the effects of seminal receptacle structure on mating strategies and the evolution of crab diversity: How did a race to be first become a race to be last? *Zoologischer Anzeiger - A Journal of Comparative Zoology* 250(4): 378–406

McNeely JA (2000) The future of alien invasive species: changing social views. In Invasive species in a changing world. Edited by H.A. Mooney and R.J. Hobbs. Island Press, Washington, D.C. pp. 171–190

Meier HEM, Hordoir R, Andersson HC, Dieterich C, Eilola K, Gustafsson BG, Höglund A, Schimanke S (2012) Modeling the combined impact of changing climate and changing nutrient loads on the Baltic Sea environment in an ensemble of transient simulations for 1961 – 2099. *Climate Dynamics* 39: 2421–2441

Melzner F, Thomsen J, Koeve W, Oschlies A, Gutowska MA, Bange HA, Hansen HP, Körtzinger A (2013) Future ocean acidification will be amplified by hypoxia in coastal habitats. *Marine Biology*. doi:10.1007/s00227-012-1954-1

Minchin D (2007) Aquaculture and transport in a changing environment: overlap and links in the spread of alien biota. *Marine Pollution Bulletin* 55: 302–313

Minchin D, Gollasch S, Cohen AN, Hewitt CL, Olenin S (2009) Characterizing Vectors of Marine Invasion. In: G. Rilov, J.A. Crooks (eds.) Biological Invasions in Marine Ecosystems.Springer 2009, pp 109-116

Mingkid WM, Awika S, Watanabe S (2006a) Morphological characteristics, pigmentation, and distribution of the sibling penicillate crabs, *Hemigrapsus penicillatus* (de Haan 1835) and

H. takanoi Asakura & Watanabe, 2005 (Decapoda, Brachyura, Grapsidae) in Tokyo bay. *Crustaceana* 79: 1107–1121

Mingkid WM, Yokota M, Watanabe S (2006b) Salinity tolerance of larvae in the penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). *La Mer* 44: 17–21

Miyajima A, Fukui Y, Wada K (2012) Agonistic and mating behavior in relation to chela features in *Hemigrapsus takanoi* and *H. sinensis* (Brachyura,Varunidae). *Crustacean Research* 41: 47–58

Müller JD, Schneider B, Rehder G (2016) Long-term alkalinity trends in the Baltic Sea and their implictaions for CO2-induced acidification. *Limnology and Oceanography* 61: 1984–2002

Nausch G, Bachor A, Petenati T, Voss J, von Weber M (2011) Nährstoffe in den deutschen Küstengewässern der Ostsee und angrenzenden Gebieten (in German). *Meeresumwelt Aktuell Nord- und Ostsee* 1:1–16

Nijland R, Beekman J (2000) *Hemigrapsus penicillatus* De Haan 1835 waargenommen in Nederland. *Het Zeepaard* 60: 169–171

Noël PY, Tardy E, d'Udekem d'Acoz C (1997) Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe? Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie/Life Science 320: 741–745

Norling P, Kautsky N (2008) Patches of the mussel Mytilus sp are islands of high biodiversity in subtidal sediment habitats in the Baltic Sea. *Aquatic Biology* 4: 75–87

Nour OM, Pansch C, Lenz M, Wahl M, Clemmesen C, Stumpp M (2020) To invade or not to invade: Impaired larval development at low salinities could limits the spread of the non-native crab *Hemigrapsus takanoi* in the Baltic Sea. *submitted to Marine Biology*

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

Occhipinti-Ambrogi A (2002) Current status of aquatic introductions in Italy. In: Leppäkoski E, Gollasch S, Olenin S (eds) Invasive aquatic species of Europe: distribution, impacts and management. Kluwer Academic, The Netherlands, pp 311–324

Occhipinti-Ambrogi A, Galil B (2004) A uniform terminology on bioinvasions: a chimera or an operative tool? *Marine Pollution Bulletin* 49: 688–694

Occhipinti-Ambrogi A, Galil B (2010) Marine alien species as an aspect of global change. *Advances in Oceanography and Limnology* 1: 199–218

Occhipinti-Ambrogi A (2007) Global change and marine communities: alien species and climate change. *Marine Pollution Bulletin* 55:342–352

Ojaveer H, Galil BS, Carlton JT, Alleway H, Philippe Goulletquer P et al (2018) Historical baselines in marine bioinvasions: Implications for policy and management. *PLoS ONE* 13(8): e0202383.https://doi.org/10.1371/journal.pone.0202383

Okamoto K, Kurihara Y (1987) Seasonal variation of population structure of *Hemigrapsus penicillatus* (De Haan) (Crustacea: Brachyura). *Japanese Journal of Ecology* 37:81–89 (in Japanese with English abstract)

Otto T, Brandis D (2011) First evidence of *Eriocheir sinensis* reproduction from Schleswig-Holstein, Northern Germany, Western Baltic Sea. Aquatic Invasions 6 (Supplement 1): S65–69

Paavola M, Olenie S, Leppäkoski E (2005) Are invasive species most successful in habitats of low native species richness across European brackish water seas? *Estuarine, Coastal and Shelf Science* 64(4): 738 –750

Paiva F, Barco A, Chen Y, Mirzajani A, Chan FT, Lauringson V, Baltazar-Soares M et al (2018) Is salinity an obstacle for biological invasions? *Global Change Biology* 24(6):2708–2720

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology and Systematic* 37: 637–669

Pauli NC, Briski E (2018) Euryhalinity of Ponto-Caspian invader in their native and introduced regions. Aquatic Invasions 13:439–447

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in marine fishes. *Science* 308: 1912–1915

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

Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of non-indigenous species in the United States. *BioScience* 50: 53–65

Poff NL, Brinson MM, Day JW (2002) Aquatic ecosystems and global climate change: potential impacts on inland freshwater and coastal wetland ecosystems in the United States. Pew Center on Global Climate Change, Arlington, Virginia, p 56

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: A physiologist's view. *Marine Ecology Progress Series* 373: 203–217

Pörtner HO (2012) Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series* 470: 273–290

Pörtner HO, Bock C, Reipschläger A (2000) Modulation of the cost of pHi regulation during metabolic depression: a 31P-NMR study in invertebrate (*Sipunculus nudus*) isolated muscle. *Journal of Experimental Biology* 203: 2417–2428

Pörtner HO, Farrell AP (2008) Ecology: Physiology and climate change. *Science* 322 (5902): 690–692

Pörtner HO, Langenbuch M, Michaelidis B (2005) Synergistic effects of temperature extremes, hypoxia, and increases in CO2 on marine animals: from earth history to global change. *Journal of Geophysics Research* 110:C09S10

Rabalais NN, Turner RE, Wiseman WJ (2002) Gulf of Mexico hypoxia, aka "The dead zone". *Annual Review of Ecology and Systematics* 33: 235–263

Rahel FJ, Olden JD (2008): Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* 22 (3): 521–533

Reise K, Buschbaum C, Büttger H, Wegner KM (2017a) Invading oysters and native mussels: from hostile takeover to compatible bedfellows. *Ecosphere*. https://doi.org/10.1002/ecs2.1949

Reise K, Buschbaum C, Büttger H, Rick J, Wegner KM (2017b) Invasion trajectory of Pacific oysters in the northern Wadden Sea. Marine Biology 164: 68. DOI 10.1007/s00227-017-3104-2

Remane A (1934) Die Brackwasserfauna. *Verhandlungen Der Deutschen Zoologischen Gesellschaft* 36: 34–37

Reusch TBH, Dierking J, Andersson HC, Bonsdorff E, Carstensen J, Casini M et al (2018) The Baltic Sea as a time machine for the future coastal ocean. *Science Advances* 4: eaar8195.doi: 10.1126/sciadv.aar 8195

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9: 981–991

Rivera-Ingraham GA, Lignot J-H (2017) Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the question for future research. *Journal of Experimental Biology* 220: 1749–1760

Ruiz GM, Fofonoff PW, Carlton JT et al (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31:481–531

Sakai T (1976) Crabs of Japan and the Adjacent Seas. Kodansha, Tokyo [In 3 volumes: (1) English text, xxix+773pp. (2) Plates volume, 16pp., 251pls. (3) Japanese text, 461pp.]

Sakai AK, Allendorf FW, Holt JS, Lodge DM et al (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332

Salisbury J, Green M, Hunt C, Campbell J (2008) Coastal acidification by rivers: a threat to shellfish? *Eos, Transactions American Geophysical Union* 89(50): 513–528

Sax DF, Brown JH, White EP, Gaines SD (2005a) The dynamics of species invasions. invasions: Insights into the mechanisms that limit species diversity. *In*, Sax, D.F., Stachowicz, J.J., and Gaines, S.D., editors. *Species Invasions: Insights into Ecology, Evolution and Biogeography.* Sinauer Associates, Sunderland, MA

Sax DF, Stachowicz JJ, Brown JH et al (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution* 22: 465–471

Schiffer M, Harms L, Lucassen M, Mark FC, Pörtner H-O, Storch D (2014) Temperature tolerance to different larval stages of the spider crab *Hyas araneus* exposed to elevated seawater PCO_2. *Frontiers in Zoology* 11: 87. DOI 10.1186/s12983-014-0087-4

Schmidt C, Morard R, Prazeres M, Barak H, Kucera M (2016) Retention of high thermal tolerance in the invasive foraminifera *Amphistegina lobifera* from the Eastern Mediterranean and the Gulf of Aqaba. *Marine Biology* 163:228. doi:10.1007/s00227-016-2998-4

Seebens H, Gastner MT, Blasius B (2013) The risk of marine bioinvasion caused by global shipping. *Ecology Letters* 16:782–790

Seebens H, Blackburn TM, Dyer EE, Genovesi P et al (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications* 8: 14435, https://doi.org/10.1038/ncomms14435

Sharifian S, Kamrani E, Safaie M, Sharifian S (2017) Population structure and growth of freshwater crab *Sodhiana iranica* from the south of Iran. *Fundamental and Applied Limnology* 189(4): 341–349

Sharma S, Jackson DA, Minns CK, Shuter BJ (2007) Will northern fish populations be in hot water because of climate change? *Global Change Biology* 13: 2052–2064

Shine C, Kettunen M, Genovesi P, Essl F, Gollasch S, Rabitsch W et al (2010). Assessment to support continued development of the EU Strategy to combat invasive alien species. Brussels, Belgium: Institute for European Environmental Policy (IEEP)

Shinji J, Strussmann CA, Wilder MN, Watanabe S (2009) Short-term responses of the adults of the common Japanese intertidal crab, *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsoidea) at different salinities: osmoregulation, oxygen consumption, and ammonia excretion. *Journal of Crustacean Biology* 29 (2):269–272

Simberloff D (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology and Systematics* 40: 81–102

Simberloff D (2011) Non-natives: 141 scientists object. *Nature* 475:36. doi:10.1038/475036a

Simberloff D, Gibbons L (2004) Now you see them, now you don't! Population crashes of established introduced species. *Biological Invasions* 6: 161–172

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

Simberloff D, Souza L, Nuñez M, Barrios-Gracia MN, Bunn W (2012) The natives are restless, but not often and mostly when disturbed. *Ecology* 93(3): 598–607

Smith A, Hewitt N, Klenk N, Bazely DR, Yan N, Wood S, Henriques I, MacLellan JI, Lipsig-Mummé C (2012) Effects of climate change on the distribution of invasive alien species in Canada: a knowledge synthesis of range change projections in a warming world. *Environmental Reviews* 20: 1–16

Smyth K, Elliott M (2016) Effects of changing salinity on the ecology of the marine environment. In: Solan M, Whiteley NM (eds), Stressors in the marine environment. Oxford University Press, Oxford, UK, pp 356, https://doi.org/10.1093/acprof:oso/9780198718826.003.0009

Snoeijs-Leijonmalm P, Schubert H, Radziejewska T (2017) Biological oceanography of the

Baltic Sea. Springer, Dordrecht, pp. 683

Soors J, Faasse M, Stevens M, Verbessem I, de Regge N, Van den Bergh E (2010) New crustacean invaders in the Schelde estuary (Belgium). *Belgian Journal of Zoology* 140: 3–10

Spitzner F (2018) Effects of environmental stress on early life history stages of the European shore crab *Carcinus maenas* Linnaeus 1758 (Brachyura: Portunidae). PhD Thesis, University of Greifswald

Spitzner F, Giménez L, Meth R, Harzsch S, Torres G (2019) Unmasking intraspecific variation in offspring responses to multiple environmental drivers. *Marine Biology* 166:112https://doi.org/10.1007/s00227-019-3560-y

Stachowicz JJ, Terwin JR, Whitlatch RB, Osman RW (2002) Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. *Proceeding of the National Academy of Sciences of the United States of America* 99: 15497–15500

Stigebrandt A (2001) Physical oceanography of the Baltic Sea. In: Wulff, F., Rahm, L.,Larsson, P. (Eds.), A systems analysis of the Baltic Sea. Springer Verlag, pp. 19–74

Storch D, Santelices P, Barria J, Cabeza, K, Pörtner H-O, Fernández M (2009) Thermal tolerance of crustacean larvae (zoea I) in two different populations of the kelp crab *Taliepus dentatus* (Milne-Edwards). *Journal of Experimental Biology* 212: 1371–1376

Takano M, Ikeda M, Kijima A (1997) Biochemical and morphological evidence of two sympatric forms, interpreted as sibling species, in the estuarine grapsid crab *Hemigrapsus penicillatus* (De Haan). *Benthos Research* 52: 111–117

Talbot P, Clark WH, Lawrence AL (1972) Light and electron microscopic studies on osmoregulatory tissue in the developing brown shrimp, *Penaeus aztecus*. *Tissue and Cell* 4: 271–286

Thieltges DW, Strasser M, Beusekom JEE van, Reise K (2004) Too cold to prosper-winter mortality prevents population increase of the introduced American slipper limpet *Crepidula fornicata* in northern Europe. *Journal of Experimental Marine Biology and Ecology* 311:375–391

Thomas CW, Crear BJ, Hart PR (2000) The effect of temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, *Jasus edwardsii*. *Aquaculture* 185: 73–84

Thomsen J, Gutowska MA, Saphörster J, Heinemann A, Trübenbach K, Fietzke J, Hiebenthal C, Eisenhauer A, Körtzinger A, Wahl M, Melzner F (2010) Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* 7: 3879–3891

Tompkins DM, Poulin R (2006) Parasites and biological invasions. *Biological Invasions in New Zealand* 186: 67–84

Tsiamis K, Palialexis A, Stefanova K, Gladan ZN, Skejić S, Despalatović M, Cvitović I et al (2019) Non-indigenous species refined national baseline inventories: a synthesis in the context of the European union’s marine strategy framework directive. *Marine Pollution Bullet* 145: 429–435

Ulman A, Ferrario J, Occhpinti-Ambrogi A, Arvanitidis C, Bandi A, Bertolino M et al (2017) A massive update of non-indigenous species records in Mediterranean marinas. *PeerJ* 5:e3954; DOI 10.7717/peerj.3954

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

van den Brink AM, Wijnhoven S, McLay CL (2012) Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta. *Journal of Sea Research* 73:126–136

Väinölä, R, Hvilsom MM (1991) Genetic divergence and a hybrid zone between Baltic and North Sea Mytilus populations (Mytilidae: Mollusca). *Biological Journal of the Linnean Society* 43: 127–148

Vuorinen I, Hännine J, Rajasilta M, Laine P, Eklund J, Montesino-Pouzols; Corona F, Junker K, Meier HEM, Dippner JW (2015) Scenario simulations of future salinity and ecological consequence in the Baltic Sea and adjacent North Sea areas-implications of environmental monitoring. *Ecological Indicators* 50: 196 –205

Wahl M, Saderne V, Sawall Y (2016) How good are we at assessing the impact of ocean acidification in coastal systems? Limitations, omissions and strengths of commonly used experimental approaches with special emphasis on the neglected role of fluctuations. *Marine and Freshwater Research* 67: 25– 36

Walther K, Anger K, Pörtner HO (2010) Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79°N). *Marine Ecology Progress Series* 417:159–170

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. *Nature* 416: 389–395

Watanabe S (2003) Structure and distribution of crustacean resources. Pp. 94–108 in J. Otomi and S. Watanabe, eds. Crustacean Resources and their Diversity. Kouseisha Kouseikaku, Tokyo. [In Japanese.]

Weis JS (2010) The role of behavior in the success of invasive crustaceans. *Marine and Freshwater Behaviour and Physiology* 43: 83–98

Witte S, Christian Buschbaum C, van Beusekom JEE, Reise K (2010) Does climatic warming explain why an introduced barnacle finally takes over after a lag of more than 50 years? *Biological Invasions* 12: 3579–3589

Williamson M (2006) Explaining and predicting the success of invading species at different stages of invasion. *Biological Invasions* 8: 1561–1568

Wood CA, Bishop JDD, Davies CJ, Delduca EL, Hatton JC, Herbert RJH, Clark PF (2015) *Hemigrapsus takanoi* Asakura and Watanabe, 2005 (Crustacea: Decapoda: Brachyura: Grapsoidea): first records of the brush-clawed shore crab from Great Britain. *BioInvasions Records* 4: 109–113

Wyban J, Walsh WA, Godin DM (1995) Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquacultur*e 138: 267–279

Yamasaki I, Doi W, Mingkid WM, Yokota M, Strüssmann CA, Watanabe S (2011) Molecular-based method to distinguish the sibling species *Hemigrapsus penicillatus* and *Hemigrapsus takanoi* (Decapoda: Brachyura:Varunidae). *Journal of Crustacean Biology* 31: 577–581

Zatisev YP (1992) Recent changes in the trophic structure of the Black Sea. *Fisheries Oceanography* 1(2): 180–188

Zettler ML, Karlsson A, Kontula T, Gruszka P, Laine AO, Heke K, Scheiel KS, Maximov A, Haldin J (2014) Biodiversity gradient in the Baltic Sea: a comprehensive inventory of macrozoobenthos data. *Helgoland Marine Research* 68: 49–58

Acknowledgement

First and foremost, I want to thank my main supervisor **Prof. Dr. Martin Wahl** for giving me the opportunity to conduct my PhD in the Benthic Ecology Group. Thanks for lots of support and for answering various kinds of questions. Thanks also for being available when needed, even with very short notice. With your enthusiasm, inspiration, and great ability to explain things clearly and simply, you broadened my perspective of marine biology. I doubt that I will ever be able to convey my appreciation fully, but I owe you my eternal gratitude. Thank you very much for everything, Martin.

I want to express my sincere gratitude to my co-advisor **Dr. Frank Melzner** for his insightful comments and encouragement, but also for critical discussions which inspired me to widen my research from various perspectives and supported interdisciplinary approaches.

I am deeply thankful to my co-advisors **Dr. Meike Stumpp** and **Dr. Christian Pansch**. Meike and Christian, you have been very good supervisors to me, and I want to thank you for your patience, motivation and immense knowledge which you were always willing to share with me. Thanks also for trusting my abilities, and for sitting and listening to every single miniscule detail, roadblock, or acute problem I might have had. Although I might sometimes have bored you, you always were understanding, putting up with those issues with big smiles on your faces and good hearts inside, encouraging my never dying passion for science. Thanks for the weekly constructive meetings, where I received useful advice for performing my experiments and good ideas for the analysis and interpretation of the data. Your great ideas, solutions, meticulous comments, and guidance helped me throughout the research and writing of the thesis. I could not have imagined having a better advisors and mentors for my PhD study.

A very special and hearty thank you is for you, **Meike**, for offering me an office beside you at the University during the last two years. Rather than just being my co-advisor, you are even more so my mentor, my tutor, my role model, and my never-ending motivation to obtain success in my endeavour. I say this with a whole heart, full esteem and gratitude for you. I could literally have never done this without you. I hope one day to make you proud of me.

I am deeply grateful for the support of **Björn** in diverse forms, particularly during laboratory work; thanks for many creative and helpful ideas for the technical setup of experiments. These inputs made this work possible. I additionally express my thanks to **Dr. Mark Lenz** for his statistical help. Furthermore, I would like to thank everybody in the Benthic Ecology Group of the GEOMAR for being great colleagues and for offering help, whenever difficulties were mentioned. The friendly environment definitely helped make long days spent in the constant temperature room or the office a much more pleasant experience. In particular, thanks to **Markus, Guido, Thea, Jahan, and Fabian** who were not only supporting me in sampling and laboratory works, but also supported me with encouraging words, creative and helpful ideas, and for always offering me an open ear. Also, it does not go without saying thank you for many people for their help during sampling work, especially: **Sonia, Laura, Jenny, Claudia, Trystan, Sophie, Christian, and Frank**.

I also would like to thank **Dr. Jonas Geburzi**, for sharing your knowledge and ideas with me throughout my PhD, as well as for your quick replying to my emails and requests. In particular, thanks for teaching me how to catch crabs and handle them at the beginning of my PhD.

I am grateful to the German Academic Exchange Service (DAAD) for providing me with a scholarship and the possibility to take part in many interesting activities. Thanks to **Avan Anita** and the Integrated School of Ocean Sciences (ISOS). I am pleased in having participated in different courses that helped me develop my PhD thesis. Thanks also for supporting my visits to conferences.

My biggest thanks go to my four best friends: **Marco, Maysa, Claudia, and Luisa.** You are the best gift that Germany gave me. Thanks for being my family in Kiel. Your friendship and love are what makes life colourful. It’s hard to express how thankful and lucky I am that we have met. You made me strong and kept me going, especially during certain difficult situations during my PhD. Thanks for the nice time and warm hosting in Pizzighettone, Cremona, Parma, and Venice. Thanks also for the many nice evening that we spent togethers playing “UNO”, chatting, and cooking. Thanks for many jokes and laughs, and for many delicious Italian and Brazilian foods. I am looking forward to the long way we will go together. Luisa, thank you very much for translating the summary of this thesis.

Finally, I am immeasurably grateful to my family for supporting and encouraging me. I am deeply and forever indebted to my father (**Salah El-Din**); thank you, dad, for being supportive in all aspect of my life. Thanks for believing in me and inspiring me to follow my dreams; without your love, encouragement and assistance, I would not have finished this thesis. Thanks to my brothers and my sisters in law, for unconditional love, support and always cheering me up despite long distances.

I dedicate this thesis to my father and the soul of my mother; Mum: long time ago since you passed away, but you are and will always be in my heart.

Declaration

I, Ola Mohamed Nour, hereby declare that the dissertation submitted, entitled "**The non-native crab *Hemigrapsus takanoi* in the south-western Baltic Sea: Population structure and sensitivity to salinity and temperature shifts**" was written independently by me and only using the sources listed. The content and design of this thesis, apart from the supervisor's guidance, is my own work. The thesis has not been submitted either partially or wholly as a part of a doctoral degree to another examining body and is my first and only doctoral procedure. Chapter I of this thesis was published in the scientific Journal of Aquatic Invasions in 2020. The authors' share of the manuscripts is explained in the section "Chapters and contributions of authors" (page 25). This work has been prepared respecting the Rules of Good Scientific Practice of the German Research Foundation. I have not been deprived of an academic degree.

Kiel, 29.06.2020 …………………………………...

Ola Mohamed Nour