



# **A CENTURY OF CHINESE MITTEN CRAB INVASION**

Lessons learnt on the ecology and behaviour  
of the species in Belgium

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## Summary

Non-native invasive species pose a significant threat to biodiversity and ecosystem services worldwide. In today's increasingly connected world, the rate at which such species are introduced and become established has outpaced our ability to manage them effectively. In an effort to turn the tide, the European Union has established a list of non-native invasive species of 'Union concern', for which member states must implement measures to prevent introduction, limit spread, and mitigate impact. One of the species on this list is the Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards 1853). The Chinese mitten crab is an example of a highly successful aquatic invader, having established populations across Northwestern Europe and North America. Nevertheless, despite its invasion success and long invasion history, much of its ecology, behaviour and impact in non-native regions remain poorly understood. This thesis examines the ecology of the Chinese mitten crab in Belgium, where the species has persisted for nearly a century.

Understanding the life cycle of a species is essential, as life history patterns drive spatiotemporal variation in abundance. This knowledge is key for assessing its ecology, evaluating its impacts, and developing effective management strategies. Monitoring data from four crab traps across Flanders (northern Belgium) were used to determine the regional timing of key phases in the species' catadromous life cycle and to estimate age and size at maturity. In addition, acoustic telemetry revealed the trajectory, behaviour, and timing of adult crabs during their downstream spawning migration.

Reproduction occurs in brackish to marine environments between October and late May, along the Belgian coast and in the lower sections of the Scheldt Estuary. Mature crabs, with recorded carapace widths ranging from 33 to 87 mm, remain in these areas until summer and die after completing reproduction. Megalopa settlement, which marks the end of the larval development, likely takes place in two periods: autumn, and from spring to summer. Both megalopae and early juvenile crabs then migrate upstream to brackish and freshwater tidal habitats, where they remain until they grow large enough to migrate further. Juveniles measuring 15 to 25 mm typically migrate further upstream to non-tidal freshwater habitats between February and June, peaking from March to May, though some upstream movement may occur year-round. In certain areas, individuals may reach maturity in tidal areas without further upstream migration. Across the longitudinal range of the river basin, our results suggest clinal variation in size and age at maturity, with upstream populations reaching larger sizes

and older ages before reproducing. We estimate that mature crabs are aged three to six years when they commence migrating downstream from September to December for their final reproductive phase.

By tracking individual crabs with acoustic telemetry, we uncovered adaptive differences in the behaviour of migrating adult mitten crabs between non-tidal and tidal environments. In shallow, non-tidal freshwater rivers, adult mitten crabs migrate primarily at night, likely as a predator-avoidance strategy. By using the unidirectional current, they can achieve migration speeds of up to 15 km day<sup>-1</sup>, with an average of 4.65 km day<sup>-1</sup>. In deeper tidal rivers, movements are less synchronised with diel rhythms but are instead linked to the tidal cycle. Chinese mitten crabs use the ebb tide to move downstream, a strategy known as selective tidal stream transport, which likely reduces the energetic cost of migration. In these tidal environments, migration is slower, averaging at 1.29 km day<sup>-1</sup>. The tracking data indicate that crabs reach the estuary in one to two months and stop their migration at the border between the mesohaline and polyhaline zone of the estuary.

Furthermore, this thesis examines the interactions between the Chinese mitten crab and its environment, highlighting the species' ecological impact. As an omnivore, the Chinese mitten crab likely impacts multiple aspects of its environment by reducing macrophyte abundance and diversity, altering benthic invertebrate communities, changing the quantity and form of organic matter, and increasing turbidity and nutrient concentrations. Furthermore, the Chinese mitten crab is a burrowing species capable of creating extensive burrow networks in tidal areas, which may influence various ecosystem processes.

An experimental setup was used to examine how their detritivorous feeding behaviour influences organic matter and water quality. Both juvenile and adult Chinese mitten crabs were shown to be effective detritivores, which can feed on diverse types of terrestrial leaf litter. Adult mitten crabs substantially accelerated leaf litter breakdown rates of all litter types tested, whereas the effect of juvenile crabs was limited. Their feeding behaviour and excretions increased the production of fine particulate organic matter, dissolved nitrogen concentrations, and the biological oxygen demand. Through this behaviour, Chinese mitten crabs have the potential to accelerate leaf litter breakdown and nutrient cycling in freshwater habitats.

Using an extensive field study in both a restored and natural freshwater tidal marsh along the Scheldt Estuary, combined with laboratory experiments, we established morphometric relationships between crabs and their burrows, and examined spatial

and temporal patterns in burrow density and size. The burrows were typically ellipsoid, with opening dimensions, length, and volume scaling positively with crab size. Burrow densities differed between the two marshes and showed temporal variation at one site, with higher burrow densities from late spring to autumn following an influx of juvenile crabs. Higher burrow densities were generally associated with steep banks and areas near the marsh inlet, while larger burrows occurred on steep slopes, in fine-grained sediments, and on banks with minimal sediment turnover. In the restored tidal marsh, high burrow densities ( $\geq 30$  burrows  $m^{-2}$ ) were linked to riverbank erosion, whereas low, temporally stable densities showed no association with erosion or sedimentation in the natural marsh. These results indicate that at high densities, Chinese mitten crab burrowing can modify marsh habitats, with implications for sediment dynamics and ecosystem functioning.

In its non-native range, little is known about the prevalence and physiological effects of the microsporidian pathogen *Hepatospora eriocheir*, a natural enemy of the Chinese mitten crab. To investigate this, adult crabs were collected during the same period from two non-native populations and subjected to identical physiological tests. Infection prevalence ranged from 27 % in the Scheldt population (Belgium) to 38 % in the Elbe population (Germany). Physical exercise trials revealed that infected crabs exhibited a reduced aerobic scope compared with uninfected individuals. Moreover, analyses of haemolymph glucose and hepatopancreas lipid content indicated depleted energy reserves in infected crabs, likely due to direct resource consumption by this intracellular parasite, which in turn reduces the capacity of its host for energetically demanding activities.

Finally, the findings of this thesis are synthesized and their relevance to the Chinese mitten crab's invasion success is discussed. In addition, the ecological and socio-economic impacts of the species in Flanders are evaluated, and recommendations for management are provided.

Collectively, the findings of this thesis provide a deeper understanding of the ecology of the Chinese mitten crab in its non-native range and highlight the biological traits underlying its invasion success and ecological impact in Belgium. Nevertheless, further field studies are needed to assess the species' broader ecological effects, and follow-up research on management measures, such as the crab trap, is essential to mitigate the impacts of this invasive species in regions where it is well-established.

## Samenvatting

Invasieve uitheemse soorten vormen wereldwijd een belangrijke bedreiging voor biodiversiteit en ecosysteemdiensten. In een wereld die steeds meer met elkaar verbonden is, gaat het tempo waarmee dergelijke soorten worden geïntroduceerd en zich vestigen sneller dan onze capaciteit om ze effectief te beheren. Om dit tegen te gaan, heeft de Europese Unie een lijst ('de Unielijst') opgesteld van uitheemse invasieve soorten die als zorgwekkend voor de Unie worden beschouwd, waarvoor de lidstaten maatregelen moeten implementeren om introductie te voorkomen, verspreiding te beperken en de impact te mitigeren. Een van de soorten op deze lijst is de Chinese wolhandkrab (*Eriocheir sinensis*, H. Milne-Edwards 1853). De Chinese wolhandkrab is een voorbeeld van een bijzonder succesvolle aquatische invasieve soort, met gevestigde populaties in Noordwest-Europa en Noord-Amerika. Ondanks het invasiesucces en de lange invasiegeschiedenis van de soort, blijft veel over haar ecologie, gedrag en impact in geïntroduceerde gebieden onduidelijk. Deze thesis onderzoekt de ecologie van de Chinese wolhandkrab in België, waar de soort al bijna een eeuw aanwezig is.

Het begrijpen van de levenscyclus van een soort is essentieel, omdat het de ruimtelijke en temporele variatie in abundantie bepaalt. Deze kennis is nodig om haar ecologie te bestuderen, de impact te evalueren en effectief beheer mogelijk te maken. Op basis van monitoringsgegevens van vier krabbenvallen in Vlaanderen werd de regionale timing van belangrijke fasen in de katadrome levenscyclus van de soort vastgesteld en werden leeftijd en grootte bij maturiteit bepaald. Daarnaast werd met akoestische telemetrie het traject, gedrag en de timing van volwassen krabben tijdens hun stroomafwaartse voortplantingsmigratie in detail in kaart gebracht.

De voortplanting van de Chinese wolhandkrab vindt plaats in brakwater- tot mariene omgevingen tussen oktober en eind mei, langs de Belgische kust en in de lagere delen van het Schelde-estuarium. Volwassen krabben met een carapaxbreedte van 33 tot 87 mm blijven in deze gebieden tot de zomer en sterven nadat de voortplanting voltooid is. De vestiging van megalopae, wat het einde van de larvale ontwikkeling markeert, gebeurt waarschijnlijk in twee perioden: in de herfst en van lente tot zomer. Megalopae en jonge juveniele krabben migreren stroomopwaarts naar brak- en zoetwatergetijdengebieden, waar ze blijven totdat ze voldoende gegroeid zijn om verder te migreren. Juveniele krabben van 15 tot 25 mm migreren vervolgens verder stroomopwaarts naar niet-getijdengebonden zoetwaterhabitats tussen februari en juni, met een piek van maart tot mei, hoewel beperkte stroomopwaartse beweging het

hele jaar door kan plaatsvinden. In bepaalde gebieden kunnen individuen hun volledige levenscyclus voltooien in getijdengebieden zonder verdere stroomopwaartse migratie. Onze resultaten suggereren variatie in grootte en leeftijd bij maturiteit over de longitudinale gradiënt van het stroomgebied, waarbij stroomopwaartse individuen doorgaans groter en ouder zijn bij hun voortplanting. We schatten dat volwassen krabben drie tot zes jaar oud zijn wanneer ze van september tot december stroomafwaarts migreren voor hun finale reproductieve fase.

Door individuele krabben te volgen met akoestische telemetrie, ontdekten we adaptieve verschillen in het gedrag van migrerende volwassen wolhandkrabben tussen niet-getijden- en getijdenomgevingen. In ondiepe, niet-getijdengebonden zoetwaterrivieren migreren volwassen krabben voornamelijk 's nachts, wat waarschijnlijk duidt op een strategie om predatie te vermijden. Door gebruik te maken van de unidirectionele stroming, kunnen ze migratiesnelheden van maximaal 15 km per dag bereiken, met een gemiddelde van 4,65 km per dag. In diepere getijdenrivieren is de migratie minder gesynchroniseerd met het dag-nachtritme en eerder gekoppeld aan de getijdencyclus. Chinese wolhandkrabben maken gebruik van de ebstroom om stroomafwaarts te migreren, een strategie die bekendstaat als 'selective tidal stream transport' en waarschijnlijk de energetische kosten van de migratie verlaagt. In deze getijdenomgevingen is de migratie trager, met een gemiddelde van 1,29 km per dag. Verder tonen de trackinggegevens aan dat de krabben het estuarium binnen één tot twee maanden bereiken en hun migratie stoppen aan de grens tussen de mesohaliene en polyhaliene zone van het estuarium.

Daarnaast belicht deze thesis de interacties van de Chinese wolhandkrab met haar omgeving en de daaruit voortvloeiende ecologische impact. Als omnivoor beïnvloedt de Chinese wolhandkrab waarschijnlijk meerdere aspecten van zijn omgeving, onder andere door de biomassa en diversiteit van waterplanten te verminderen, bentische macro-invertebratengemeenschappen te veranderen, de hoeveelheid en vorm van organisch materiaal te beïnvloeden en de troebelheid en nutriëntconcentraties te verhogen. De Chinese wolhandkrab is bovendien een gravende soort die uitgebreide holennetwerken in getijdengebieden kan creëren, met mogelijke effecten op verschillende ecosysteemprocessen.

Een experimentele opzet werd gebruikt om te onderzoeken hoe de consumptie van detritus door de Chinese wolhandkrab de kwantiteit en vorm van organische materie en waterkwaliteit beïnvloedt. Zowel juveniele als volwassen Chinese wolhandkrabben bleken efficiënte detrivoren, die verschillende soorten bladmateriaal van bomen kunnen verwerken. Volwassen wolhandkrabben versnelden het afbraakproces van

bladafval van alle onderzochte types aanzienlijk, terwijl het effect van jonge krabben eerder beperkt bleef. Door hun voedingswijze en excreties verhoogde de productie van fijn partikelvormig organisch materiaal, de stikstofconcentraties en het biologisch zuurstofverbruik. Door dit gedrag kunnen Chinese wolhandkrabben de afbraak van bladmateriaal en de nutriëntenkringloop in zoetwaterhabitats versnellen.

We combineerden een uitgebreide veldstudie, in zowel een hersteld als een natuurlijk zoetwaterschor langs de Schelde, met laboratoriumexperimenten om morfometrische relaties tussen krabben en hun holen vast te stellen, en om ruimtelijke en temporele patronen in holendichtheid en -grootte te onderzoeken. De holen waren typisch ellipsvormig, waarbij de afmetingen van de opening, de lengte en het volume positief schaalden met de grootte van de krab. Holendichtheden verschilden tussen de twee schorren en vertoonden temporele variatie op één locatie, met hogere dichtheden van laat in de lente tot de herfst, na een nieuwe instroom van juveniele krabben in het gebied. Hogere dichtheden kwamen vooral voor op steile oevers en in de buurt van de inlaat van het schor, terwijl grotere holen vaak op steile hellingen, in fijnkorrelige sedimenten en op oevers met weinig sedimentverplaatsing werden aangetroffen. In het herstelde schor waren hoge holendichtheden ( $\geq 30$  holen  $m^{-2}$ ) geassocieerd met oevererosie, terwijl lage, stabiele dichtheden geen verband vertoonden met erosie of sedimentatie in het natuurlijke schor. Deze resultaten tonen aan dat bij hoge dichtheden het graafgedrag van Chinese wolhandkrabben schorren kan beïnvloeden, met gevolgen voor de sedimentdynamiek en de werking van het ecosysteem.

In het uitheemse verspreidingsgebied is weinig bekend over de prevalentie en fysiologische effecten van de pathogeen *Hepatospora eriocheir*, een microsporidium en natuurlijke vijand van de Chinese wolhandkrab. Om dit te onderzoeken, werden volwassen krabben in dezelfde periode verzameld uit twee invasieve populaties en onderworpen aan dezelfde fysiologische testen. De infectieprevalentie liep van 27 % in de Scheldepopulatie (België) tot 38 % in de Elbepopulatie (Duitsland). Fysieke tests toonden aan dat geïnfecteerde krabben een verminderde aerobe capaciteit hadden in vergelijking met niet-geïnfecteerde krabben. Bovendien gaven analyses van het glucosegehalte in het hemolymfe en het lipidengehalte in de hepatopancreas aan dat geïnfecteerde krabben uitgeputte energiereserves hadden, waarschijnlijk door directe benutting van hulpbronnen door deze intracellulaire parasiet, wat op zijn beurt de capaciteit van de gastheer voor intense activiteit vermindert.

Tot slot worden de bevindingen van deze thesis samengebracht en wordt hun relevantie voor het invasiesucces van de Chinese wolhandkrab besproken. Daarnaast

worden de ecologische en socio-economische effecten van de soort in Vlaanderen geëvalueerd, en worden aanbevelingen voor het beheer gegeven.

Gezamenlijk dragen deze bevindingen bij tot een beter begrip van de ecologie van de Chinese wolhandkrab in zijn uitheemse verspreidingsgebied en benadrukken ze de adaptieve eigenschappen die zijn invasiesucces en ecologische impact in België faciliteren. Toch zijn verdere veldstudies nodig om de bredere ecologische effecten van de soort in kaart te brengen, en vervolgonderzoek naar beheersmaatregelen, zoals de krabberval, is essentieel om de impact van deze invasieve soort in gebieden waar ze wijdverspreid is te beperken.

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# 1

## General introduction

## 1.1 What are non-native invasive species?

### 1.1.1 Terminology

Non-native invasive species have become a defining feature of the Anthropocene, and now stand alongside habitat loss and degradation, overexploitation, pollution, and climate change as one of the most urgent global threats to biodiversity, ecosystem services, and food security (Bellard et al., 2022; IPBES, 2023). But what exactly defines a non-native invasive species, and are all non-native species inherently invasive?

To address this, it is important to first clarify what constitutes a native species. **Native species** are those species that have evolved within a specific geographic region or have arrived there naturally (i.e., range expansion), and thus without human intervention (Richardson et al., 2011). In contrast, **non-native species** are organisms that originated elsewhere and were introduced to a region through human assistance (Richardson et al., 2011). This introduction can either be intentional, such as species brought for hunting, cultivation, or ornamental purposes, or unintentional, such as seeds hitching a ride on shoes or sessile organisms attached to boats. While there are many terms synonymous with non-native, such as exotic, alien, allochthonous and non-indigenous, these cannot be used interchangeably with “invasive” (Soto et al., 2024).

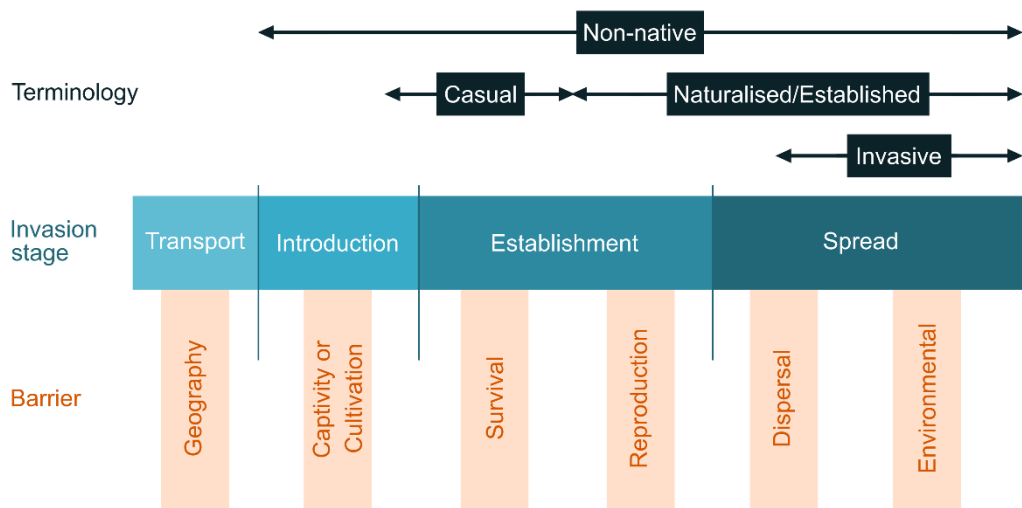
Both native and non-native species can be considered invasive. Native species can become invasive when natural obstacles to their proliferation disappear, allowing them to spread and dominate within ecosystems (Valéry et al., 2008). This shift is often the result of environmental changes due to human disturbance, for example habitat modification or eutrophication (Valéry et al., 2009). For instance, the native clonal grass *Elymus athericus* has expanded across salt marshes in northwestern Europe, likely due to changes in grazing practices, increased sedimentation, and elevated nitrogen inputs, often replacing characteristic vegetation with dense monospecific stands (Koop-Jakobsen et al., 2021; Valéry et al., 2004). However, invasion biology has traditionally focused on non-native species, and the broader interpretation remains debated (Courchamp et al., 2017). In this thesis, we will focus on non-native invasive species.

According to the definition that is widely adopted by international organisations and governance bodies, **non-native invasive species** are organisms that have been introduced outside of their native geographic range, which successfully establish, proliferate, and cause, or are likely to cause, significant economic or environmental harm, or harm to human health (CBD, 2024; European Commission, 2014; IUCN, 2025;

Richardson et al., 2011). In contrast, within invasion biology research, the definition of an invasive species typically does not include impact (Richardson et al., 2011; Soto et al., 2024). Instead, a non-native species is considered invasive if it successfully establishes self-sustaining populations and can spread within its new environment from the point of introduction (Blackburn et al., 2011; Soto et al., 2024). In this thesis, we will adhere to this latter definition.

### 1.1.2 Stages of the biological invasion process

The process of biological invasion is typically described as a series of stages (Fig. 1.1), which all have their own barriers that a non-native species must overcome in order to become invasive (Blackburn et al., 2011).



**Fig. 1.1** Conceptual framework for biological invasions, adapted from Blackburn et al. (2011). The scheme illustrates the sequential stages of invasion and the barriers that need to be overcome at each stage. Terminology commonly used to describe non-native species at different invasion stages is indicated along the top of the framework.

The journey of a non-native invasive species begins in its native range. From there, human activities transport the species beyond its natural geographic boundaries, overcoming the geographical barrier (Fig. 1.1). Common intentional introduction pathways include the ornamental plant and animal trade, agriculture, aquaculture, and the aquarium and pet trade (Hulme et al., 2008). Unintentional pathways typically involve the transport of organisms as contaminants (e.g., seeds or insects in agricultural produce) or as unintended hitchhikers in shipping, for example via ballast

water or biofouling (Hulme et al., 2008). While some organisms are directly introduced into their new environment, others may have to overcome additional barriers, i.e. to escape from captivity or cultivation (**Fig. 1.1**).

Upon arrival in a new environment, the first challenge for an introduced species is to survive (**Fig. 1.1**). Due to differences in climate, habitat characteristics, food availability, and the presence of predators, many species fail to persist after introduction. Secondly, they must be able to reproduce and establish a self-sustaining population. A non-native species that survives but does not establish a self-sustaining population is considered casual (**Fig. 1.1**). However, when a species manages to survive and successfully reproduce for multiple generations, it is considered established (Soto et al., 2024). If these established populations also spread over long distances and form new, self-sustaining populations, the species is classified as invasive (Soto et al., 2024).

Over 39,000 non-native species have been recorded worldwide, and at least 5,000 of these non-native species have been identified as invasive (IPBES, 2023). The likelihood of a species progressing through each stage of the invasion process is highly taxon- and habitat-dependent (García-Berthou et al., 2005; Jeschke and Heger, 2018). On average, it is estimated that around 25 % of non-native plants and invertebrates, and 50 % of non-native vertebrates, successfully move through these consecutive stages (Jeschke and Heger, 2018). Furthermore, non-native freshwater species are generally more successful than their terrestrial counterparts in progressing through the successive stages of invasion (Jeschke and Heger, 2018).

### **1.1.3 Impact and policy**

The establishment of non-native species has increased continuously in the past centuries, and is expected to keep rising (IPBES, 2023; Sardain et al., 2019; Seebens et al., 2017). In parallel, the number of documented ecological and socio-economic impacts has steadily increased as well (IPBES, 2023). Non-native invasive species have been implicated in approximately 60 % of recorded global animal and plant extinctions, either as a primary cause or contributing factor, with islands being particularly vulnerable (Clavero et al., 2009; IPBES, 2023). Positive effects of non-native species have been observed, such as creating habitat for other species (e.g. Pacific oyster *Magallana gigas* (Martin et al., 2025)), providing nectar for pollinators (Kovács-Hostyánszki et al., 2022), or supplying resources for humans, including timber and food (Shackleton et al., 2019). However, the vast majority of documented ecological impacts are negative, often resulting in local population declines of native

species (Gallardo et al., 2016; IPBES, 2023; Mollet et al., 2017; Pyšek et al., 2020). The type and severity of impacts vary greatly among organisms and habitats. Common mechanisms by which they affect native species include habitat alteration, both physical and chemical, and trophic interactions, including competition, predation, and herbivory (IPBES, 2023). In this way, non-native species exert additional pressure on ecosystems already stressed by human-induced disturbances such as climate change, habitat loss and modification, altered nutrient cycles, pollution, and overexploitation. Moreover, it has been established that these anthropogenic disturbed habitats facilitate the establishment of non-native species (i.e. the ‘disturbance hypothesis’) (Jeschke and Heger, 2018). Furthermore, the presence of non-native invasive species facilitates the establishment of new non-native species, a phenomenon known as ‘invasion meltdown’ (Jeschke and Heger, 2018).

Beyond ecological consequences, non-native invasive species can have severe socio-economic impacts, especially in developing countries (Pyšek et al., 2020). The types of impacts are again very diverse. They can impact food supply, for instance by reducing agricultural production, or affect natural resources such as timber (Shackleton et al., 2019). In addition, they can pose risks to human health, for example through the spread of zoonotic diseases or by inducing allergic reactions (Shackleton et al., 2019). Although estimates vary widely, the global costs associated with non-native species typically amount to several billions of euros annually (IPBES, 2023; Pyšek et al., 2020).

In an increasingly connected world, it is highly challenging to bring the introduction of non-native species to a halt. However, growing awareness has led to the development of new legislation and regulatory frameworks worldwide, aimed at reducing the unintentional and intentional translocation of species. For example, the International Maritime Organization (IMO) implemented the Ballast Water Management Convention (2004, entered into force in 2017), aimed at minimising the transfer of aquatic organisms via ship ballast water (IMO, 2004). Among other requirements, ships must manage their ballast water and sediments according to a ballast water management plan, which includes measures to remove or render harmless aquatic organisms before the ballast water is discharged into a new location. In another example, in the Antarctic region, the Protocol on Environmental Protection to the Antarctic Treaty explicitly prohibits the introduction of non-native species and includes strict provisions on the control of organic waste and other materials, such as cargo, equipment or food supplies, that could introduce non-native species (e.g., seeds or insects) (ATS, 2025; Leihy et al., 2025).

In 2014 the European Union adopted Regulation (EU) No. 1143/2014 on invasive alien species, which establishes a list of species of Union concern ('the Union list') and outlines measures that need to be taken across the EU (European Commission, 2014). Member States are required to take action to stop the introduction and spread of these species, establish early detection and rapid eradication measures, and effectively manage invasive species that have already become widespread (European Commission, 2014). The listed species are, or have the potential to become, invasive within the EU under current or foreseeable environmental conditions and are likely to cause adverse effects on ecosystems (European Commission, 2014). The list is regularly updated and currently includes 114 species (European Commission, 2025). Among them is the Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards 1853), which was included in the first version of the Union list, which entered into force in 2016 (European Commission, 2016). Its place on the list comes as no surprise, given that the species was ranked among the world's 'worst invasive species' (Luque et al., 2014). Therefore it is safe to say that the Chinese mitten crab is a notoriously invasive and impactful, non-native species.

## 1.2 Description and distribution of the Chinese mitten crab

### 1.2.1 The genus *Eriocheir* and its native range

This crab species is part of the order Decapoda (decapods), infraorder Brachyura (true crabs), family Varunidae and the genus *Eriocheir*. The genus *Eriocheir* contains three other species aside from *E. sinensis*: *Eriocheir japonica* (Japanese mitten crab, De Haan 1835), *Eriocheir hepuensis* (Hepu mitten crab, Dai 1991) and *Eriocheir ogasawarenis* (Ogasawara mitten crab, Komai et al., 2006). However, the taxonomic status of these species remains contentious due to their close morphological and genetic similarities (Sui et al., 2009).

All species of the genus *Eriocheir* are native to East Asia, have characteristic hairy claws (**Fig. 1.2**) and a catadromous life cycle, which means they live in freshwater but migrate to the sea to spawn. The Chinese mitten crab (**Fig. 1.2A**) is native to the area surrounding the Yellow Sea, including most of the northern and central coastal region of China and western coastal region of the Korean Peninsula (Hymanson et al., 1999). The Japanese mitten crab (**Fig. 1.2B**) inhabits the Japanese mainland, the Ryukyu Islands, Taiwan, the eastern coast of the Korean Peninsula, and the southeastern tip of Russia (Kobayashi and Vazquez Archdale, 2021). The other two species have a more restricted distribution. The Hepu mitten crab inhabits the southern coastal regions of China and the Ogasawara mitten crab is endemic to the Japanese Ogasawara (Bonin)

Islands, although its full distribution may not yet be fully understood (Komai et al., 2006; Sui et al., 2009). Although the Japanese mitten crab is actually more common in East Asia, it is the Chinese mitten crab that is the most widespread globally.



**Fig. 1.2** Adult males of the two most common species of the genus *Eriocheir* shown side by side: (A) Chinese mitten crab (*Eriocheir sinensis*), and (B) Japanese mitten crab (*Eriocheir japonica*).

In its native range, the Chinese mitten crab is known as the ‘Shanghai hairy crab’ and is the centre of an important culinary, and by extension cultural, tradition especially in the Yangtze region (Naser et al., 2012; Wang et al., 2006; Yan and Zhang, 2025). The hepatopancreas, gonads and meat are the three primary edible parts of adult crabs, and because particularly the gonads are considered a delicacy, mature crabs are sold at a higher price (Cheng et al., 2018). Traditionally, the mature adult crabs are harvested in autumn, in time for the mid-autumn festival (Yan and Zhang, 2025).

However, since the 1960s the wild population of mitten crabs in China has greatly diminished due to habitat degradation, blocking of migration routes, pollution and overfishing (Cheng et al., 2018; Hymanson et al., 1999; T. Zhang et al., 2023). The demand for mitten crabs is therefore met through aquaculture. Initially, wild larvae were harvested each year to produce crabs, but as wild populations declined, new hatchery techniques have been developed and refined over the last decades (Cheng et al., 2018). Nowadays the full life cycle of the Chinese mitten crab can be successfully completed in aquaculture. The high and growing demand for mitten crabs is evident from FAO data, which shows a significant increase in aquaculture production: from 202.5 thousand tonnes in 2000 to 775.9 thousand tonnes in 2020. This growth has made the Chinese mitten crab the third most cultivated crustacean in global aquaculture, following the whiteleg shrimp (*Penaeus vannamei*) and the red swamp crayfish (*Procambarus clarkii*) (FAO, 2022).

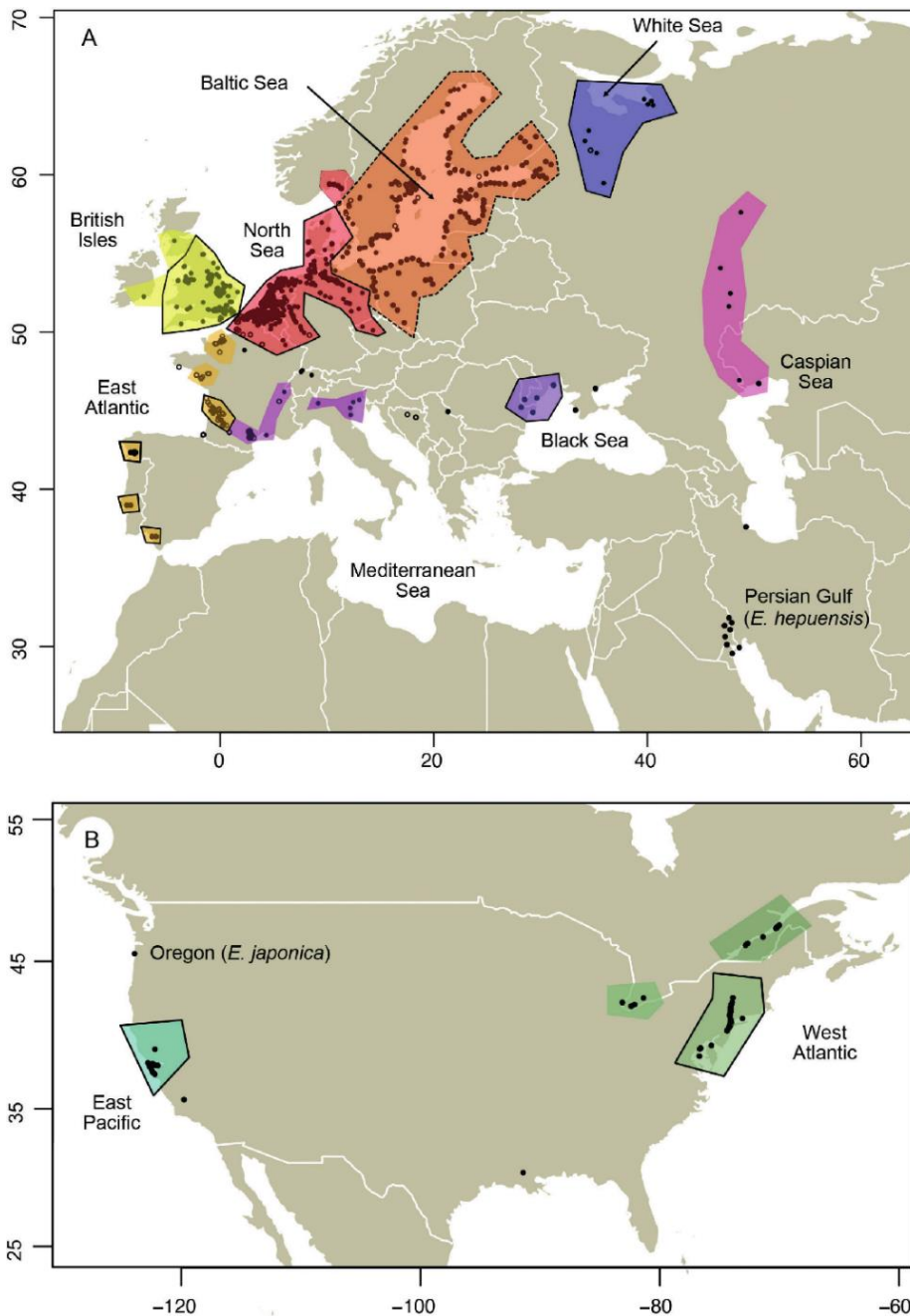
## 1.2.2 Invasion history and non-native range

The species' popularity in Asian cuisine has likely contributed to some deliberate introductions in new regions (Cohen and Carlton, 1997; Ewers et al., 2023; Veldhuizen, 2001). However, its first appearance in Europe in the early 1900s is believed to result from unintentional introductions through ballast water of ships returning from East Asia, likely facilitated by the expansion of international shipping during that period. The first documented observation dates from September 1912, in the Aller River, a tributary of the Weser River in Germany (Peters and Panning, 1933). In the following years, multiple sightings were reported annually, culminating in 1925 with the capture of the first egg-bearing females in the Elbe Estuary (Peters and Panning, 1933). From that point onward, the species began to spread rapidly, and its population grew sharply.

Soon after, the species was observed across Northern Europe (**Fig. 1.3A**) in Denmark (1927), Poland (1927), the Netherlands (1930), Sweden (1932), Latvia (1932), Belgium (1933), Estonia (1933), Finland (1933), Lithuania (1935), the UK (1935) and France (1945) (Ewers et al., 2023). In countries like Germany, Belgium and the Netherlands, the species became very abundant throughout the 1930s and 1940s. In the following decades they were reported in Norway (1976), Russia (1980), Portugal (1986), and Spain (1987).

In North America (**Fig. 1.3B**), the species was first observed in the Laurentian Great Lakes in 1965. Afterwards an established population was reported in San Francisco Bay after the first observation in 1992. Other reports include observations in the St. Lawrence River (2004), Chesapeake Bay and Delaware Bay (2005), and the Hudson River (2007), but it has not been confirmed whether these are established populations (Dittel and Epifanio, 2009; Ewers et al., 2023).

There are few reports of the Chinese mitten crab in Japan (Takeda and Koizumi, 2005), where the species is regulated under the Invasive Alien Species Act (Ministry of the Environment Japan, 2004). However, even though large numbers of the species are imported regularly from China to Japan, both for human consumption and aquaculture in northern Japan, it has failed to establish itself until now (Takeda and Koizumi, 2005; Z. Zhang et al., 2019b).



**Fig. 1.3** Non-native distribution of the Chinese mitten crab in (A) Europe and (B) North America, reproduced from Ewers et al. (2023). Each dot represents an individual record: closed dots indicate GBIF records from after 2000, while open dots indicate records prior to 2000. Coloured areas highlight regions with frequent reports, and black-outlined areas indicate established populations.

Currently, the Chinese mitten crab is in the top ten of the most widespread non-native invasive crustacean species worldwide (IPBES, 2023). However, based on habitat suitability, models have predicted that the species could potentially extend its non-native range further in both Europe and North America, and could possibly settle in new areas including parts of Japan, South America, Australia, and New Zealand (Herborg et al., 2007a; Z. Zhang et al., 2019a).

### 1.2.3 Morphological description

The names ‘mitten crab’ or ‘hairy crab’ refer to the brown fur-like setae that grow on the white-tipped claws (chelae) of the crab (**Fig. 1.2**). The characteristic is especially well-developed in adult male crabs. Interestingly, these setae have been found to serve as a microhabitat for a wide range of epibionts, including nematodes and bivalves (Normant et al., 2007).

The hairy claws spark curiosity and therefore the most frequently asked question throughout my period as a PhD student was “*Why do they have hair?*”. To my knowledge, there has been no published research on this specific question. However, there are a few possible hypotheses as to why the ‘hair’ is so extensively developed. (1) Sensory function: like other types of setae, the hairs may serve a sensory role, helping to detect prey or environmental stimuli (Chen et al., 2023; Peters and Panning, 1933). (2) Sexual selection: since there is sexual dimorphism, it could be a result of sexual selection where males with more developed setae patches have a higher mating success (Kobayashi and Satake, 2017). (3) Size illusion: when submerged, the voluminous setae create the illusion of larger chelae and maybe an overall larger body size. This could intimidate competitors or deter predators, which could benefit reproductive success and survival. For instance, a competition experiment has shown that differences in size among individuals from the genus *Eriocheir* reduces the likelihood of agonistic interactions for resources (Z. Zhang et al., 2019b).

Other characteristics to distinguish the crabs are the four pairs of lateral spikes on the carapace and the four frontal spikes between the eyes. Although the crab can reach a carapace size of 10 cm, the adult Chinese mitten crab generally has a carapace width of 4 to 8 cm in Europe.

## 1.3 Ecology of the Chinese mitten crab

### 1.3.1 Life cycle

Similar to the well-known European eel (*Anguilla anguilla*), the Chinese mitten crab is a catadromous species, meaning that it spends most of its life in freshwater but migrates to saltwater to mate and reproduce. Therefore, the life cycle of the Chinese mitten crab is characterised by two migration events between freshwater and saltwater. The general life cycle of the Chinese mitten crab is well documented. However, the life history of the Chinese mitten crab is still largely unknown in Belgium and much of Europe.

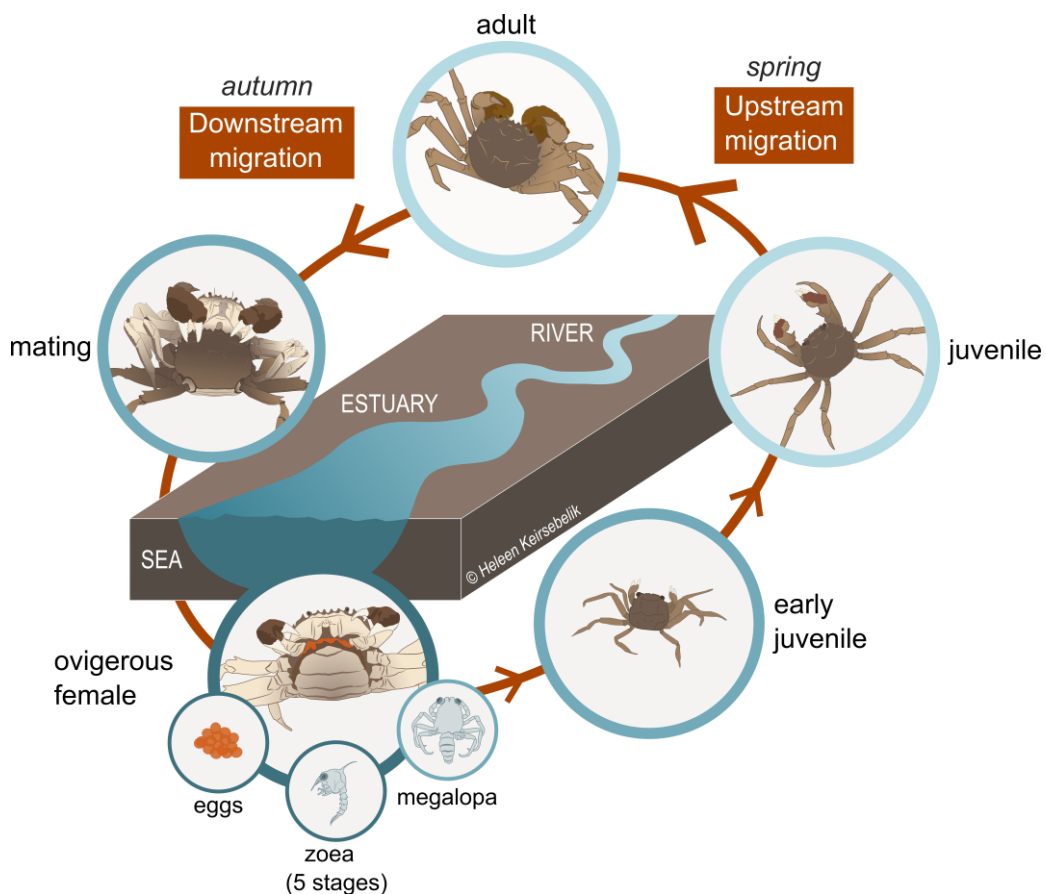
Following their final (puberty) moult, adult crabs typically migrate downstream from freshwater habitats to brackish estuarine waters between late August to December (**Fig. 1.4**) (Panning, 1938; Rudnick et al., 2005b; Schoelynck et al., 2021). Panning (1938) suggested adult crabs move downstream at rates of 8 to 12 km day<sup>-1</sup>. It has been hypothesized that Chinese mitten crabs move with the flow or ebb tide to reach the estuary, however this behaviour has not been investigated (Dittel and Epifanio, 2009).

Unlike many other brachyuran crabs, which mate after the female has moulted and her shell is soft, mitten crabs mate when the female has a hard shell (Herborg et al., 2006; Peters and Panning, 1933). Mating is preceded by fighting and holding behaviour (Herborg et al., 2006; Peters and Panning, 1933). Laboratory experiments suggest there is no long-distance pheromone involved in mate recognition, instead, recognition occurs only after direct physical contact (Herborg et al., 2006). Additionally, a peak in mating activity has been observed during full and new moons, suggesting a semi-lunar rhythm (Herborg et al., 2006).

After mating (**Fig. 1.4**), eggs are extruded within 24 hours (Peters and Panning, 1933). The brood consists of clusters of eggs that are attached to the abdominal pleopods by a cement-like substance (Peters and Panning, 1933). The number of eggs per brood ranges from 20,000 to 1 million (Anastácio et al., 2018; Hymanson et al., 1999; Kobayashi, 2001; Lee and Yamazaki, 1990; Peters and Panning, 1933). The large variation is explained by environmental conditions, the female's size, and the number of broods she produces (Anastácio et al., 2018; Kobayashi, 2001; Peters and Panning, 1933). Larger females tend to have larger broods (Anastácio et al., 2018; Kobayashi, 2001; Peters and Panning, 1933). Since the Chinese mitten crab reproduces only once in its lifetime, its size at maturity significantly impacts its fecundity (Kobayashi, 2001). A single female can produce multiple broods during this single reproductive period, with research on *E. japonica* indicating a maximum of three (Kobayashi, 2001; Rudnick

et al., 2005b). However, the number of eggs decreases with each successive brood (Kobayashi, 2001; Rudnick et al., 2005b).

Ovigerous females are observed between October and June, though the exact timing varies by region and is often limited to a few months (Anastácio et al., 2018; Clark, 2011; Czerniejewski et al., 2012a; Herborg et al., 2006; Hymanson et al., 1999). The regional variation is likely influenced by temperature (Anastácio et al., 2018). After the reproductive period, the adult crabs die (Kobayashi and Matsuura, 1995a, 1994; Rudnick et al., 2005b). Reproducing adults are typically found in the estuary until July (Clark, 2011; Peters and Panning, 1933; Rudnick et al., 2003).



**Fig. 1.4** Graphical presentation of the life cycle of the Chinese mitten crab, showing the different life stages according to habitat.

The survival and development of the embryos within the eggs is known to be affected by water temperature, salinity, and infections (Anger, 1991; Wang et al., 2019).

Experimental studies suggest that the optimal salinity range for embryonic development ranges from 5 to 20 ‰, with higher salinities being less favourable (Wang et al., 2019). Anger (1991) observed that multiple broods of females collected from the Elbe Estuary were infected by fungi, leading to low larval survival. Generally, larvae hatch from the eggs after approximately one month, but lower temperatures can slow down development (Rudnick et al., 2005b). It has been suggested that ovigerous females move to deeper waters during winter, carrying their eggs until more favourable conditions return in spring (Rudnick et al., 2003).

Planktonic larvae (**Fig. 1.4**) hatch in spring in brackish water, progressing through a short non-feeding prezoa stage, five zoeal stages and a final megalopa stage (Anger, 1991). Interestingly, additional larval stages can occur in case of unfavourable environmental conditions, such as the combination of low temperature and salinity (Anger, 1991). Optimal larval development occurs at warm water temperatures (15-18°C), while lower temperatures (< 12°C) significantly increase mortality. Each larval stage has specific salinity requirements for development. The first zoeal and final megalopa stages tolerate a broad salinity range (10-30 ‰) and develop best in low-salinity estuarine waters (Anger, 1991; Wang et al., 2019). However, the intermediate zoeal stages require high salinities and have low tolerance for reduced salinity (Anger, 1991; Wang et al., 2019).

After completing the megalopa stage (**Fig. 1.4**), larvae metamorphose into juvenile crabs. This metamorphosis can occur anywhere between saltwater and freshwater, provided the megalopa has had sufficient time to acclimate to the reduced salinity (Anger, 1991; Peters and Panning, 1933). It has been suggested that megalopae migrate upstream using selective tidal stream transport, taking advantage of flood tides (Anger, 1991; Peters and Panning, 1933). After a few moults, the juvenile crabs are large enough to move against the current and continue their migration upstream, typically in spring between March and June (Peters and Panning, 1933; Rudnick et al., 2005b; Schoelynck et al., 2021). These juvenile crabs then disperse in search of suitable freshwater habitats, where they will reside until they reach maturity (**Fig. 1.4**). Depending on regional differences in factors like temperature and food availability, mitten crabs complete their life cycle within one to six years (Hymanson et al., 1999; Kamps, 1937; Panning, 1938; Rudnick et al., 2005b).

### **1.3.2 Habitat preferences**

In their native range, Chinese mitten crabs are commonly found in rice fields, ponds, lakes and rivers (Dittel and Epifanio, 2009). Similarly, in their non-native range, they

inhabit a broad range of aquatic habitats from stagnant water including ponds and lakes, to small streams, concrete canals and large rivers (Rudnick et al., 2003). Mitten crabs require shelter in their habitat, which may include both natural or artificial structures such as macrophytes, debris or rocks (Gilbey et al., 2008; Rudnick et al., 2003; Veldhuizen, 2001). Mitten crabs particularly thrive in environments with macrophyte stands, which improve water quality and provide shelter and food (Cheng et al., 2018; Jin et al., 2001b).

In the absence of suitable shelters, mitten crabs are known to dig burrows, particularly in tidal areas where they seek refuge during low water levels (Hymanson et al., 1999; Rudnick et al., 2005a; Veldhuizen, 2001). Pools in these areas can also serve as refuges. Additionally, mitten crabs have been observed using burrows created by other species, such as the Harris mud crab (*Rhithropanopeus harrisi*) (Veldhuizen, 2001).

### **1.3.3 Diet**

Like most brachyuran crabs, Chinese mitten crabs are omnivores and highly opportunistic, meaning they eat almost anything they can get a hold of (Rosewarne et al., 2016). Food items include, but are not limited to; bivalves (e.g. blue or zebra mussels), gastropods, small crustaceans like isopods, amphipods or copepods, oligochaetes, polychaetes, insect larvae (e.g. chironomids and caddisflies), rotifers, protozoa, fish, fish eggs, macrophytes (e.g. species of *Potamogeton* or *Elodea*), algae, carrion and detritus (Czerniejewski et al., 2010; Jin et al., 2003; Koester et al., 2022; Mills et al., 2016; Rosewarne et al., 2016; Schoelynck et al., 2020; Webster et al., 2015; Wójcik-Fudalewska et al., 2019; Wójcik et al., 2015; Zeng et al., 2018). In captivity, they have been shown to be cannibalistic as well, preying on soft-shelled or injured conspecifics (Peters and Panning, 1933; Zeng et al., 2018). Interestingly, they do not seem to feed on tadpoles or frogs when offered, dead or alive (Peters and Panning, 1933).

Most studies examining the stomach contents of Chinese mitten crabs have found that their diet mainly consists of plant material and detritus (Czerniejewski et al., 2010; Jin et al., 2003; Koester et al., 2022; Mao et al., 2016; Rogers, 2000; Rudnick et al., 2000; Rudnick and Resh, 2005). However, a food preference experiment suggested a preference for animals, and a few studies that used stable isotope analyses indicated that animal-based food is in fact the primary food source (Koester et al., 2022; Mao et al., 2016; Rogers, 2000; Rudnick and Resh, 2005). This discrepancy is usually attributed to the differing timeframes considered by the two methods: stomach content analysis provides a snapshot of recent feeding activity, whereas stable isotope

analysis reflects the diet over a longer period (Koester et al., 2022; Rudnick and Resh, 2005). Additionally, differences in digestibility between plant fibres and animals may play a role (Koester et al., 2022; Rudnick and Resh, 2005). However, it is very likely that feeding differences among life stages and regions or habitats account for a significant portion of the observed variation between studies (Cui et al., 2021; Rudnick and Resh, 2005; Wójcik-Fudalewska et al., 2019).

Marine zooplankton has been identified as the primary food source for megalopae and early juvenile stages of the Chinese mitten crabs (Cui et al., 2021; Weng et al., 2025). Aquaculture studies further indicate that larvae and juveniles are also capable of filter feeding on phytoplankton (Zeng et al., 2023). As juvenile mitten crabs grow, their diet appears to shift toward a greater contribution of benthic algae and macrophytes, alongside zoobenthos (Cui et al., 2021; Jin et al., 2001). In contrast, it has been suggested that adult Chinese mitten crabs predominantly feed on animal-based food sources, however, there is no conclusive evidence to support this claim (Rudnick and Resh, 2005; Wójcik-Fudalewska et al., 2019).

A common fear of naturalists and water managers is that the Chinese mitten crab predate on small native fish species. Multiple studies have found remains of fish in their stomach and its known from fishermen that the crabs predate on fish when they are trapped together in a net or cage (Jin et al., 2003; Panning, 1938; Wójcik-Fudalewska et al., 2019). Additionally, laboratory studies indicate that when given the opportunity, the crabs feed on fish eggs (Culver, 2005; Rosewarne et al., 2016; Soes et al., 2007; Webster et al., 2015). However, to my knowledge, there is no documented case in literature of a Chinese mitten crab catching a live (and healthy) fish in the field. On the contrary, it has been described that in aquaria, mitten crabs show little to no interest in fish passing nearby (Panning, 1938; Peters and Panning, 1933). Generally it is assumed that mitten crabs only consume dead or injured fish, which they seem to be particularly drawn to (Panning, 1938; Peters and Panning, 1933; Wójcik-Fudalewska et al., 2019).

#### **1.3.4 Predation on the Chinese mitten crab**

There is limited literature available on predators of the Chinese mitten crab in its native range. Soes et al. (2007) note that China holds a great diversity of large freshwater fish that are potential predators, and suggest that additionally Yangtze river dolphins, Asian giant softshell turtles and Chinese alligators could also prey on mitten crabs. In semi-natural aquaculture ponds, snakehead fish (e.g. Northern snakehead) and catfish (e.g. Yangtze catfish) have been reported to feed on mitten crabs (Luo et al., 2014).

Additionally, other fish such as river chub, Chinese minnow, gobies and dark sleeper have been documented preying on juvenile specimens of the closely related Japanese mitten crab (Suzuki et al., 1998).

Although there were no freshwater crabs in Belgium before, many native predators have been observed eating Chinese mitten crabs. Especially small crabs seem to be on the menu. The evidence for predation is however mostly anecdotal.

According to Flemish recreational fishermen, small mitten crabs are commonly found in the stomachs of European eels (*Anguilla anguilla*). Zanders (*Sander lucioperca*) are often observed regurgitating crabs when caught, while pike (*Esox lucius*) and other predatory species have been observed with crabs in their mouths. This aligns with an earlier report by Fladung (2000), which also identified other fish species, including river trout (*Salmo trutta*), common barbel (*Barbus barbus*), European perch (*Perca fluviatilis*), common chub (*Squalius cephalus*), Atlantic cod (*Gadus morhua*), ruffe (*Gymnocephalus cernua*), burbot (*Lota lota*) and common bream (*Abramis brama*) preying on mitten crabs in Germany. While mitten crabs are thought to be merely an occasional food source for most of these fish species, they appear to be a significant part of the diet of eels and (large) perch (Fladung, 2000). In North America, white sturgeon (*Sinosturio transmontanus*), striped bass (*Morone saxatilis*), black bass (*Micropterus* spp.) and catfish were reported to feed on mitten crabs (Veldhuizen, 2001).

Some examples of birds for which photographic evidence was obtained, via citizen science platforms, news articles, and camera traps for wildlife monitoring, include; grey heron (*Ardea cinerea*), Eurasian coot (*Fulica atra*), scaup (*Aythya marila*) (HLN, 2021), carrion crows (*Corvus corone*) (**Fig. 1.5A**), great crested grebe (*Podiceps cristatus*) (**Fig. 1.5B**) (Clark, 2011), and various loon species (*Gavia* spp.) (**Fig. 1.5C**). In literature also other birds are mentioned, such as white stork (*Ciconia ciconia*), various species of ducks, gulls (e.g. great black-backed gull (*Larus marinus*) and European herring gull (*Larus argentatus*)) and mergansers (*Mergus* spp.) in Europe, and loons and egrets in North America (Clark, 2011; Fladung, 2000; Veldhuizen, 2001). In Germany, a study in a heron colony estimated, based on pellets, that on average 15 to 20 % of their diet consisted of mitten crabs (Fladung, 2000).



**Fig. 1.5** Various bird species consuming Chinese mitten crabs, A: carrion crow (*Corvus corone*), B: great crested grebe (*Podiceps cristatus*), C: yellow-billed loon (*Gavia adamsii*) (source A: waarnemingen.be, B: bnnvara.nl, C: waarneming.nl).

Also mammals are known to eat the species. For instance, since the establishment of mitten crabs in Belgium, brown rats (*Rattus norvegicus*) have been reported to prey on them (Leloup, 1938a). This behaviour has also been observed more recently in Merelbeke (Belgium), where rats were seen consuming crabs as they emerged from the crab trap (M. Bats, pers. comm.). Also black rats (*Rattus rattus*) and European polecat (*Mustela putorius*) are predators, and the remains of Chinese mitten crabs were previously found in otter (*Lutra lutra*) spraints in Germany (Fladung, 2000; Weber, 2008). Similarly, remains of mitten crabs were found in (presumably) raccoon (*Procyon lotor*) scat in North America (Rudnick et al., 2000).

Lastly, American bullfrogs (*Lithobates catesbeianus*) have been reported to consume mitten crabs in the San Francisco Estuary (Veldhuizen, 2001).

Despite the various observations of native animals preying on Chinese mitten crabs, the high catch numbers of these crabs in Belgium and in other European countries show that predators are not able to keep the population density to a low level.

### 1.3.5 Pathogens

Numerous pathogens, including bacterial, viral, fungal, and other eukaryotic organisms, have been documented in the native range of the Chinese mitten crab (Ding, 2023). These pathogens are especially prevalent in aquaculture, where crabs are kept at high densities, which increases the risk of outbreaks and raises associated mortality (Coates and Rowley, 2022; Ding, 2023). This section gives a short summary of documented diseases based on the review by Ding (2023).

*Spiroplasma eriocheiris* is a bacterial pathogen that causes tremor disease in Chinese mitten crabs. The pathogen likely interferes with nerve transmission, resulting in uncontrollable shaking of the pereopods (i.e. walking legs) (Ding, 2023). The disease

weakens the immune system, often leading to death. Other bacterial infections caused by various *Vibrio* species, including *V. parahaemolyticus*, *V. anguillarum*, and *V. alginolyticus*, have also been documented in aquaculture. Infected larvae often show diminished appetite, which can lead to mortality (Ding, 2023).

White spot syndrome virus (WSSV) causes the white spot disease, a disease that affects and is transmitted by multiple crustacean species, and has caused particularly high mortality in shrimp aquaculture (Coates and Rowley, 2022; Ding, 2023). The infection can spread to multiple tissues, including the stomach, gills, cardiac muscle, and pleopods (Ding, 2023). During an epidemic in Xinghua city, eastern China, this virus caused mortality rates of 80 to 100 % in cultured crabs (Ding et al., 2015). Additional viruses reported include a newly discovered reovirus and ronivirus (Zhang et al., 2004; Zhang and Bonami, 2007).

Microsporidia are obligate intracellular parasites, related to fungi. In the Chinese mitten crab, the species *Hepatospora eriocheir* has been described, which typically infects the hepatopancreas of its host (Stentiford et al., 2011; Wang and Chen, 2007). The pathogen directly exploits the host's energy reserves, alters its lipid metabolism, and can severely damage the hepatopancreas (Ding, 2021; Ding et al., 2018; Hou et al., 2023). Some studies suggest it is associated with the hepatopancreatic necrosis disease, which causes high mortality in aquaculture (Ding et al., 2016; Hou et al., 2023, 2022). However, this is debated as other studies suggested the disease is caused by environmental factors (Huang et al., 2021; Pan et al., 2017; Shen et al., 2017, 2021). The milky disease in Chinese mitten crabs is the result of a yeast infection caused by *Metschnikowia bicuspidate*. Weakness, opaque or whitish muscles, and milky haemolymph are typical symptoms according to Ding (2023).

Finally, the Chinese mitten crab is parasitised by *Polyascus gregaria*, a highly specialised parasitic barnacle that affects the immune response and hepatopancreas of its host (Yang et al., 2021; Zhao et al., 2023).

Although several pathogens have been identified in the non-native range of the Chinese mitten crab, there is limited knowledge about pathogen prevalence and impact. In the Thames Estuary, the bacteria *Vibrio parahaemolyticus* was detected in Chinese mitten crabs between 2003 and 2006, and was particularly abundant during summer (Wagley et al., 2009). Histological screening also revealed the presence of *Hematodinium* spp., a parasitic dinoflagellate, in crabs from the Thames in 2018 (Coates and Rowley, 2022; Kerr et al., 2025). Furthermore, the microsporidium *Hepatospora eriocheir* was detected in various countries within the European range of

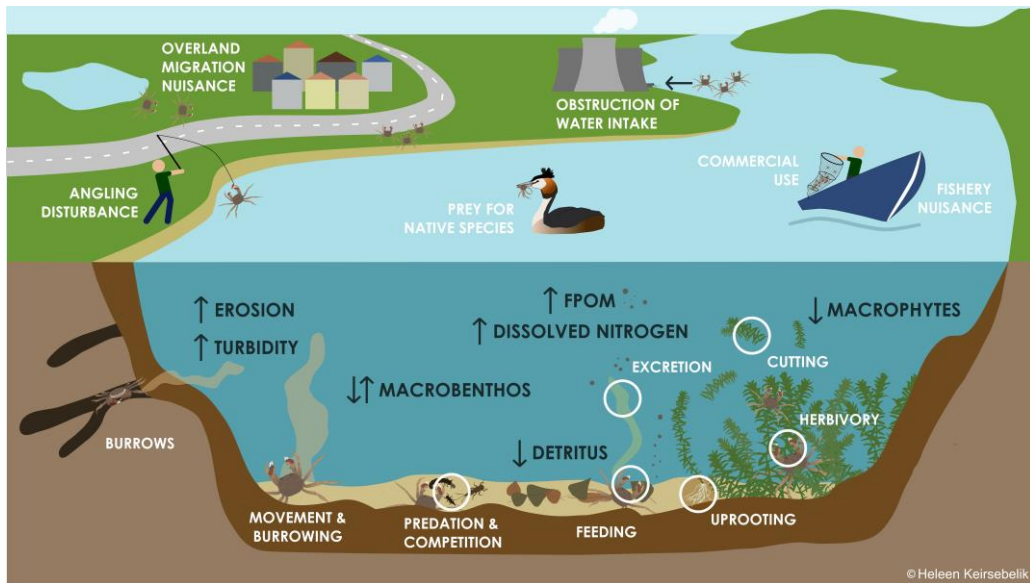
the Chinese mitten crab, with prevalence ranging from 33 to 70 % (Bateman et al., 2016; Normant-Saremba et al., 2024; Stachnik et al., 2025; Stentiford et al., 2011). Finally, laboratory experiments established the Chinese mitten crab as a vector for the crayfish plague pathogen (*Aphanomyces astaci*) (Schrimpf et al., 2014), which was also confirmed in wild-captured mitten crabs in the Netherlands (Tilmans et al., 2014).

#### **1.4 Impact of the Chinese mitten crab**

In Belgium, and in large parts of Europe, no native freshwater or catadromous crabs have been present. As a result, the Chinese mitten crab represents a novel species in these systems, with the potential to affect multiple trophic levels and ecosystem processes. The diet of the Chinese mitten crab, as discussed in Section 1.3.3, reveals that they prey on a wide range of organisms. Laboratory experiments have shown that mitten crabs exhibit higher predation intensity on macroinvertebrates compared to native crayfish (Rosewarne et al., 2016). When they occur in high densities, their predation is expected to have a significant impact on benthic communities. However, the full extent of these effects remains poorly understood due to a lack of field research. Some insight can be drawn from historical reports during the mitten crab's peak abundance in the 1930s and 1940s in Germany, where several species that were once abundant in the Elbe River, such as snails, mussels, and amphipods, largely disappeared following their arrival (Schiemenz, 1942). However, as this is only a correlation, other environmental stressors and anthropogenic pressures at the time may also have contributed to these declines. In addition to predation, increased competition for resources such as food or shelter poses additional challenges for native fauna (**Fig. 1.6**). For instance, the Chinese mitten crab can outcompete the native European shore crab for shelter (Gilbey et al., 2008). However, they are unable to exclude size-matched Japanese mitten crabs from their shelter (Z. Zhang et al., 2019b).

Chinese mitten crabs are known to consume large amounts of detritus, particularly terrestrial leaf litter. This suggests they may affect leaf litter breakdown and nutrient cycling within aquatic ecosystems. Furthermore, by consuming and transforming the leaf litter into fine particulate organic matter, they could indirectly shape the benthic food web by altering the availability of coarse and fine particulate matter, as well as dissolved nutrients (Rudnick and Resh, 2005) (**Fig. 1.6**). Earlier research has shown that large Chinese mitten crabs can consume and transform detritus at a higher rate than native and non-native crayfishes (Doherty-Bone et al., 2018b). Furthermore, it has been demonstrated that they can consume leaf litter of both native and non-native

riparian plants (Doherty-Bone et al., 2018a). However, many aspects of this potential ecological impact remain poorly understood, ranging from small-scale factors such as the effects of crab size or leaf litter type, to broader questions about how mitten crab activity influences nutrient availability and detritus dynamics in natural field conditions.



**Fig. 1.6** Conceptual illustration depicting how the Chinese mitten crab affects ecosystems and human activities beyond its native range.

The Chinese mitten crab poses a significant threat to native flora as well. Macrophytes have been demonstrated to be a beneficial component in the habitat of mitten crabs, however, several studies have shown that mitten crabs can cause significant damage to macrophyte stands (Guo-zhang et al., 1999; Jin et al., 2001b; Schoelynck et al., 2020; Wang et al., 2017, 2006; Zeng et al., 2018) (**Fig. 1.6**). Their impact on aquatic plants is influenced by their body size and density, and is time dependent (Guo-zhang et al., 1999; Jin et al., 2001b; Schoelynck et al., 2020; Wang et al., 2017). The smallest crabs (carapace width < 1.0 cm) have no measurable effect, regardless of their density (Jin et al., 2001b). Once crabs reach a carapace width of 1.8 cm, their impact becomes noticeable, and intensifies as density increases (Jin et al., 2001b). Large crabs (> 3.5 cm) can have a significant impact even at low densities (Jin et al., 2001b; Schoelynck et al., 2020). At high densities, the effects of these crabs can be drastic, sometimes leading to the complete eradication of macrophytes (Jin et al., 2001b; Schoelynck et al., 2020). The damage to the plants is primarily caused by severing or cutting of the stems, leaves and roots, and to a smaller extent through actual

consumption (Jin et al., 2001b; Schoelynck et al., 2020). Healthy and dense macrophyte stands are resilient to disturbance by mitten crabs, with little to no short-term effects. However, if crab densities increase or remain high for several years, they are prone to decline as well (Wang et al., 2017).

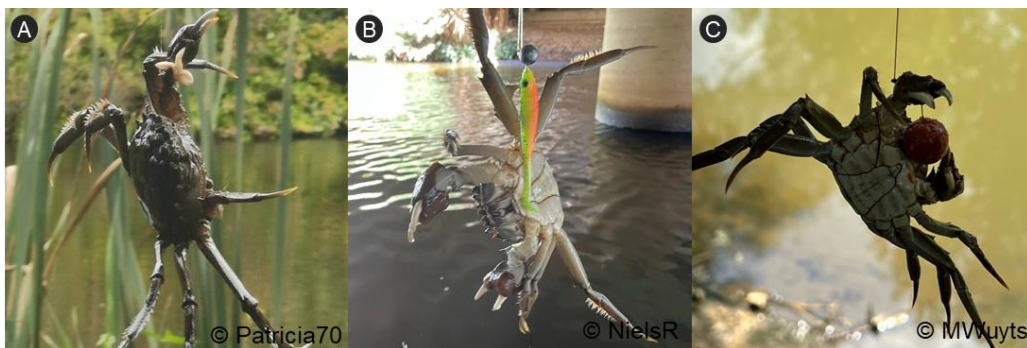
The decline in macrophytes is further accelerated by additional environmental stressors, such as pollution and light limitation (Schoelynck et al., 2020). The latter is often also affected by the presence of mitten crabs (Wang et al., 2017). Through bioturbation, i.e. when the crabs forage, burrow into the top layer of the sediment, or construct horizontal burrows in riverbanks, they stir up the sediment and increase turbidity (**Fig. 1.6**). In addition to reducing water clarity, the resuspension of sediment could also lead to an increased release of pollutants from the riverbed (Soes et al., 2007). Beyond superficial bioturbation, the mitten crab also actively creates burrows.

The burrowing behaviour of the Chinese mitten crab has a very visual impact on the environment. The crabs dig horizontal down-sloping burrows in clay-rich sediment of creek and riverbanks (Panning, 1938; Rudnick et al., 2005a) (**Fig. 1.6**). The burrows are primarily created by juvenile crabs and are mostly confined to tidal areas, where the crabs use the burrows as a shelter during low tide (Hymanson et al., 1999; Kamps, 1937; Rudnick et al., 2005a). The crabs are less likely to burrow when other shelters such as rocks or submersed macrophytes are present (Hymanson et al., 1999). This shelter offers protection against predators and desiccation, which they are especially vulnerable to when they are moulting (Hymanson et al., 1999). The burrows are generally less than 50 cm deep and range from a single tunnel to highly complex networks (Kamps, 1937; Rudnick et al., 2005a). Burrow densities vary widely, from just a few burrows per m<sup>2</sup> up to 80 burrows per m<sup>2</sup> (Kamps, 1937; Peters and Panning, 1933; Rudnick et al., 2005a; Veldhuizen, 2001). These high burrow densities turn riverbanks into ‘Swiss cheese’, which is thought to affect bank erosion rates and levee stability (Harvey et al., 2019). In Germany, the United Kingdom and San Francisco Bay, high burrow densities have been linked to increased erosion and slumping of riverbanks (Morritt et al., 2013; Panning, 1938; Rudnick et al., 2005a). However, despite similarly high burrow densities in the Netherlands, no significant erosion has been reported (Adema, 1991; Kamps, 1937).

Next to these effects that mostly affect ecosystems, there are more direct impacts to people as well, particularly during migration peaks. The most common damage is to fisheries. Literature from Germany and the Netherlands often refer to big swarms of crabs filling commercial fishermen’s nets (Adema, 1991; Kamps, 1937). When caught, mitten crabs often damage both the catch and the nets, decreasing the number of fish

caught but also the commercial value of the catch (Adema, 1991; Kamps, 1937) (**Fig. 1.6**). In some locations and periods, mitten crabs far exceed the catch of fish, thereby significantly increasing the handling time of the catch (Kamps, 1937; Schiemenz, 1942). In the Netherlands, commercial fishery on the Chinese mitten crab is permitted (CLO, 2023), offering a modest economic benefit (**Fig. 1.6**). Though the market value has declined in recent years from approximately 20 euro kg<sup>-1</sup> in 2021, to 10 euro kg<sup>-1</sup> in 2024 (Moerkerke, 2025). In Belgium, however, there are no major inland fisheries, and fyke nets, the type of fishing gear that is most affected by Chinese mitten crabs, are no longer permitted.

In addition to commercial fishermen, recreational anglers have also expressed frustration over the high numbers of crabs infesting various fishing locations (Sneyers, 2024; Veldhuizen, 2001; VRTnws, 2023). The crabs frequently steal their bait or damage their lines (**Fig. 1.6, Fig. 1.7**) (Adema, 1991; Kamps, 1937). In some cases, they pose such a big problem that angler competitions have to be shut down (VRTnws, 2023).



**Fig. 1.7** Chinese mitten crab catching the bait of recreational anglers (source A, C: waarnemingen.be, B: waarneming.nl).

Both scientific and grey literature from different countries indicate that migrating crabs can clog important water-intake infrastructures (**Fig. 1.6**). In the San Francisco Estuary for instance, a boom in the Chinese mitten crab population led to severe fish mortality and escalated operational and engineering expenses at the US Bureau of Reclamation’s pumping facility and fish bypass systems (Rudnick et al., 2005b; Veldhuizen, 2001; White et al., 2000).

Lastly, periodic but significant issues can arise when Chinese mitten crabs move onto land during their migrations (**Fig. 1.6**). From August through December, adult crabs often leave the water to travel overland when migrating from isolated ponds or lakes to rivers. Similarly, in spring, large groups of juvenile crabs sometimes emerge onto land

when they encounter underwater migration barriers. Mitten crabs are exceptionally good climbers and can move up stone walls. The resulting problems range from minor inconveniences, such as a single crab appearing in a garden or house, to more disruptive situations, such as swarms of crabs overrunning a restaurant terrace for weeks or large numbers of crabs accumulating in basements of houses (Devlieger, 2023; HLN, 2019, 2016).

On the plus side, Chinese mitten crabs serve as a food source for many native predators, as discussed in Section 1.3.4. They have also been used in various ways, including as feed for livestock such as chickens, ducks and pigs, fish food, fertilizer, souvenirs, and even as food for octopuses in a public aquarium (Adema, 1991; Panning, 1938). In the past and up till now, they have been sold, both legally and illegally, for human consumption (Zaalmink and Rijk, 2018). More recently, they have been fed to various zoo animals (Pakawi Park, 2021), and there have been trials exploring the extraction and use of chitin from their carapaces (Vandecasteele et al., 2021).

However, none of these applications have been widely adopted, and as a result their use and economic value in non-native regions remains minimal. In general, the production of fish or livestock feed from mitten crabs is not competitive with existing alternatives. Another drawback is their tendency to accumulate pollutants. Research has shown that Chinese mitten crabs can carry significant amounts of contaminants such as per- and polyfluoroalkyl substances (PFAS), trace metals, polychlorinated biphenyls (PCBs) and dioxins, potentially posing health risks to both humans and animals which consume them (Clark et al., 2009; Groffen et al., 2024; Hoogenboom et al., 2015). While human consumption may generally be safe at low intake levels, some fishing areas in the Netherlands have been closed for mitten crab fisheries due to unsafe pollution levels. Additionally, the peak period for capturing adult mitten crabs coincides with the migration of the endangered European eel, raising concerns about accidental bycatch and potential harm to this protected species (Clark et al., 2017). Furthermore, within the European Union, the commercial exploitation of species listed as invasive alien species of Union concern is generally prohibited (European Commission, 2014), as it could promote the spread of the species or encourage the maintenance of existing populations for continued commercial use.

## 1.5 Outline of this thesis

Despite being listed as a ‘species of Union concern’ within the European Union, its long-established presence in Belgium, and its high abundance in the Flemish region, fundamental aspects of the Chinese mitten crab’s ecology are unknown (D’hondt et al., 2021; European Commission, 2014). This thesis aims to investigate the species’ life history, environmental interactions, and ecological impact in Belgium, thereby contributing to a broader understanding of its global invasion success. **Chapters 2** and **3** focus on the species’ life cycle, followed by **Chapters 4** and **5**, which examine its ecological impact. **Chapter 6** explores the impact of a prevalent pathogen on the fitness of the species.

In **Chapter 2**, the general ecology of the species in Belgium is examined, based on up to seven years of catch and biometric data from four freshwater locations across Flanders. While the general life cycle of the Chinese mitten crab is well documented, the regional timing of its key life history stages remains poorly understood. We investigate the distribution and abundance of different size classes, sex ratios, autotomy incidence, timing of the two different migrations and reproduction, and size and age at maturity. This chapter provides substantial new information on the species’ life history in Belgium and offers an important update to our knowledge of the species in Northwestern Europe.

**Chapter 3** delves deeper into the movement behaviour of adult Chinese mitten crabs during their downstream spawning migration. Using acoustic telemetry, applied to this species for the first time, we tracked individuals from non-tidal freshwater habitats to tidal brackish estuarine environments. We reveal their movement rates, migration distance and duration, and adaptive behavioural strategies across the longitudinal river gradient in function of diel and tidal cycles, providing fundamental insights into the species’ ecology.

In **Chapter 4**, we examine how crab size and type of leaf litter influence the detritivorous feeding behaviour of the Chinese mitten crab, and how this in turn affects water quality. Previous stomach content analyses have shown that terrestrial leaf litter is a major component of their diet, while experimental studies confirm their effectiveness as detritivores. Since leaf litter breakdown is a critical process in freshwater nutrient cycling, this chapter sheds light on the ecological role and potential impact of the species in invaded ecosystems.

**Chapter 5** investigates the burrowing behaviour of the Chinese mitten crab in tidal freshwater marshes in Belgium. Although the species is known to construct extensive burrow networks that may contribute to riverbank erosion, relatively few studies have investigated these burrows. Based on experimental investigations, we define morphometric relations between crab size and burrow dimensions. Based on field observations, we explore the relation between burrow density and size to environmental factors, as well as temporal variation in burrow characteristics. Using year-long monitoring data, we evaluate the association between mitten crab burrowing activity and patterns of sedimentation and erosion along riverbanks.

The prevalence of pathogens and their impact on the fitness of non-native Chinese mitten crabs has received little attention, while it could be an important factor in their invasion success. In **Chapter 6**, we examine the prevalence of the microsporidian parasite *Hepatospora eriocheir* (Wang & Chen, 2007) and test for the first time how it affects the physiological condition and performance of non-native Chinese mitten crabs.

Finally, **Chapter 7** summarises the main findings presented throughout this thesis, which are then discussed in the broader context of the species' global invasion success. In addition, it synthesises current knowledge on the ecological and socio-economic impact of the Chinese mitten crab in Belgium and offers recommendations for management.



# 2

## **Life history and ecology of the long-established invasive Chinese mitten crab in Belgium**

*Heleen Keirsebelik, Machteld Bats, Pieter Boets, Paul Van Loon, Dan Sloodmaekers and Jonas Schoelynck*

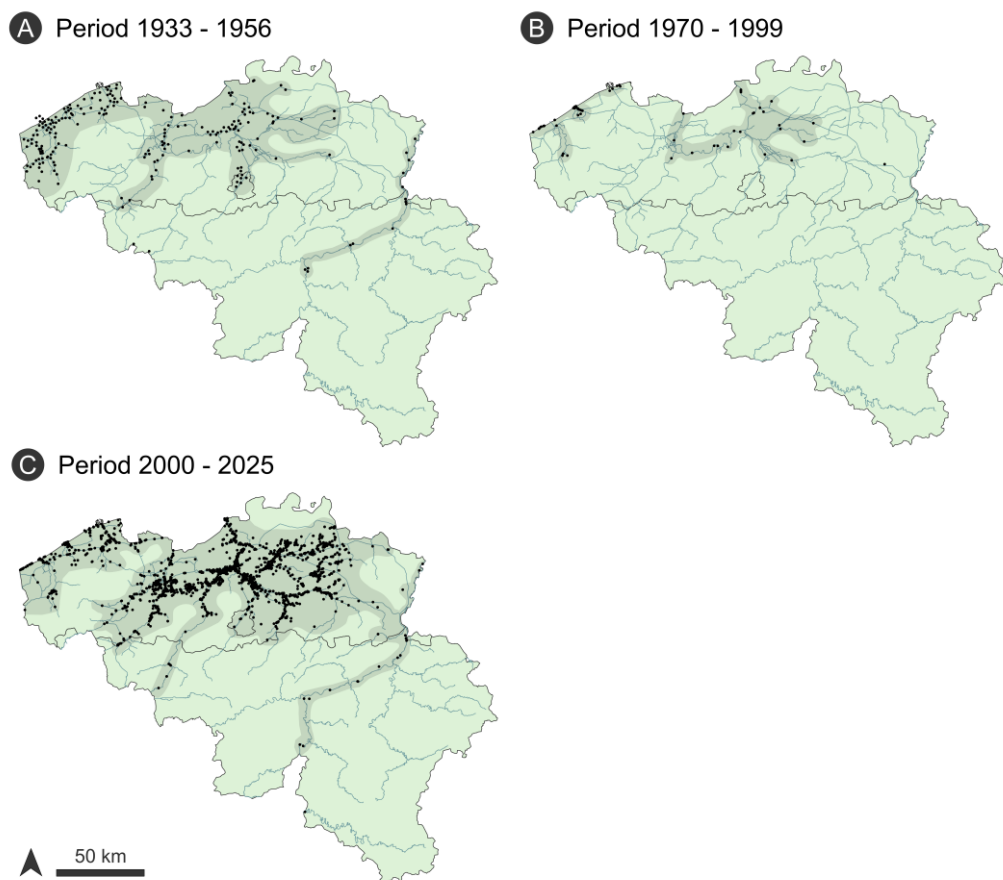
## Abstract

The Chinese mitten crab is a widespread, non-native invasive species with established populations in Europe and North America. In Belgium, the species was first recorded in 1933 and has become particularly abundant over the last three decades. The ecological impact and nuisance caused by the species, along with the implementation of European legislation on invasive species, have prompted active management efforts in Flanders, northern Belgium. However, significant knowledge gaps regarding the life history of the species in Belgium remain. Between 2018 and 2024, four permanent crab traps were installed across Flanders to catch Chinese mitten crabs, which provided the opportunity to investigate spatiotemporal patterns in abundance as well as key aspects of the species' life history and ecology. Catch data reveal substantial inter-annual fluctuations in abundance, as well as variation in spatiotemporal abundance of different size classes. Based on size-frequency analysis, we estimate the species is aged three to six years at maturity. The migration of juvenile crabs occurs between February and June, but peaks from March until May. At some locations, there might be upstream migration year-round. Adult mitten crabs start migrating downstream from late August to December, generally reaching brackish environments in autumn and winter. Ovigerous females are typically observed between October and May, in the lower reaches of the Scheldt Estuary, downstream of northern Antwerp, and along the Belgian coast. The size-mass relationship indicates mostly isometric growth in male crabs, while female crabs showed slightly negative allometric growth. Autotomy incidence varied seasonally, ranging from 23 to 44 %, with lower rates observed in winter compared to summer. The majority of crabs had lost at most one appendage. These findings contribute valuable baseline data on the species' local ecology, which can inform future research and support more targeted management strategies.

## 2.1 Introduction

The Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards 1853) is a catadromous crustacean native to East Asia, primarily found in freshwater and estuarine systems in the coastal regions of China and Korea (Hymanson et al., 1999). However, it has also become one of the world's most notorious aquatic invaders, with populations in both Europe and North America (Dittel and Epifanio, 2009). It has been listed as one of the world's worst invasive species and as a species of Union Concern by the regulation of the European Union on invasive species (European Commission, 2014; Luque et al., 2014).

The species was first recorded outside of its native range in 1912 in Germany (Peters and Panning, 1933). Within the next two decades, the crabs were observed across a whole list of European countries, including, in chronological order, Denmark, Poland, the Netherlands, Sweden, Latvia, Belgium, Estonia, Finland, Lithuania, the United Kingdom, and France (Ewers et al., 2023). In Belgium specifically, the first Chinese mitten crab was caught by an angler in 1933 near Antwerp (Lestage, 1935). The species spread across Belgium primarily via two invasion routes: (1) the North Sea, from where they colonised watercourses in the coastal zone, and (2) the Scheldt Estuary, from where they spread to tributaries and connected canals further inland (Leloup, 1937). By the late 1930s, it had become widespread throughout the region (**Fig. 2.1A**), covering the entire coastal zone as well as the Scheldt River, its main tributaries, and associated canal systems (Leloup, 1943, 1939, 1938b, 1937; Lestage, 1937). This sharp increase in abundance and distribution throughout the 1930s mirrored patterns observed in neighbouring countries including Germany and the Netherlands (Ewers et al., 2023; Kamps, 1937; Peters and Panning, 1933). During this period, exceptionally large numbers of crabs were caught, for instance, 100,000 kg of juvenile crabs were caught in 1936 in the Havel River in Germany (Panning, 1938), and reports of their impact accumulated. Interestingly, populations declined by the mid-1950s, presumably leading to near local extinction, with only sporadic sightings throughout the 1970s and 1980s (**Fig. 2.1B**) (Adema, 1991; Ewers et al., 2023; Mares, 1995). From the 1990s onwards, a significant population recovery was observed, and both their numbers and geographic range increased again (**Fig. 2.1C**) (Ewers et al., 2023; Fladung, 2000; Mares, 1996). This recovery of mitten crab populations has been linked to improvements in riverine water quality and rising sea surface temperatures, but secondary cryptic invasions and genetic introgression might have contributed as well (Ewers et al., 2023; Homberger et al., 2022; Ironside et al., 2025).



**Fig. 2.1** Distribution of the Chinese mitten crab in Belgium during three time periods: (A) 1933 – 1956, (B) 1970 – 1999, and (C) 2000 – 2025. Each dot represents a recorded observation. No map is shown for the period 1956–1970 due to the limited number of observations. Data sources (listed in full: Suppl. Mat. 8.1 **List S. 1**): (A) Adapted from Leloup (1943), with additional records from Wouters (2002), RBINS, and GBIF (2025); (B) Adapted from Wouters (2002), with additional records from RBINS and GBIF (2025); (C) Based on different datasets from GBIF (2025).

Nowadays, Chinese mitten crabs are believed to inhabit nearly all riverine waterbodies in Flanders, northern Belgium (**Fig. 2.1C**). In Wallonia, southern Belgium, there are only sporadic observations of the crab, primarily in the rivers Scheldt and Meuse. The abundance of the species has become apparent through their periodic visibility during their seasonal migrations, when large groups of crabs frequently come on land, as well as through the nuisance they cause to anglers at many different locations. However, it was not until the installation of a new crab trap system on the Kleine Nete River in 2018

that the true magnitude of the population was revealed (Schoelynck et al., 2021). Chinese mitten crabs pose a significant challenge for water managers, as they can disrupt local ecosystems by burrowing into riverbanks, damaging macrophytes, preying on native species, and competing with local fauna for resources (Rosewarne et al., 2016; Rudnick et al., 2005a; Schoelynck et al., 2020). Due to their ecological and economic impacts in invaded habitats (Dittel and Epifanio, 2009), understanding the details of the life history of the Chinese mitten crab is crucial, particularly given the persistence of the species in Belgium. Studying patterns in their abundance and key life history traits in invaded regions provides valuable insights into their population dynamics and supports the development of effective management strategies.

As a catadromous species, the life cycle of the Chinese mitten crab is complex and includes freshwater, brackish and marine phases (Rudnick et al., 2005b). In autumn, adult crabs migrate downstream from freshwater habitats to estuaries or marine environments to reproduce (Chapter 3; Keirsebelik et al., 2025; Panning, 1938; Rudnick et al., 2005b). Typically, males migrate first and are followed by females. After mating, female crabs carry the fertilized eggs for several weeks up to a few months, before releasing the larvae into brackish or marine waters. A single female can have up to three broods, however, after reproduction is completed, the adult crabs die (Kobayashi, 2001; Rudnick et al., 2005b). The larvae develop in marine environments, passing through several planktonic stages before settling as a megalopa and migrating upstream (Anger, 1991; Peters and Panning, 1933). In tidal areas, the megalopae metamorphose into juvenile crabs (Anger, 1991), which continue their journey further upstream in spring. Once they are settled in suitable freshwater habitats, the crabs grow and mature, often far upstream in the catchment (Panning, 1938). Estimations of their life span vary by region, but generally range from one to six years (Hymanson et al., 1999; Kamps, 1937; Peters and Panning, 1933; Rudnick et al., 2005b).

This paper investigates the life history and ecology of the non-native Chinese mitten crab in Belgium, where the species has been abundant for over a century but has not been studied in detail. We focus primarily on Flanders (northern Belgium), because of the limited number of observations in Wallonia (southern Belgium). Specifically, we address five key questions: (1) what is the timing of the migratory and reproductive phases, (2) is there spatial and temporal variation in size, abundance and sex ratio, (3) at what age do individuals reach sexual maturity, (4) what is the relation between carapace width and body mass, (5) what is the incidence of autotomy (i.e. missing appendages)? Over up to seven years, mitten crabs were captured year-round at four locations across Flanders to examine temporal and spatial patterns. Subsets of these

crabs were measured to examine mass-size relationships, autotomy and age at maturity through size-frequency analysis. This study updates our understanding of the ecology of non-native Chinese mitten crabs in Northwestern Europe and provides a foundation for effective management strategies.

## 2.2 Materials and Methods

### 2.2.1 Study area

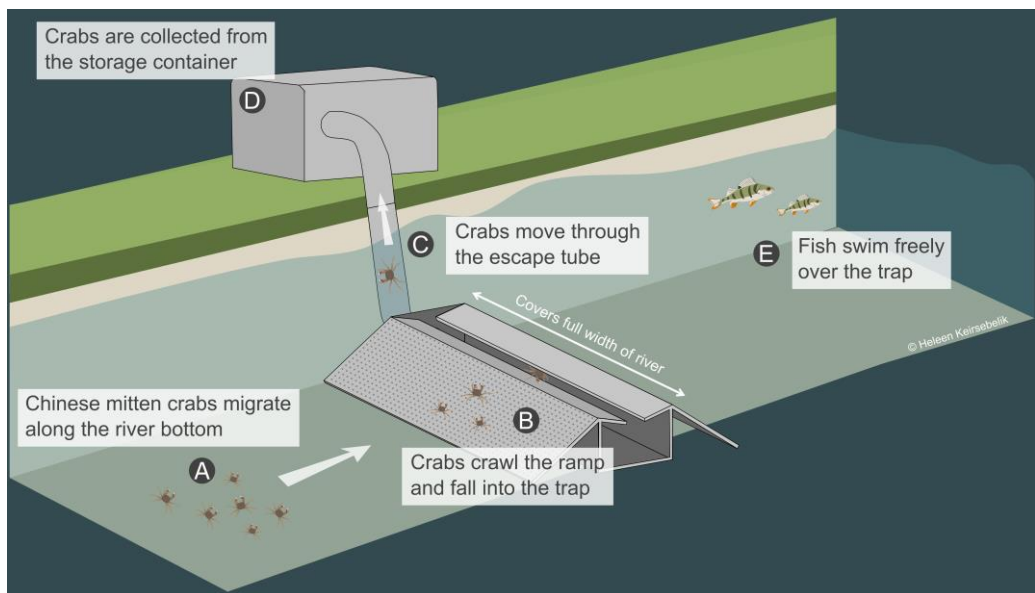
This study was performed in the region of Flanders, in northern Belgium, and focused primarily on the Scheldt River (**Fig. 2.2**) and its tributaries. The Scheldt River is the main connection between the North Sea and inland waterbodies, and therefore represents an important migration route for Chinese mitten crabs in Flanders (Keirsebelik et al., 2025; Stevens et al., 2009). The Scheldt River originates in Saint-Quentin (northern France) and flows through Belgium before entering the North Sea near Vlissingen (the Netherlands). The river is approximately 355 km long and drains a catchment area of 21,863 km<sup>2</sup> (Meire et al., 2005). The Scheldt Estuary is macrotidal and covers a full salinity gradient from freshwater to marine conditions. The Scheldt River is tidally influenced up to Ghent, beyond which tidal action is reduced by sluices and weirs. The Scheldt experiences highly variable discharge with a peak in winter (Meire et al., 2005).



**Fig. 2.2** Map of the Scheldt Estuary, located on the border of Belgium and the Netherlands (see inset). The location of the four permanent crab traps, (A) Grobbendonk, (B) Kalken, (C) Lippenbroek, and (D) Merelbeke, are indicated along the Scheldt River and its tributaries.

## 2.2.2 Catch data crab traps

Data were primarily collected from four permanent traps that were specifically designed to catch Chinese mitten crabs. Each trap consists of a rectangular steel box, submerged and secured to the riverbed (**Fig. 2.3**). The trap covers the full width of the river. On the topside, there is a 15 cm wide longitudinal opening. On each side of the trap, there are perforated ramps leading up to the top of the trap. Due to their limited swimming abilities, migrating mitten crabs climb the ramps while moving along the river bottom and fall through the top opening into the trap (**Fig. 2.3**). Once inside, their only exit is through PVC tubes, lined with steel mesh, which lead them to storage containers on the riverbanks. From these storage containers, the crabs cannot escape, but can be collected with ease. The traps are highly effective during migration periods, capturing both downstream migrating adults and upstream migrating juveniles. The primary purpose of the traps is to prevent upstream migrating crabs from entering sensitive areas (located upstream of the trap). Detailed information on the design and functionality of these traps is available in Schoelynck et al. (2021).



**Fig. 2.3** Illustration of the functioning of the submerged permanent trap used to capture Chinese mitten crabs. Labels indicate (A) crab migration, (B) entry into the trap via ramps, (C) movement through escape tube to (D) storage container on the riverbank, where crabs are collected, and (E) fish passage over the trap.

### 2.2.2.1 Grobbendonk

The trap is located in the upstream reaches of the Nete River Basin, which is part of the Scheldt River Basin, at approximately 125 km from the mouth of the Scheldt Estuary. The trap was installed on a fish ladder at Grobbendonk (**Fig. 2.2A**) in the Kleine Nete River (51°11'35.3" N, 4°45'23.1" E) in February 2018. The trap was monitored one to three times per week during the spring migration period (when the majority of crabs are caught) and every two weeks or less frequently outside this period. Each time the trap is monitored, the containers on the riverbank are emptied, and the bulk weight of all the crabs is determined. The number of crabs within one kilogram is counted, and this ratio (number of crabs kg<sup>-1</sup>) is used to extrapolate the total number of crabs in the catch. Approximately biweekly (based on monitoring frequency), a random subsample of the total catch (about 100 crabs) was collected for individual biometric measurements. In total 100 subsamples were analysed.

### 2.2.2.2 Kalken

The trap (51°01'02.4" N, 3°55'32.7" E) is located on a fish ladder on the Driesesloot, a small watercourse that drains into the Scheldt River (**Fig. 2.2B**), and was installed in 2020. The trap is located at approximately 135 km from the mouth of the Scheldt Estuary. The Driesesloot connects to numerous smaller watercourses within the Kalkense Meersen, a restored wetland nature reserve that covers approximately 600 ha. In contrast to Grobbendonk, the trap was not continuously operational. In the first three years after installation (2020-2022), it was closed from July through December, and in 2022 also during most of spring. In 2023-2024, the trap was operational during the two migration periods (February-June and September/October-December). When operational, the trap was monitored weekly to biweekly. The total number and weight of crabs were determined using the same method as in Grobbendonk. Additionally, random subsamples (n = 24) were collected biweekly for biometric measurements.

### 2.2.2.3 Lippenbroek

The trap was installed in November 2021, in the restored freshwater tidal marsh Lippenbroek, located along the Scheldt River at approximately 100 km from the mouth of the estuary (**Fig. 2.2C**). The area (10 ha) is surrounded by a ring dyke and experiences a reduced semidiurnal tidal regime that is controlled through two separate sluices. The trap (51°05'10.3" N, 4°10'19.5" E) is located near the inflow/outflow of the area, within the main creek. The storage container on the riverbank was checked every two weeks. Because this trap is located in a dynamic tidal area, the trap itself was cleaned out

once a month, to remove any buildup sediment or organic material. During this clean-up all crabs within the trap are collected as well. Due to the low number of crabs, every individual crab is counted and measured, instead of taking subsamples. In total, 57 samples were collected.

#### 2.2.2.4 Merelbeke

The trap was installed in 2021 in the Melsenbeek, a small tidal watercourse that is connected to the Scheldt River, at approximately 160 km from the mouth of the Scheldt Estuary (**Fig. 2.2D**). This trap (51°00'04.1" N, 3°44'35.8" E) lacks storage containers like the other traps and instead has tubes that return the crabs back to the Scheldt River. Therefore, the crabs are normally not caught. In this way, the trap prevents the crabs from migrating upstream, protecting nature areas while minimising the management costs (D'hondt et al., 2021). From January 2023 onwards, the number of crabs that pass through the return system were periodically monitored. The tubes were disconnected every week to every two weeks, and all crabs passing through over 24 hours were collected in a container and weighed. From this, a subsample (n = 101) of about 100 crabs was collected each time for biometric measurements. The catch between sampling dates was interpolated linearly based on the daily catch rates, to estimate the total number of crabs per month and year.

### 2.2.3 Biometric measurements

For each crab in every (sub)sample (n = 282 samples), carapace width (CW, mm) was measured just behind the fourth lateral spines, using a digital calliper with a precision of 0.01 mm. Individual wet mass (g) was recorded using a digital balance with accuracy up to 0.01 g. Sex was determined based on the shape of the pleon. Crabs with a CW smaller than 7 mm were classified as 'unsexed juveniles', as sex cannot be unambiguously determined below this size (Rudnick et al., 2003).

Autotomy incidence was assessed using samples (n = 22 samples) collected from Merelbeke in 2023, as these specimens were well-preserved and not damaged during the freezing process. For each individual, the number of missing walking legs and chelae was recorded.

Sexual maturity in female individuals was assessed in a subset of samples collected at the end of 2023 (Grobbendonk (n = 2 samples)) and at the end of 2024 (Grobbendonk (n = 2 samples), Kalken (n = 3 samples), and Lippenbroek (n = 4 samples)). Ovaries were examined macroscopically and classified as either immature or mature, following the

developmental stages described by Wu et al. (2017), with stage I considered immature and all subsequent stages (II to V) considered mature.

#### **2.2.4 Data analysis**

All analyses were performed in R version 4.2.2 (R Core Team, 2022) and graphs were created using the R package ggplot2 (Wickham, 2016). The significance level for statistical analyses was set at  $p < 0.05$ .

A generalized linear model (GLM) with negative binomial distribution and log link function (glmmTMB R package (Brooks et al., 2017)) was used to assess differences in annual crab catch between catch locations and years. Both location and year were included as fixed effects in the model. The model was used as input for an analysis of variance test (ANOVA, car R package (Fox and Weisberg, 2019)). For each significant factor, pairwise comparisons between levels were performed with Tukey adjustment (emmeans R package (Lenth, 2017)). To further explore monthly variation in crab catches per location, we used GLMs with a negative binomial distribution and log link function. Separate models were fitted for each location, and month and year were included as fixed effects. In the case of Grobbendonk, a constant zero-inflation term (ziformula = ~ 1) was specified to account for the zero counts.

To assess the overall difference in average crab size between locations and sexes, a generalized linear mixed model (GLMM) was applied with a gamma distribution and a log link function. Crab size, as carapace width in mm, was fitted in function of location as fixed effect. Month and year were included as random effects to account for temporal variability. To explore further differences in monthly average crab size within and between locations, a GLMM (gamma distribution, log link) was used. Crab size was modelled in function of location, month, and their interaction as fixed effects. Year was included as random effect to account for interannual variation. Pairwise comparisons between months for each location, and between locations for each month were performed with Tukey adjustment.

Cohort analysis was conducted using FISAT II software (Gayanilo et al., 2005). Consistent with Kobayashi (2011), the term 'cohort' refers to a group of crabs that settled during the same period and year, as identified through cohort analysis, rather than to a year class. The analysis was performed using carapace width measurements, which were rounded to the nearest millimetre. The Bhattacharya's method was applied to each monthly size-frequency dataset to identify distinct cohorts and to calculate the mean  $\pm$  standard deviation (SD) for each cohort.

Separate models were run for each location to assess monthly differences in the catch proportions of male and female crabs. A generalized linear model (GLM) with a binomial error distribution (stats R package (R Core Team, 2022)) was used, with the response variable representing the number of males and females per month. A significant deviation from the intercept-only model indicates a sex ratio that differs significantly from the expected 50:50 distribution. Measurements of unsexed juveniles were not included in the models.

Linear mixed-effects models (LMM) were used to examine the allometric relationship between the individual mass and carapace width of Chinese mitten crabs (lme4 and lmerTest R package (Bates et al., 2015; Kuznetsova et al., 2017)). Separate models were fitted for each location, with log-transformed mass as dependent variable and log-transformed carapace width, sex, and their interaction as fixed effects. Year was included as a random effect to account for temporal variability. Measurements from unsexed juveniles were excluded from the analysis. The estimates from these models were used to establish relationships between individual mass (g) and carapace width (CW, in mm) for each sex and location, based on the following equation:

$$\log(Mass) = \log(a) + b \log(CW)$$

$$Mass = a \times CW^b$$

If the value of the slope ( $b$ ) is equal to 3, the mass scales proportionally with carapace width, indicating isometry. If the 95 % confidence interval for  $b$  included 3, growth was considered isometric.

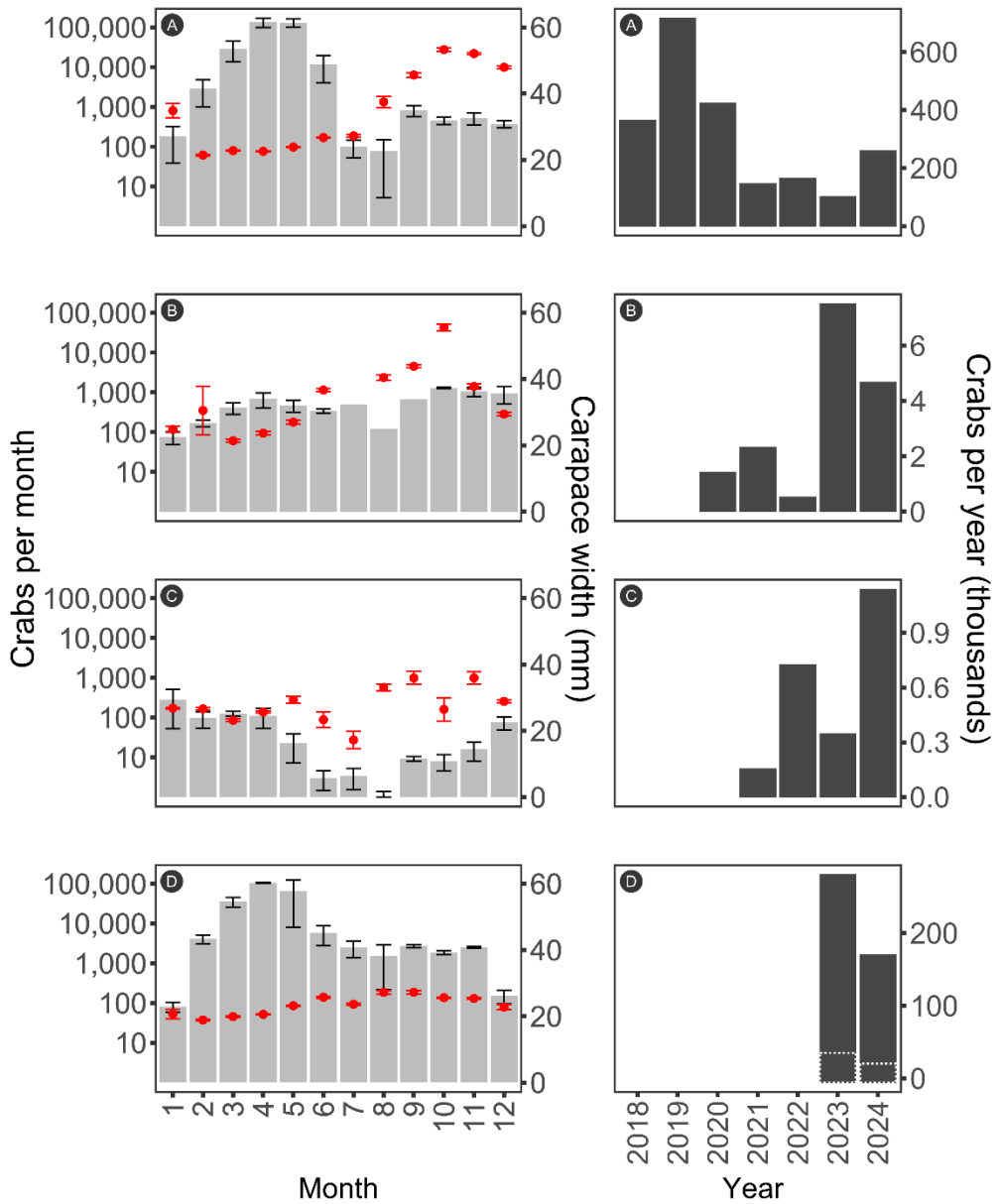
To assess whether the proportion of injured (autotomised) individuals varied across months, seasons, sex, and size classes, separate chi-square tests were conducted (stats R package (R Core Team, 2022)). When a significant effect was detected, pairwise Fisher's exact tests (rstatix R package (Kassambara, 2023)) were used to identify differences between specific levels (e.g., between months). Size classes were determined based on carapace width, beginning with a class for individuals smaller than 15 mm, followed by successive 5 mm intervals (15-19.99 mm, 20-24.99 mm, etc.), with the final class including all individuals larger than 45 mm.

## 2.3 Results

### 2.3.1 Interannual trends in crab catches

Total annual catches varied across years within locations (**Fig. 2.4**, right panels), but no consistent overall differences between years were observed ( $\chi^2 = 11.87$ ,  $p = 0.065$ ). Annual catch numbers differed between locations ( $\chi^2 = 325.31$ ,  $p < 0.001$ ), and the highest yearly catches were recorded at Grobbendonk and Merelbeke, which did not differ significantly from each other ( $p = 0.954$ ). These were followed by Kalken (all  $p < 0.001$ ), and all three locations had significantly higher catches than Lippenbroek (all  $p < 0.001$ ).

Grobbendonk (**Fig. 2.4A**) offers the longest time series. Catch numbers were highest in the first three years after trap installation, peaking in 2019 with over 700,000 crabs captured. Since 2021, annual catches have been lower and range between 100,000 and 260,000 crabs per year. Lippenbroek (**Fig. 2.4C**) shows similar interannual variation in catch numbers as Grobbendonk, although yearly catches were substantially lower, ranging from 348 to 1,140 crabs per year. Note that the trap was only installed in November 2021, meaning data for that year is incomplete. At Kalken (**Fig. 2.4B**), interannual variation was influenced by intermittent closing periods of the trap. As a result, reported catch numbers (500 to 7,500 crabs per year) do not fully reflect actual yearly totals. Although the trap at Merelbeke (**Fig. 2.4D**) was installed in 2022, estimates of yearly catch numbers are only available for 2023 and 2024. The actual catch numbers ranged from 28,000 to 44,000 crabs, while extrapolated yearly totals suggest catch numbers between 170,000 and 280,000 crabs. Interestingly, the interannual variation of this location followed a similar trend as Kalken.



**Fig. 2.4** Bar plots show the average number of crabs caught per month (left panels,  $\pm$  SE) and the total number of crabs caught per year at each location (right panels). Note that the average number of crabs caught per month is shown on a log scale. The average carapace width (mm,  $\pm$  SE) of crabs caught each month is indicated in red. Locations: (A) Grobbendonk, (B) Kalken, (C) Lippenbroek, (D) Merelbeke. The white dotted line represents the actual number of crabs caught in Merelbeke (D) and the bar represents the estimated number of crabs per year.

### 2.3.2 Monthly variation in crab catches

Crab catches varied throughout the year, with distinct seasonal peaks observed at all study locations (**Fig. 2.4**, left panels).

At Grobbendonk (**Fig. 2.4A**, Suppl. Mat. 8.1 **Table S. 1**), monthly crab catches showed pronounced seasonal and moderate interannual variation (month:  $\chi^2 = 390.3$ ,  $p < 0.001$ ; year:  $\chi^2 = 26.6$ ,  $p < 0.001$ ). The highest numbers were recorded between February and June, peaking in April-May. A smaller, secondary increase occurred in autumn (September-December). Overall, monthly catches were significantly higher in 2019 ( $p < 0.05$ ) compared to other years.

At Kalken (**Fig. 2.4B**, Suppl. Mat. 8.1 **Table S. 2**), monthly catches were more consistent but followed a broadly similar seasonal pattern (month:  $\chi^2 = 88.1$ ,  $p < 0.001$ ). The lowest numbers of crabs were caught in January, while higher catches were recorded during spring (March – June) and autumn (September-December). However, the number of crabs caught per month in spring was notably lower compared to Grobbendonk, whereas autumn catches were comparable between both sites. Monthly crab catches also exhibited moderate interannual variation (year:  $\chi^2 = 29.1$ ,  $p < 0.001$ ), with overall significantly higher values observed in 2023 ( $p < 0.01$ ) compared to all other years except for 2022 ( $p = 0.999$ ).

In contrast, Lippenbroek (**Fig. 2.4C**, Suppl. Mat. 8.1 **Table S. 3**) showed a different seasonal pattern (month:  $\chi^2 = 170.7$ ,  $p < 0.001$ ). Catches were highest during winter and early spring (December-April), peaking in January. Monthly catches remained low in summer and autumn, with lowest catch numbers occurring from June to August. Although total catches were substantially lower compared to other locations, January catches exceeded those at all other locations during the same month. Interannual variation in monthly catches was moderate (year:  $\chi^2 = 14.1$ ,  $p < 0.01$ ), with significantly lower catches in 2023 ( $p < 0.05$ ) compared to all other years except 2022 ( $p = 0.155$ ).

At Merelbeke (**Fig. 2.4D**, Suppl. Mat. 8.1 **Table S. 4**), catches remained consistently high throughout most of the year but still showed significant seasonal variation (month:  $\chi^2 = 428$ ,  $p < 0.001$ ). Similar to Grobbendonk and Kalken, a peak in catches occurred in spring, though the peak started slightly earlier, lasting from March until May. While monthly catches declined in December and January, numbers remained comparatively high from June through November and in February, making Merelbeke the only location with elevated catches year-round. Interannual differences in monthly catches were also observed (year:  $\chi^2 = 428$ ,  $p < 0.01$ ), with higher catches in 2023 than in 2024.

### 2.3.3 Monthly variation in crab size per location

As expected, average carapace width varied considerably across months and locations, with a significant interaction between location and month ( $\chi^2 = 6694.1$ ,  $p < 0.001$ ).

At Grobbendonk (**Fig. 2.4A**, Suppl. Mat. 8.1 **Table S. 5**), average crab size was intermediate in January (mean = 37.18 mm) but decreased sharply toward early spring, reaching minimum values in March-April (mean = 21.82-22.41 mm,  $p < 0.001$ ). From late spring onward, average size increased significantly until June (mean = 26.74 mm) and remained relatively large through summer. The largest individuals were observed in autumn (October-November: mean = 51.69-53.10 mm,  $p < 0.001$ ), after which sizes declined slightly in December (mean = 48.98 mm).

At Kalken (**Fig. 2.4B**, Suppl. Mat. 8.1 **Table S. 5**), average crab sizes remained relatively small and variable from January through March (mean = 21.27 mm), with no significant differences between these months ( $p > 0.05$ ). As spring progressed, crab size gradually increased, reaching an estimated average of 26.99 mm in May. During summer, significantly larger crabs were observed (June-August: mean = 39.06-41.11 mm,  $p < 0.001$ ), with peak sizes recorded in early autumn (September-October: mean = 50.65-55.26 mm). Average size declined again toward the end of the year (December: mean = 29.80 mm,  $p < 0.001$ ).

At Lippenbroek (**Fig. 2.4C**, Suppl. Mat. 8.1 **Table S. 5**), average sizes were small and stable in January-February (mean = 23.81-23.87 mm). In the following months, average sizes were highly variable. Sizes decreased slightly in March (mean = 21.52 mm), then increased until May (mean = 27.11 mm), followed by a marked drop in July, when the smallest individuals were recorded (mean = 15.37 mm,  $p < 0.001$ ). Average sizes fluctuated substantially in the remaining months, ranging from 24.79 mm in October to 33.69 mm in September.

At Merelbeke (**Fig. 2.4C**, Suppl. Mat. 8.1 **Table S. 5**), average crab sizes were smallest from January until April ( $p > 0.05$ ), with a minimum of 18.86 mm in March and a maximum of 20.47 mm in January. From April onward, monthly average size gradually increased until June (mean = 24.50 mm). A temporary decline in size occurred in July (mean = 22.79 mm), followed by the largest average sizes in August and September (mean = 25.63-26.58 mm). Sizes then gradually decreased toward winter (December: mean = 20.90 mm), returning to values similar to those of early in the year ( $p > 0.05$ ).

### 2.3.4 Differences in crab size across locations

Average crab size varied significantly among locations ( $\chi^2 = 2498.5$ ,  $p < 0.001$ ). The largest individuals were found in Grobbendonk and Kalken (**Table 2.1**), where sizes were comparable ( $p = 0.633$ ). Crabs in Lippenbroek were on average smaller than those at these two sites but still significantly larger than those in Merelbeke, where consistently smaller individuals were found ( $p < 0.001$ ).

**Table 2.1** Overview of carapace width (mm) per location. With n = sample size, and SD = standard deviation, and group depicting significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey's adjustment. Groups sharing the same letter are not significantly different from each other.

Location	n	Minimum	Mean $\pm$ SD	Maximum	Group
Grobbendonk	7295	9.87	30.88 $\pm$ 13.81	86.88	a
Kalken	1448	9.27	33.72 $\pm$ 11.59	68.95	a
Lippenbroek	2345	3.89	26.56 $\pm$ 7.42	52.08	b
Merelbeke	6045	7.24	23.63 $\pm$ 6.51	74.71	c

However, these size differences varied substantially across months ( $\chi^2 = 6694.1$ ,  $p < 0.001$ ). In January, average crab size was lowest in Kalken and Merelbeke, with no significant difference between them ( $p = 0.732$ ). Crab sizes slightly increased in Lippenbroek ( $p < 0.05$ ) and were substantially larger in Grobbendonk ( $p < 0.001$ ). In February, Merelbeke had significantly smaller crabs ( $p < 0.001$ ) compared to Lippenbroek and Grobbendonk (Suppl. Mat. 8.1 **Table S. 6**), which did not differ from each other ( $p = 0.996$ ). At Kalken, sizes were highly variable and not significantly different from other locations ( $p > 0.05$ ).

In spring, variability in crab sizes between locations decreased. In March, Merelbeke continued to have the smallest crabs ( $p < 0.001$ ). Lippenbroek and Kalken showed intermediate and statistically indistinct sizes ( $p = 0.942$ ), while the largest crabs were found in Grobbendonk ( $p < 0.05$ ). In the next two months, April and May, patterns were consistent (Suppl. Mat. 8.1 **Table S. 6**). Merelbeke remained the location with the smallest crabs ( $p < 0.001$ ), followed by intermediate crabs at Grobbendonk ( $p < 0.01$ ) and the largest crabs were found at Lippenbroek and Kalken (April:  $p = 0.609$ , May:  $p = 0.999$ ).

During summer, spatial differences in size became more pronounced. In June, crabs were smallest in Merelbeke and Lippenbroek ( $p = 0.053$ ), intermediate in size at Grobbendonk ( $p < 0.001$ ), and largest in Kalken ( $p < 0.001$ ). Crabs were substantially

smaller in Lippenbroek in July compared to all other locations ( $p < 0.001$ ), followed by Merelbeke ( $p < 0.001$ ), and with the largest crabs recorded in Grobbendonk ( $p < 0.001$ ). There was no data available for Kalken for the month of July. In August, Merelbeke showed again the smallest individuals, followed by Grobbendonk ( $p < 0.01$ ) and Kalken ( $p < 0.001$ ), which also differed from each other ( $p < 0.05$ ). Average crab size at Lippenbroek was not significantly different from other locations ( $p > 0.05$ ).

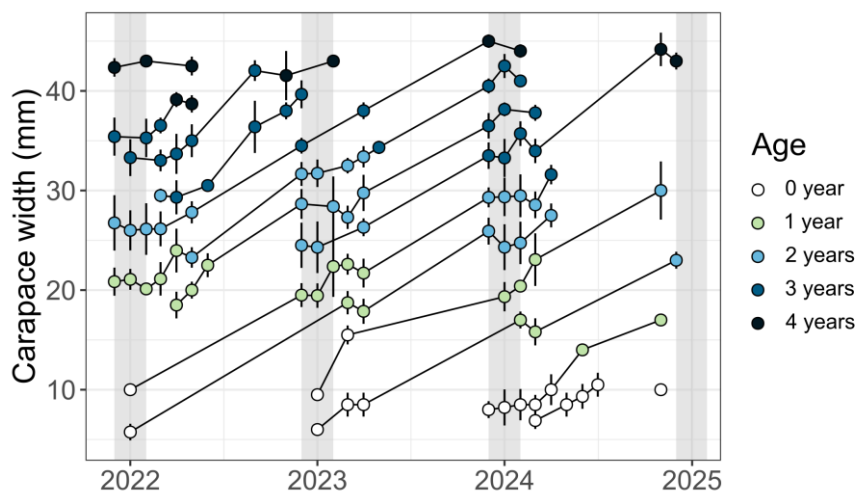
By September, large differences between locations became apparent, smallest crabs were found in Merelbeke ( $p < 0.001$ ), followed by Lippenbroek ( $p < 0.001$ ), and Kalken and Grobbendonk, which were similar but statistically different ( $p < 0.001$ ). In October, the pattern shifted slightly, average crab size was again smallest in Merelbeke and Lippenbroek (Suppl. Mat. 8.1 **Table S. 6**), with no significant difference between them ( $p = 0.608$ ). In contrast, crabs at Kalken and Grobbendonk were substantially larger ( $p < 0.001$ ), with no significant difference between them ( $p = 0.166$ ).

Finally, in November and December, the pattern resembled that of August, with smallest sizes recorded in Merelbeke ( $p < 0.001$ ), followed by Lippenbroek ( $p < 0.05$ ), then Kalken ( $p < 0.001$ ) and largest in Grobbendonk ( $p < 0.001$ ).

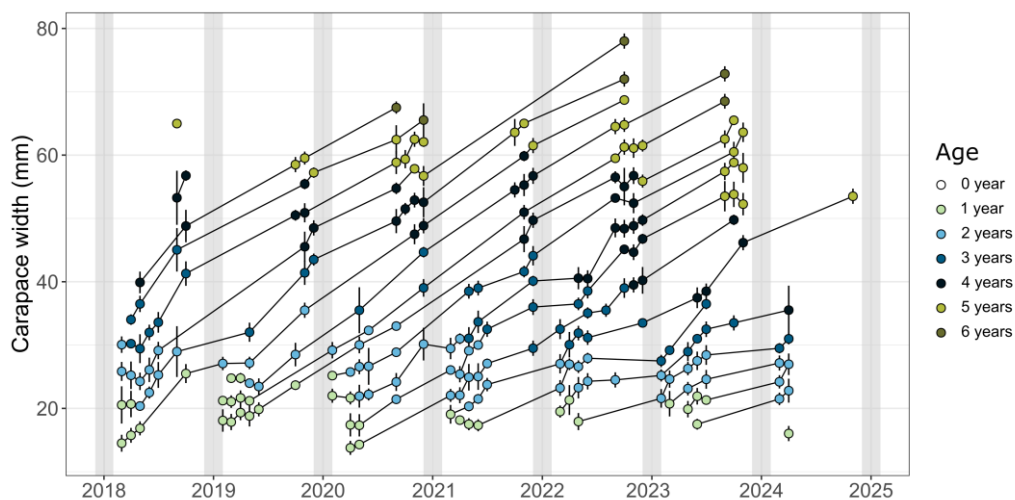
### 2.3.5 Cohort analysis

At the tidal marsh Lippenbroek, 15 cohorts were identified (**Fig. 2.5**) over the span of three years using monthly size-frequency data (Suppl. Mat. 8.1 **Fig. S. 1**), based on 25 subsamples comprising 2,306 crabs. At the beginning of each year two new cohorts could be distinguished. Age-0 crabs range on average from 6 to 16 mm in carapace width. One-year-old crabs range from 14 and 24 mm, two-year-olds from 23 to 33 mm, three-year-olds from 29 to 42 mm, and four-year-old crabs range from 39 to 45 mm.

At the upstream, non-tidal freshwater site, Grobbendonk, 23 cohorts were identified over the span of seven years (**Fig. 2.6**, Suppl. Mat. 8.1 **Fig. S. 2**, **Fig. S. 3**, **Fig. S. 4**), based on 52 subsamples comprising 7,179 crabs. No age-0 crabs were observed at this site. One-year-old crabs enter the area at the beginning of the year and range on average from 14 to 26 mm in carapace width. Two-year-olds from 20 to 36 mm, three-year-olds from 27 to 45 mm, four-year-olds from 36 to 60 mm, five-year-olds from 52 to 69 mm, and six-year-old crabs range from 66 to 78 mm in carapace width.



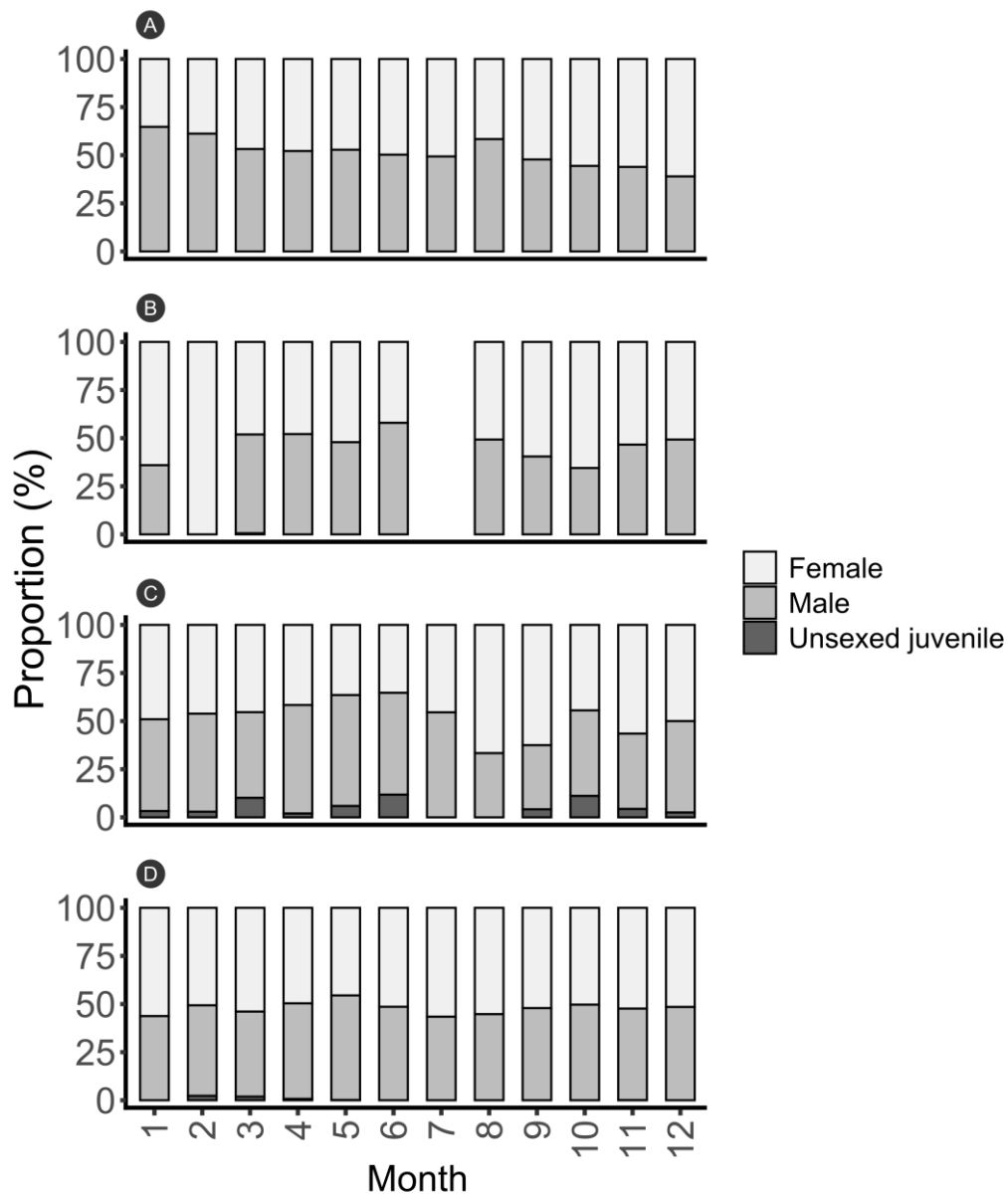
**Fig. 2.5** Temporal changes in mean carapace width (circles  $\pm$  SD) for each cohort identified using Bhattacharya’s method for location Lippenbroek. Cohort age estimates are indicated by colour. Grey shaded areas denote winter periods (December – February) during which mitten crab growth is likely minimal.



**Fig. 2.6** Temporal changes in mean carapace width (circles  $\pm$  SD) for each cohort identified using Bhattacharya’s method for location Grobbendonk. The estimated age of each cohort, counting from megalopa settlement, is indicated by colour. Grey shaded areas denote winter periods (December – February) during which mitten crab growth is likely minimal.

### 2.3.6 Seasonal variation in sex ratio

In Grobbendonk (**Fig. 2.7A**), a significant male bias was observed during February ( $p < 0.001$ ) and March ( $p < 0.05$ ), while females dominated the catch in October ( $p < 0.05$ ), November ( $p < 0.01$ ), and December ( $p < 0.001$ ). No significant differences in the sex ratio were found in other months. At Kalken (**Fig. 2.7B**), females were generally more abundant than males. However, significant differences in sex ratio were only observed in January ( $p < 0.05$ ), September ( $p < 0.01$ ), and October ( $p < 0.05$ ), when females outnumbered males. In June, males were more abundant ( $p < 0.05$ ). The sex ratio at Lippenbroek (**Fig. 2.7C**) fluctuated throughout the year, however, significant differences were only found in April ( $p < 0.01$ ) and May ( $p < 0.05$ ), when females were slightly more abundant than males. In Merelbeke (**Fig. 2.7D**), the male-to-female ratio remained balanced throughout the year, except in March, when females were slightly more abundant ( $p < 0.01$ ), and in May, when males were more numerous ( $p < 0.05$ ).



**Fig. 2.7** Stacked bar plots show the proportion of female, male and juvenile (unsexed) crabs caught per month at each location (dark grey). Locations: (A) Grobbendonk, (B) Kalken, (C) Lippenbroek, (D) Merelbeke.

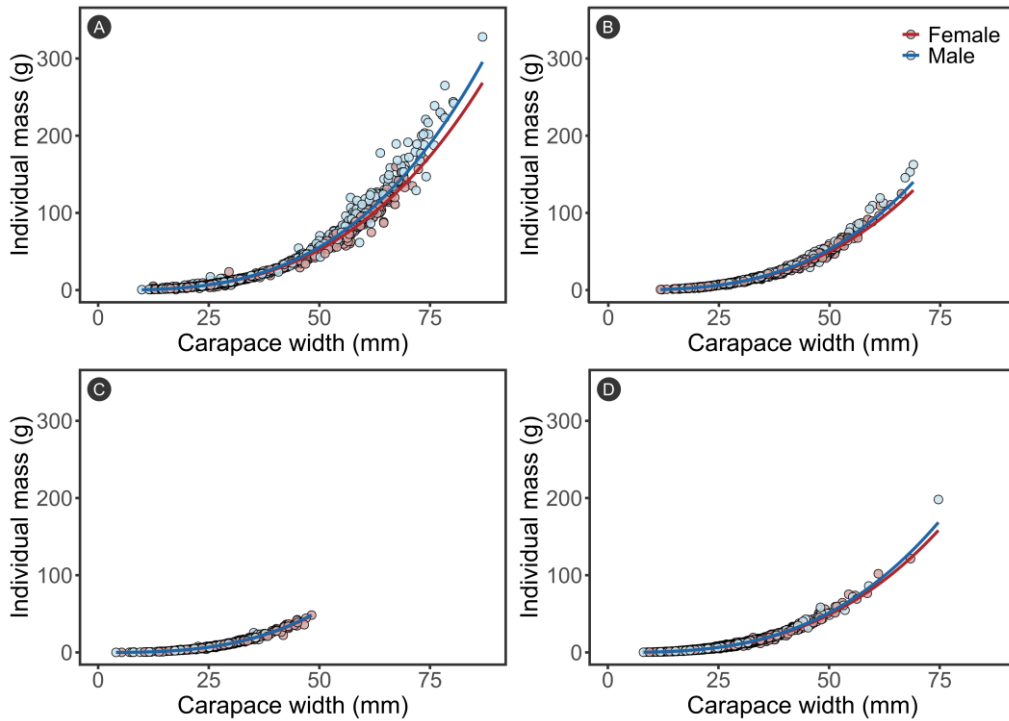
### 2.3.7 Relation between carapace width and mass

A total of 10,887 measurements of individual Chinese mitten crabs were included in the linear-mixed effects models for the locations Grobbendonk (n = 3,462), Kalken (n =

766), Lippenbroek (n = 1,224), and Merelbeke (n = 5,435) (**Table 2.2**). At locations Grobbendonk, Kalken and Merelbeke, both sex ( $p < 0.001$ ) and the interaction between log-transformed carapace width and sex ( $p < 0.001$ ) had significant effects, indicating that the mass-carapace width relationship differs between sexes. The relationships are similar, but males have at these locations slightly steeper slopes ( $b$  value), meaning their mass increases more rapidly with carapace width compared to females (**Fig. 2.8**). However, females have a slightly higher intercept ( $a$  value), meaning that at smaller carapace widths, females might be heavier than males. At Lippenbroek, no significant interaction between log-transformed carapace width and sex ( $p = 0.175$ ) was detected, nor was there a significant main effect of sex ( $p = 0.058$ ). Growth was isometric for both sexes at this location. At Grobbendonk and Kalken, female growth was slightly negative allometric, while male growth was isometric. In contrast, at Merelbeke growth of both male and female crabs was negative allometric (**Table 2.2**). Random effects analysis showed significant but minimal interannual variability (range  $\sigma^2$ :  $2.24 - 4.35 \times 10^{-4}$ ), suggesting that year-to-year variation had little influence on the overall mass-carapace width relationship.

**Table 2.2** Estimated parameters that describe the mass-carapace width relationship for male and female crabs per location. With  $a$  = intercept,  $b$  = slope, 95 % CI = 95 % confidence interval, and types of growth (I = isometry, A+ = positive allometry, A- = negative allometry).

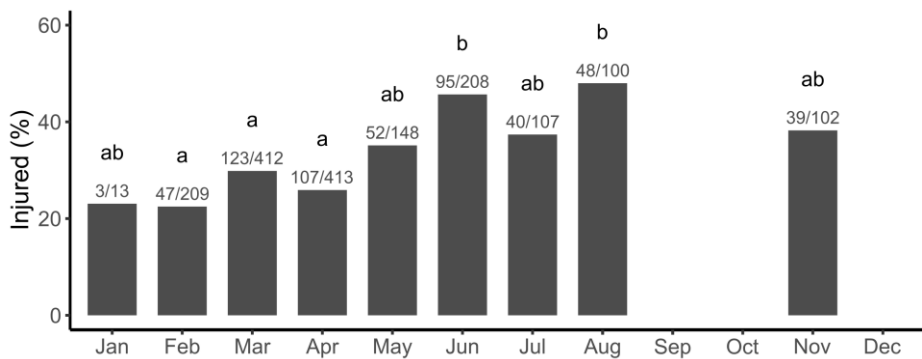
Location	Sex	$a$ (95% CI)	$b$ (95% CI)	Growth	n
Grobbendonk	Female	$4.73 \times 10^{-4}$ ( $4.54 - 4.92 \times 10^{-4}$ )	2.97 (2.96 - 2.98)	A-	1697
	Male	$4.14 \times 10^{-4}$ ( $3.77 - 4.56 \times 10^{-4}$ )	3.02 (2.99 - 3.05)	I	1765
Kalken	Female	$4.42 \times 10^{-4}$ ( $4.06 - 4.82 \times 10^{-4}$ )	2.97 (2.95 - 2.99)	A-	412
	Male	$3.71 \times 10^{-4}$ ( $3.13 - 4.41 \times 10^{-4}$ )	3.03 (2.99 - 3.08)	I	354
Lippenbroek	Female	$4.47 \times 10^{-4}$ ( $4.14 - 4.83 \times 10^{-4}$ )	2.99 (2.96 - 3.01)	I	604
	Male	$4.94 \times 10^{-4}$ ( $4.12 - 5.93 \times 10^{-4}$ )	2.97 (2.91 - 3.02)	I	620
Merelbeke	Female	$5.33 \times 10^{-4}$ ( $5.14 - 5.52 \times 10^{-4}$ )	2.92 (2.91 - 2.93)	A-	2775
	Male	$4.86 \times 10^{-4}$ ( $4.49 - 5.26 \times 10^{-4}$ )	2.96 (2.93 - 2.98)	A-	2660



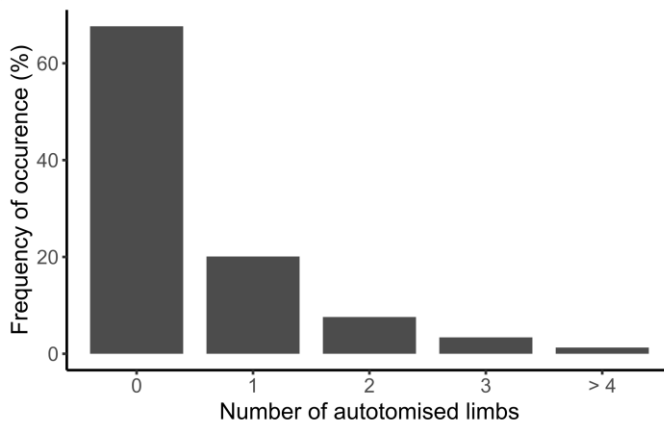
**Fig. 2.8** Individual wet mass (g) in function of carapace width (mm) for both female (red) and male (blue) Chinese mitten crabs per location: (A) Grobbendonk, (B) Kalken, (C) Lippenbroek, (D) Merelbeke. The relationships estimated by linear mixed-effects models are plotted as lines.

### 2.3.8 Autotomy incidence

Patterns in autotomy incidence were analysed for the location Merelbeke in 2023. No significant differences were found between sexes ( $\chi^2 = 0.107$ ,  $df = 1$ ,  $p = 0.743$ ) or among size classes ( $\chi^2 = 8.79$ ,  $df = 7$ ,  $p = 0.268$ , Suppl. Mat. 8.1 **Fig. S. 5**) of Chinese mitten crabs. However, the proportion of injured individuals varied significantly across months ( $\chi^2 = 50.24$ ,  $df = 8$ ,  $p < 0.001$ ; **Fig. 2.9**) and seasons ( $\chi^2 = 42.61$ ,  $df = 3$ ,  $p < 0.001$ ). Autotomy incidence was high during autumn and summer (38 – 44 %), peaking in June (46 %) and August (48 %). In winter and spring, lower levels were observed ( $p < 0.001$ ), ranging between 23 and 29 %. The majority of autotomised crabs had lost only a single limb (20 %, 344 out of 1,712), with the frequency of occurrence decreasing as the number of autotomised limbs increased (**Fig. 2.10**). Fewer than 1.5 % of individuals (22 out of 1,712) had lost four or more limbs.



**Fig. 2.9** Percentage of injured crabs (i.e. individuals with missing appendages) per month in 2023. Numbers above each bar represent the number of crabs with autotomy and the total number of crabs analysed. Letters indicate significance groups; months sharing the same letter are not significantly different from each other ( $p > 0.05$ ).



**Fig. 2.10** Frequency of occurrence (%) of per category of number of autotomised limbs in Chinese mitten crabs in 2023.

### 2.3.9 Sexual maturity

The gonads of 62 female crabs were analysed, across three different locations in autumn 2023 and 2024. In Grobbendonk, mature females ( $n = 24$ ) had a carapace width ranging from 46.9 to 63.8 mm (mean  $\pm$  SD:  $55.3 \pm 4.3$  mm), while only one immature individual was observed (51.8 mm). At Kalken, mature females ( $n = 13$ ) had a similar size (mean  $\pm$  SD:  $53.2 \pm 7.5$  mm; range: 39.7–63.9 mm) as mature individuals at Grobbendonk, and also at this location only one immature individual was observed (27.4 mm). Mature female crabs captured at Lippenbroek ( $n = 14$ ) ranged between 33.7 and 46.8 mm (mean  $\pm$  SD:  $42.7 \pm 4.0$  mm), while the carapace width of immature crabs ( $n = 9$ ) ranged between 9.7 and 33.0 mm (mean  $\pm$  SD:  $20.5 \pm 8.5$  mm).

## 2.4 Discussion

### 2.4.1 Life history in Belgium

The life history of the Chinese mitten crab has been studied extensively at the beginning of its invasion in neighbouring countries such as the Netherlands and Germany (Kamps, 1937; Panning, 1938; Peters and Panning, 1933). However, despite its long-established presence and high abundance, its life cycle in Belgium has never been examined in detail. In this section, the different life stages of the species are reviewed, starting with the larval stages.

Based on the findings of this study, Chinese mitten crabs in Belgium are estimated to have a lifespan of three to six years, starting from the megalopa stage. Ovigerous females are observed from autumn to spring, and embryonic development is estimated to last between one and two months. The subsequent larval development until the megalopa stage takes approximately 15 to 74 days, depending on both water temperature and salinity (Anger, 1991; Rudnick et al., 2005b). However, the exact timing remains uncertain, as no research on larval development has been conducted in Belgian waters to date.

The presence of two new cohorts of juvenile crabs at the beginning of the year at the site Lippenbroek suggests there are two distinct megalopa settlement periods. Settlement likely occurs in spring (April – June) and early summer (July), with a second settlement period in autumn (October – November). In some cases, it appeared there were three cohorts from the same year, this likely reflects early and late settlers from the spring settlement period. In literature, the timing of megalopa settlement has not been clearly defined, largely because megalopae were rarely observed in the field (Kamps, 1937; Rudnick et al., 2005b).

For most non-native populations settlement is generally estimated to occur from late spring through summer (**Table 2.3**) (Dittel and Epifanio, 2009; Rudnick et al., 2005b). Panning (1938) however, suggested that in Germany megalopae settle between July and August in years with a warm spring, and not until October in years with unfavourable weather. In the Yangtze Estuary (China), megalopa recruitment primarily occurs in June (Hymanson et al., 1999; Wu et al., 2024). For the Japanese mitten crab (*Eriocheir japonica*) two distinct megalopa settlement periods have been described in its native range; one in late spring (May to June) and another in mid-autumn (October), with small numbers settling in November and between January and February (Kobayashi, 2016, 2011, 1998).

**Table 2.3** Overview of the timing of life history traits in native and non-native populations of the Chinese mitten crab, as well as the Japanese mitten crab (in italics). Adapted from Dittel & Epifanio (2009) and Rudnick et al. (2005b).

Region	First record of species	Period of larval settlement	Period of upstream migration	Period of downstream migration	Breeding period	Size of sexually mature adults (mm CW)	Suggested life span	Sources
China	Native	June	Year-round, peak May-June	August-November	October-April	30-90	1 – > 3 years	Hymanson et al., 1999; Jin et al., 2001a
Belgium	1933	April-July ?, October-November ?	Year-round, peak March-May	September-December	October-May	33-77 (likely up to 87)	3 – 6 years	Present study; Lestage, 1937; Leloup, 1938, 1939
California (USA)	1992	April-June + ?	Spring-early summer	September-December	November-June	30-95	2 – 4 years	Rudnick et al., 2005b
France	1930	April-July	n.d.	August-October	n.d.	50-90	n.d.	As cited in Rudnick et al., 2005b
Germany	1912	May-August, October	March-July	August-October	October-May	38-84	4 – 6 years	Peters and Panning, 1933
the Netherlands	1931	July ?	March-May	September-December	October-May	38-80	2 – 4 years	Kamps, 1937
the United Kingdom	1936	n.d.	February-July	August-November	September-June	38-50	n.d.	Clark, 2011; Clark et al., 2008; Morritt et al., 2013
Japan	Native	May-June, October-November	n.d.	September-February	September-May	36-70	n.d.	Kobayashi, 1999, 2003

Future research should be focused on investigating larval stages of non-native Chinese mitten crab populations to confirm the timing and duration of larval stages in the field and explore the potential impact of introgression of the Chinese and Japanese mitten crab (Hayer et al., 2019; Homberger et al., 2022; Ironside et al., 2025) on larval development.

Early juvenile crabs (3 – 5 mm) were found in the upper tidal reaches of the Scheldt Estuary, approximately 115 to 135 km (H.K. unpublished data) upstream. These findings support the notion that upstream migration from brackish to freshwater habitats begins as early as the megalopa and first juvenile stages, likely facilitated by selective tidal stream transport (Gilbey et al., 2008; Kobayashi, 2016; Panning, 1938). Yet, the distribution of megalopae and early juveniles within the lower estuary was not investigated, but could in the future improve our understanding of the timing and duration of such upstream movement.

Similar to findings in San Francisco Bay (Rudnick et al., 2005b), the smallest crabs observed at the upstream, non-tidal freshwater site Grobbendonk were larger than 10 mm. This suggests that smaller crabs are likely not strong enough to move further upstream against the unidirectional current (Panning, 1938). Chinese mitten crabs caught during the upstream migration typically have a carapace width of 15 – 25 mm, which corresponds to individuals of approximately one to two and a half years old, counting from megalopa settlement. Mark-recapture experiments suggest these crabs move at rates between 0.2 and 2.8 km day<sup>-1</sup> (Schoelynck et al., 2021). The upstream migration (**Table 2.3**) occurs between February and June, and typically peaks between March and May. Nevertheless, catch data at the tidal, freshwater site Merelbeke suggests that upstream movement continues throughout the year, though in lower numbers. Observations indicate that the sex ratio of upstream migrating crabs is close to 1:1. These patterns largely confirm observations in both the native and non-native range (Dittel and Epifanio, 2009; Hymanson et al., 1999; Panning, 1938; Rudnick et al., 2005b). Although the general timeframe is consistent, the onset of the spring migration varies between years, which could be related to environmental factors such as water temperature or river discharge (Rudnick et al., 2005b).

In the tidal freshwater marsh, Lippenbroek, mitten crabs with a carapace width of approximately 40 mm were observed almost year-round. The maximum size recorded in the area was 52 mm. These observations suggest that at least some individuals remain and grow until maturity within this tidal zone rather than migrating further upstream (Rudnick et al., 2005b). Crabs remaining in the tidal marsh tend to be smaller than their upstream conspecifics and are likely three to four years old when they

commence their spawning migration. Visual inspection of gonads confirmed that most female individuals with a carapace width of 34 – 47 mm leaving the area in autumn were sexually mature. These larger crabs were particularly abundant in catches during November and December, suggesting this is the period they start their downstream migration. A large number of immature, smaller crabs were also observed leaving the tidal marsh during winter. These crabs likely move to deeper waters during winter, while some may continue moving upstream to complete their development (Gilbey et al., 2008; Rudnick et al., 2000).

In upstream freshwater, non-tidal habitats, Chinese mitten crabs reside longer before eventually moving downstream for spawning. We estimate that sexually mature crabs are typically between four and six years old, with carapace widths generally ranging from 45 to 75 mm. The largest observed individual measured 86.9 mm. Inspection of gonads confirmed that female individuals with carapace width of 40 to 64 mm, captured in autumn, were sexually mature. These crabs commence their spawning migration from late Augusts onwards, but typically from mid-September to December (**Table 2.3**). It has been suggested that male crabs start migrating first, followed by female crabs (Peters and Panning, 1933). Although we did not observe male-biased sex ratios in August or September, female crabs predominated in catches from October to December at some locations. Earlier research in the catchment of the Scheldt River showed that these adult crabs can reach the estuary within one to two months, moving on average  $4.65 \text{ km day}^{-1}$  in unidirectional rivers and  $1.29 \text{ km day}^{-1}$  in tidal rivers (Chapter 3; Keirsebelik et al., 2025).

Ovigerous females have primarily been recorded in the Scheldt Estuary and at the Belgian coast, particularly in the Yser Estuary, from October to the end of May (**Table 2.4**). Interestingly, a high number of ovigerous females was observed in or near the outflow of cooling water from nuclear power plants in the Scheldt Estuary, i.e. Borssele and Doel. While this pattern may reflect a sampling bias, it is also possible that ovigerous females are attracted to these areas due to the localised warming effect of the cooling water discharge (Stevens and Van den Bergh, 2010). The size of ovigerous females varied between 36 and 77 mm (**Table 2.4**), which supports the findings on the size at sexual maturity. Acoustic telemetry suggested adult crabs remain present within the lower reaches of the Scheldt Estuary until July (Chapter 3; Keirsebelik et al., 2025). Occasional observations, mainly in June and July, of adult crabs overgrown by barnacles and of dead mitten crabs washed up along beaches appear to confirm this (Mares, 1995; Vanhaelen, 1995).

**Table 2.4** Overview of occurrence records of ovigerous (egg-bearing) female Chinese mitten crabs in Belgium and the Scheldt Estuary (the Netherlands). Records were compiled from historical literature, citizen science platforms, unpublished MSc thesis data (Broeren, 2013), and bycatch data of monitoring activities. When available, comments on carapace width (CW) and egg colouration are included.

Date	Month	Location	River	Number	Source	Comments
1936-10	October	Nieuwpoort	Yser	8	Lestage, 1937	
1937-10-29/30	October	Nieuwpoort	Canal Plassendale-Nieuwpoort, Yser	4	Leloup, 1938	60-70 mm CW
1937-11-02	November	Heist		1	Leloup, 1938	60 mm CW
1937-11-02	November	Nieuwpoort	Yser	10	Leloup, 1938	65-75 mm CW
1937-11-4/6	November	Nieuwpoort	Canal Plassendale-Nieuwpoort, Yser	16	Leloup, 1938	65-75 mm CW
1937-11-15	November	Nieuwpoort	Canal Plassendale-Nieuwpoort	1	Leloup, 1938	70 mm CW
1937-11-23	November	Nieuwpoort	North Sea	1	Leloup, 1938	70 mm CW
1937-12-17	November	Nieuwpoort	North Sea	1	Leloup, 1938	70 mm CW
1938-05-06	May		Lovaart	1	Leloup, 1939	70 mm CW
1938-12-10	December	Sint-Jan-in-Eremo	Boerekreek	1	Leloup, 1939	68 mm CW
2012-09-28	September	Steendorp	Scheldt Estuary	1	Broeren, 2013	49 mm CW
2012-10-29	October	Liefkenshoek	Scheldt Estuary	2	Broeren, 2013	42-47 mm CW
2012-10-31	October	Liefkenshoek	Scheldt Estuary	4	Broeren, 2013	41-54 mm CW
2012-11-07	November	Liefkenshoek	Scheldt Estuary	6	Broeren, 2013	37-48 mm CW
2012-12-18	December	Liefkenshoek	Scheldt Estuary	2	Broeren, 2013	36-46 mm CW
2013-04-26	April	Zandvliet	Scheldt Estuary	2	Broeren, 2013	48-77 mm CW
2013-05-02	May	Liefkenshoek	Scheldt Estuary	1	Broeren, 2013	62 mm CW
2019-05-24	May	Liefkenshoek	Scheldt Estuary	5	Broeren, 2013	51-62 mm CW

2018-03-21	March	Oostende	North Sea	1	waarnemingen.be	brown eggs
2022-03-17	March	Nieuwpoort	Yser (De Ganzepoot)	1	INBO	orange eggs
2022-03-23	March	Nieuwpoort	Yser (De Ganzepoot)	4	INBO	47-59 mm CW, orange/brown eggs
2025-01-31	January	Borssele	Scheidt Estuary	1	waarneming.nl	orange eggs
2025-01-31	January	Borssele	Scheidt Estuary	1	iNaturalist.org	brown eggs
2025-02-02	February	Borssele	Scheidt Estuary	1	waarneming.nl	brown eggs
2025-02-08	February	Borssele	Scheidt Estuary	1	waarneming.nl	brown eggs
2025-03-05	March	Oostende	Canal Gent-Brugge- Oostende	1	INBO	
2025-03-15	March	Borssele	Scheidt Estuary	1	waarneming.nl	brown eggs
2025-04-29	April	Doel	Scheidt Estuary	3	INBO	
2025-04-30	April	Doel	Scheidt Estuary	3	INBO	

The estimated age at maturity varies between three to six years in this study, which corresponds to the findings for invasive Chinese mitten crabs in Germany, which were estimated to live up to four to six years (Fladung, 2000), but is longer than earlier estimations in the Netherlands and California (**Table 2.3**) (Kamps, 1937; Panning, 1938; Rudnick et al., 2005b). In its native region, the age at maturity varies between one to more than three years, and in aquaculture crabs complete their entire life cycle in one to two years (Hymanson et al., 1999; Jin et al., 2001a). The size at maturity on the other hand does not appear to vary, and is similar in both the native and invasive range: typically between 38 to 90 mm, but sometimes as small as 30 mm (**Table 2.3**). This suggests that non-native populations perform as well as native populations, but that differences in environmental conditions likely lead to slower development until maturity. Although larger crabs are generally older, there is considerable overlap in size between age classes, and in addition, interannual variation in growth is expected due to differences in water temperature and food availability (Yuan et al., 2017). Future research using field enclosures to monitor the growth of individual crabs could help validate the present findings and refine growth estimates.

#### **2.4.2 Spatiotemporal differences in crab size and abundance**

Collectively, our results support the presence of clinal variations in size and age at maturity, a pattern that was previously observed in populations from the species' native range (Hymanson et al., 1999) and has been well-documented in the closely related Japanese mitten crab (Kobayashi, 1999; Kobayashi and Matsuura, 1995b). Chinese mitten crabs inhabit a broad range of freshwater environments, from large tidal rivers to smaller, slower-flowing streams. The present study suggests size and age at maturity tend to increase with distance upstream along the river's longitudinal gradient. As fecundity increases with size in the genus *Eriocheir* (Anastácio et al., 2018; Kobayashi, 2001; Peters and Panning, 1933), and mitten crabs only have one reproductive period in their life, this size difference directly affects their lifetime fecundity, i.e. the total number of offspring an individual produces over its entire lifespan (Kobayashi, 2001). Mitten crabs that mature early and at smaller sizes are more likely to survive and reproduce, but they tend to produce fewer offspring (Hymanson et al., 1999). In contrast, crabs in upstream regions spend a longer time growing, which means they have to survive for a longer period to be able to reproduce. However, when they reproduce, they achieve a higher lifetime fecundity. Adult crabs living both downstream and upstream in the catchment ultimately migrate to brackish waters to mate, where these distinct groups likely come together and mix (Kobayashi, 1999). According to Hymanson et al. (1999) this strategy promotes reproduction among individuals of different cohorts and ensures that different parts of the

population reproduce each year. The factors affecting the size at maturity are unknown, it may be genetically determined or arise from phenotypic plasticity in response to environmental conditions such as water temperature, food availability, and population density (Kobayashi, 2001, 1999).

We observed substantial interannual variation in Chinese mitten crab abundance over the seven-year study period. At the upstream, non-tidal site Grobbendonk, for example, annual catch numbers fluctuated between 100,000 and 700,000 individuals. Such large year-to-year variation has been documented in several non-native mitten crab populations, including those in San Francisco and the Elbe Estuary (Fladung, 2000; Rudnick et al., 2003). The interannual variation in abundance is most likely related to environmental and biotic conditions during the reproductive and/or larval phases (Fladung, 2000; Rice, 2006; Z. Zhang et al., 2019c). Although no direct relationships have yet been established, different factors have been suggested to play a role, particularly during winter and spring, including water temperature, salinity, fluvial freshwater discharge, and planktivore and predator abundance (Blumenshine et al., 2012; Fladung, 2000; Z. Zhang et al., 2019c). Long-term quantitative monitoring of population abundance could help to validate these hypotheses in future studies.

Aside from environmental and biotic factors, part of the observed interannual variation could reflect the effects of trapping itself. However, as the vast majority of annual catches consist of incoming juvenile crabs rather than outgoing adults, the impact of upstream depletion on total catch numbers is expected to be minimal. Nevertheless, extensive removals could reduce the number of reproducing crabs and thereby affect recruitment. Such a system-wide effect would require that the adult crabs captured at the trapping locations represent a substantial fraction of the total Belgian population, which remains unknown at this stage. If similar traps removing adult crabs were to be applied more widely across the river network, such large-scale removals could potentially contribute to a reduction in the overall breeding population.

The fact that annual variation in abundance was not consistent across locations suggests that, in addition to fluctuations in overall population size, there may also be annual shifts in migration patterns. Several factors have been proposed as drivers, including population density, food availability, flow velocity, fluvial freshwater discharge, and water temperature (Fladung, 2000; Panning, 1938). However, the preferences of migrating juvenile crabs remain poorly understood and warrant further research, both to advance our ecological understanding and to enable effective management.

### 2.4.3 Size-mass relationship and autotomy incidence

Although juvenile crabs of both sexes generally have similar masses at the same size, males become progressively heavier than females as they grow larger. Overall, the allometric coefficient  $b$  ranged between 2.92 and 3.03, which is well within the range of reported values for other non-native mitten crab populations (**Table 2.5**). The size-mass relationship indicated mostly isometric growth for males, but negative allometric growth for females. This difference may result from sexual dimorphism or comparatively better condition in males, as previously suggested (Czerniejewski and Wawrzyniak, 2006; Fladung, 2000). The increasing mass difference between sexes can be explained by sexual dimorphism in chelae size, which has been documented in both Chinese and Japanese mitten crabs (Kobayashi and Satake, 2017). Males have significantly larger chelae, which can account for 30 % of their body mass compared to 15 % in females, and their chelae are also more densely covered with setae (Czerniejewski and Wawrzyniak, 2006; Kobayashi and Matsuura, 1993). Males also have a higher pinch strength relative to body mass than females (Schoelynck et al., 2020), which may allow them to access a broader range of food sources or spend less energy handling prey (Mills et al., 2016).

Autotomy is a survival strategy that can result from agonistic interactions with conspecifics, predation attempts, or complications during moulting (Juanes and Smith, 1995; Sun et al., 2024; Yuan et al., 2018). Autotomy is common in decapods, with incidence ranging between 0 and 80 %, but generally below 35 % (Juanes and Smith, 1995; Lindsay, 2010). A high incidence of limb loss was observed in Chinese mitten crabs in the Scheldt River, reaching up to 44 % in summer and autumn, and decreasing to 23–29 % during winter and spring. The vast majority of autotomised crabs had lost a single appendage. The seasonal pattern is likely related to variation in population density of resident crabs, which is typically lower in winter (Gilbey et al., 2008; Rudnick et al., 2000), and possibly higher predation pressure. A similarly high autotomy incidence (44 %) and pattern in the number of missing limbs was recorded in the Tagus Estuary (Portugal) (Anastácio et al., 2018). Limited information is available for wild native populations of Chinese mitten crabs, but in aquaculture limb loss can be high (30 %) when mitten crabs are stocked at high densities (Zhao et al. 2015 as cited by He et al., 2016; Yuan et al., 2018). The species can regenerate its limbs in two moults, and the more appendages are lost, the shorter the duration of the first moult (He et al., 2016). However, until the second moult, feeding rates, moult increment, and reproductive success are often reduced compared to healthy crabs, and could thus affect individual fitness (He et al., 2016; Juanes and Smith, 1995; Kamps, 1937).

#### **2.4.4 Conclusion**

This study provides important baseline information on the life history and ecology of the Chinese mitten crab in Belgium. The observed timing of the different life stages generally aligns well with both native and other non-native populations. Overall, size at maturity and the size-mass relationship suggest that the crabs are in a good condition and perform as well as native populations. However, the incidence of autotomy is relatively high. Estimates of age at maturity indicate late maturity compared to native populations and require further research. Furthermore, little is known about the larval stages of the Chinese mitten crab in Belgium, and by extension in other non-native populations, indicating a critical research gap for future research, especially in light of recent findings on genetic introgression with the Japanese mitten crab (Hayer et al., 2019; Homberger et al., 2022; Ironside et al., 2025).

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**Table 2.5** Overview of the mass ( $g$ ) – carapace width ( $CW$ ,  $mm$ ) relationships for Chinese mitten crabs across different countries, locations, and years, presented separately for females and males. Parameters of the power-law relationship ( $Mass = a \times CW^b$ ) are given, with  $a$  as the intercept and  $b$  as the slope. The sample size ( $n$ ) and literature sources are provided for each dataset.

Country	Location	Year	Sex	$a$	$b$	$n$	Source
Germany	Elbe and Havel Rivers	1995-1998	Female	0.000238	3.127	1458	Fladung, 2000
Germany	Elbe and Havel Rivers	1995-1998	Male	0.000208	3.176	3078	Fladung, 2000
Poland	Lake Dąbie	1998	Combined	0.0045	2.45	47	Normant et al., 2000
Poland	Szczecin Lagoon	2001	Female	0.0005-0.0013	2.7362-2.9586	344	Czerniejewski & Wawrzyniak, 2006
Poland	Szczecin Lagoon	2001	Male	0.0003-0.0006	2.9524-3.1176	312	Czerniejewski & Wawrzyniak, 2006
Poland	Odra Estuary	1999-2007	Female	0.0005-0.001	2.8009-3.001	22-712	Czerniejewski, 2010
Poland	Odra Estuary	1999-2007	Male	0.0003-0.0018	2.662-3.105	26-751	Czerniejewski, 2010
Poland	Gulf of Gdansk	1999-2007	Combined	0.0008	2.9	171	Wójcik-Fudalewska & Normant-Saremba, 2016
Poland	Vistula Lagoon	2008-2014	Combined	0.001	2.8	296	Wójcik-Fudalewska & Normant-Saremba, 2016
Belgium	Kleine Nete River (Grobendonk)	2018-2024	Female	0.000473	2.97	1697	Current study
Belgium	Kleine Nete River (Grobendonk)	2018-2024	Male	0.000414	3.02	1765	Current study
Belgium	Scheldt River (Kalken)	2020-2024	Female	0.000442	2.97	412	Current study
Belgium	Scheldt River (Kalken)	2020-2024	Male	0.000371	3.03	354	Current study
Belgium	Scheldt River (Lippenbroek)	2021-2024	Female	0.000447	2.99	604	Current study

Belgium	Scheldt River (Lippenbroek)	2021-2024	Male	0.000494	2.97	620	Current study
Belgium	Scheldt River (Merelbeke)	2023-2024	Female	0.000533	2.92	2775	Current study
Belgium	Scheldt River (Merelbeke)	2023-2024	Male	0.000486	2.96	2660	Current study



# 3

## **From non-tidal to tidal environments: movement behaviour of Chinese mitten crabs on downstream spawning migration**

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## Abstract

The Chinese mitten crab (*Eriocheir sinensis*) is a widespread species that is both threatened and commercially valuable in its native range, but considered invasive in various other parts of the world. Being catadromous, their downstream spawning migration to the sea marks the crucial final step in their life. Yet, little is known about their behaviour during this migration. In this study we investigated the migration of mitten crabs from non-tidal freshwater rivers to the tidal estuarine mouth over a distance of 125 km using acoustic telemetry. During a three-year period, a total of 34 adult mitten crabs were equipped with acoustic tags. Six were equipped with tags that also had an accelerometer and pressure sensor to record the activity and depth of the crabs. All mitten crabs migrated downstream, primarily residing within the deeper parts of the rivers. They were detected until the border between the mesohaline and polyhaline zone of the estuary, suggesting that this area serves as their spawning habitat. Migration speeds were significantly higher in non-tidal freshwater rivers (on average  $4.65 \pm 3.51$  km day<sup>-1</sup>, range: 0.06-15.37 km day<sup>-1</sup>) compared to the tidal estuary (on average  $1.29 \pm 1.22$  km day<sup>-1</sup>, range: 0.05-8.19 km day<sup>-1</sup>). Mitten crabs migrated primarily during the darker hours of the day, however this pattern diminished in the estuary. In tidal rivers migratory activity was largely driven by the tidal cycle, with crabs selectively moving downstream during the ebb tide. No behavioural differences between male and female crabs were observed. During their spawning migration, adult mitten crabs reveal movement behaviour that maximises their fitness. In shallow non-tidal rivers, migrating at night likely reduces predation risk. In tidal rivers, this behaviour largely disappears, which could be linked to increased depth and turbidity, or the prevalence of the tidal migration cue. Based on detection and acceleration data, this study provides the first evidence that adult mitten crabs use selective tidal stream transport during their migration. As a slow-moving species, this behaviour helps to preserve energy for spawning during the challenging final phase of their life cycle.

### 3.1 Introduction

While most brachyuran crabs (Crustacea: Decapoda) spend their entire lives in marine environments, primary freshwater crabs complete their full life cycle in freshwater or terrestrial habitats (Yeo et al., 2008). A distinct group, known as secondary freshwater crabs, are fully adapted to freshwater or terrestrial environments but still require the marine environment at some stage of their life cycle (Kawai and Cumberlidge, 2016). These crabs often undergo seasonal migrations, sometimes covering great distances to reproduce (Carr et al., 2004; Hicks, 1985; Hill, 1994; Sant'Anna et al., 2012; Schubart et al., 2002).

One well-known example of a crab species that undertakes long-distance spawning migrations from fresh to salt water, is the catadromous Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards 1853). The Chinese mitten crab is native to the coastal regions of the Yellow Sea, including northern and central China, and Korea. However, the species has a global distribution as a result of multiple successful invasions following (accidental) human introductions through e.g. ballast water or escapes from markets (Dittel and Epifanio, 2009). Within its native range, the species is commercially valuable, but under threat due to overfishing, habitat degradation and obstructed migration routes (Cheng et al., 2018; Hymanson et al., 1999; T. Zhang et al., 2023). Conversely, in its non-native range the species can be highly abundant, as evidenced by large numbers in Northwestern Europe, and especially Belgium (Ewers et al., 2023; Schoelynck et al., 2021). Here, the species is managed because of concerns about its ecological impact through e.g. predation on native species, damaging of macrophytes and destabilizing river banks through burrowing (Gilbey et al., 2008; Rosewarne et al., 2016; Rudnick et al., 2000; Schoelynck et al., 2020; Wang et al., 2017).

The species' life cycle starts in saline waters at sea or in estuaries, where the larvae hatch from eggs and develop through five stages into a megalopa larva and subsequent juvenile crab (Anger, 1991; Kim and Hwang, 1995). These juvenile crabs move into the estuary and generally reside in brackish to freshwater tidal areas (Gilbey et al., 2008; Rudnick et al., 2003). In spring, the juvenile crabs migrate upstream and swarm out, searching for suitable habitat up to hundreds of kilometres inland (Panning, 1938; Rudnick et al., 2005a). Once they reach maturity, aged one to five years, the adult crabs venture back seaward to reproduce (Dittel and Epifanio, 2009). Typically, male adult crabs start their spawning migration in early autumn, followed by the female crabs (Panning, 1938). After mating in brackish water, the female crabs carry the fertilized

eggs to more saline water. One brood can contain up to a million eggs (Panning, 1938; Peters, 1938; Peters and Panning, 1933).

The timing of their downstream migration appears to be consistent across both their native and non-native range, starting in late August or September and lasting until December (Rudnick et al., 2005b). Conversely, the timing of mating, brooding and larval release differs between regions (Dittel and Epifanio, 2009). Mitten crabs make this spawning migration only once in their lives and perish soon after the eggs hatch (Panning, 1938). This is a result of the high energetic cost of the migration and reproduction itself (Kobayashi and Matsuura, 1995c; Panning, 1938). They are exposed to predation and dramatic shifts in environmental conditions (Kobayashi, 2003; Normant et al., 2012). On top, in highly regulated water systems they encounter barriers that can force them to temporarily leave the river with the added risk of desiccation (Cohen and Weinstein, 2001; and references therein; Fialho et al., 2016).

Although the general life cycle of the Chinese mitten crab is fairly well understood, little is known about their behaviour during their spawning migration. General migration speeds have been derived from mark-recapture studies and range between 0.2 and 12 km day<sup>-1</sup> (Panning, 1938; Schoelynck et al., 2021). However, it is not known whether there are differences between sexes, whether they migrate continuously or take breaks, or whether their movement rates and behaviour change across non-tidal and tidal environments. To maximise survival and conserve energy for reproduction, mitten crabs likely adjust their migratory behaviour. For instance, to alleviate the energetic costs of movement, it is assumed that mitten crabs ‘go with the flow’. This is continuous in non-tidal rivers, but the direction of the current changes over time in a tidal environment. In the latter, mitten crabs could use selective tidal stream transport (STST), only migrating during the favourable conditions of the ebbing tide. This energetic-efficient behaviour is common among marine and estuarine animals, such as anadromous and catadromous fish, and has been demonstrated for many larval stages of brachyuran crabs (Forward and Tankersley, 2001; Gibson, 2003). Additionally, predator avoidance behaviour can obviously increase survival. Therefore, mitten crabs likely migrate primarily at night, which has been suggested based on catch data (Rudnick et al., 2003).

Much of our current understanding of the migration and spawning behaviour of mitten crabs has been derived from catch data (Clark, 2011; Clark et al., 1998; Morritt et al., 2013; Panning, 1938; Wójcik-Fudalewska and Normant-Saremba, 2016). While valuable, this data offers only a coarse resolution of their behavioural patterns. With the emergence of acoustic telemetry, it is possible to study the movement behaviour

of individual aquatic animals and thus to investigate their spatial ecology in detail (Hussey et al., 2015). A growing body of literature has proven the use of this technique to track decapods (Florko et al., 2021; and references therein).

In this study, adult Chinese mitten crabs were tracked for the first time using acoustic telemetry. We investigated their behaviour during their downstream spawning migration from upstream freshwater areas to brackish zones near the river mouth. Individual crabs were tracked to (1) identify the temporal and spatial scale of the migration, (2) investigate circadian and circatidal patterns in behaviour such as STST, and (3) assess movement rates. By elucidating their migratory behaviour, this study contributes significantly to the current knowledge of the ecology of an economic and ecologically important species, and by extension, of adult stages of catadromous brachyuran crabs.

## **3.2 Methods**

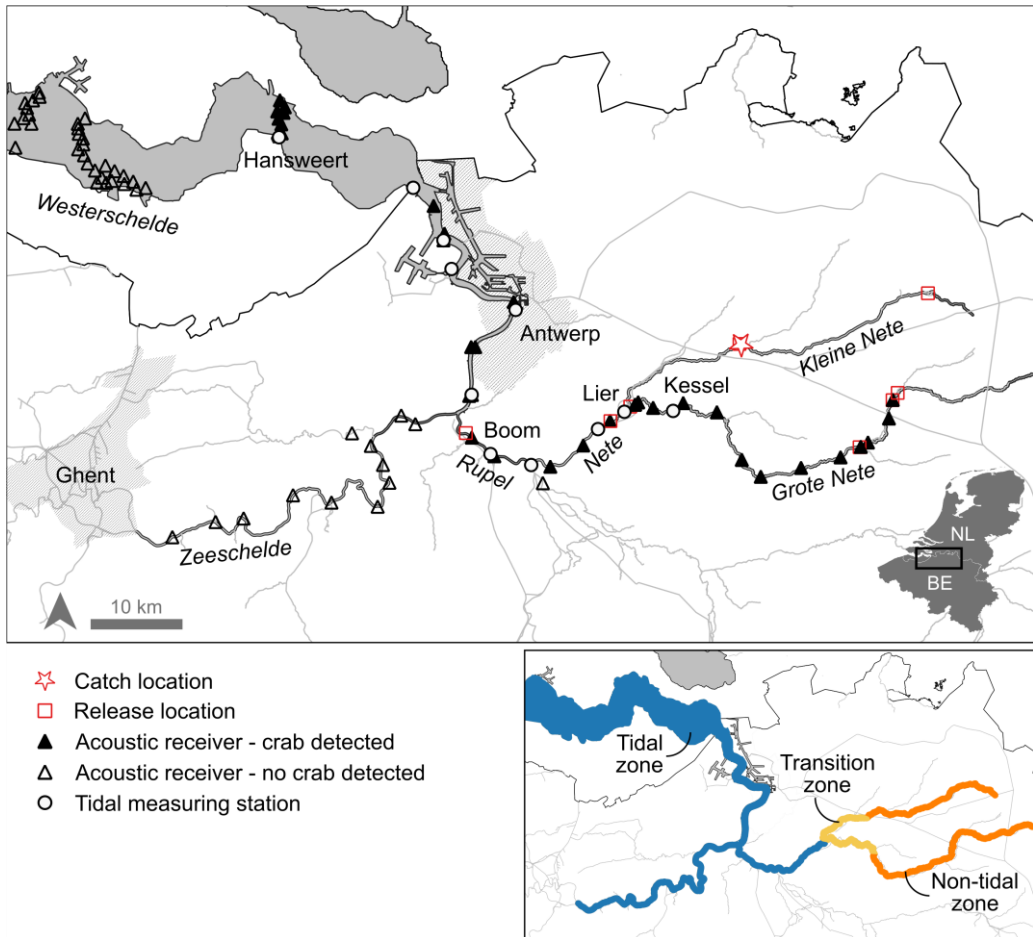
### **3.2.1 Study area**

The field study was performed in the Scheldt River basin in 2020 to 2023. The Scheldt Estuary is a principal migratory route of the Chinese mitten crab and its many tributaries and surrounding (tidal) areas provide optimal habitats for the crabs' different life stages. The River Scheldt is a 355-kilometre-long river that flows from Saint-Quentin (northern France) through Belgium to the North Sea near Vlissingen (The Netherlands). The surface area of the Scheldt river basin is approximately 21,863 km<sup>2</sup>. The Scheldt Estuary extends over two different zones: (1) the Westerschelde (58 km, depth 13-50 m) between the Belgian-Dutch border and Vlissingen and (2) the Zeeschelde (105 km, depth 6-20 m) between Ghent and Antwerp (**Fig. 3.1**).

Based on tidal influence, the study area can be divided in three classes (**Fig. 3.1**). First, a tidal zone ( $\pm$  125 km) comprising Rivers Nete and Rupel, and the Scheldt Estuary, which are all under strong tidal influence, ranging from meso- (average tidal range between 2 and 4 m) to macro-tidal (average tidal range exceeding 4 metres). This tidal zone covers the whole salinity gradient from fresh to salt water. The rivers are rain-fed, leading to highly variable discharge among seasons, with a peak in discharge during winter. Therefore, the transition zone between fresh and salt water shifts throughout the year (Meire et al., 2005). Generally, the zone between the river mouth of the Scheldt Estuary and Hansweert (the Netherlands) is polyhaline (> 30 PSU), while the zone between the Belgian-Dutch border and the vicinity of Antwerp is mesohaline (5-18 PSU). The zone from Antwerp until the confluence with the River Rupel, and the

River Rupel itself, are oligohaline (0.5-5 PSU). Second, a transition zone ( $\pm 11$  km) between this tidal zone and unidirectional rivers without tidal influence, comprising the lower reaches of Rivers Grote and Kleine Nete. The lower reaches of these rivers experience limited tidal influence (micro-tidal), with an average tidal range of less than 2 meters (Suppl. Mat. 8.2 **Fig. S. 7**, Kessel). This tidal influence is presumably affected by a culvert near their confluence and gradually fades out about 14-17 km upstream. This tidal transition zone is strictly fresh water. Thirdly, a non-tidal zone ( $\pm 30$  km), comprising the upstream reaches of Rivers Grote and Kleine Nete, which are shallow, rain-fed, freshwater rivers with no tidal influence.

Within the tidal zone, the duration of the tidal cycle is on average ( $\pm$  SD)  $12.4 \pm 0.4$  hours, but there is considerable variation in the duration of each tidal phase within the study area (Suppl. Mat. 8.2 **Fig. S. 7**). Ebb and flood are almost symmetrical in the Westerschelde (e.g. Hansweert:  $6.3 \pm 0.3$  hours ebb,  $6.1 \pm 0.2$  hours flood) and become increasingly asymmetrical upstream. This results in a longer ebb tide and shorter flood tide in the Zeeschelde (e.g. Antwerp:  $6.9 \pm 0.3$  hours ebb,  $5.5 \pm 0.3$  hours flood) and River Rupel (e.g. Boom:  $7.1 \pm 0.3$  hours ebb,  $5.3 \pm 0.3$  hours flood) up until the most upstream tidal station of the Nete River around Lier ( $8.4 \pm 0.4$  hours ebb,  $4.0 \pm 0.4$  hours flood).



**Fig. 3.1** Map of the study area with the names of the rivers and estuary indicated in italics. Solid triangles indicate acoustic receivers where crabs were detected, hollow triangles indicate acoustic receivers in the study area where crabs were not detected. The map below shows the three tidal classes: a tidal zone with meso- to macrotidal influence in blue, a tidal transition zone with microtidal influence in yellow, and non-tidal river sections in orange.

### 3.2.2 Acoustic network

In 2014, the Permanent Belgian Acoustic Receiver Network (PBARN) was set up in the Scheldt river basin and the Belgian part of the North Sea as part of the Belgian LifeWatch project (**Fig. 3.1**) (Reubens et al., 2019). The detection range of the receivers depends on different environmental variables, and ranges between < 300 m and 1005 m (Bruneel et al., 2023). In the Westerschelde, the acoustic receivers (model VR2W, InnovaSea Systems Inc., USA) are deployed on marine navigational buoys in

three separate lines over the full width of the river. With a 3 – 5 m long chain and a 10 – 17 kg weight at the end, hydrophones are pointed downwards. Due to the dependency on these buoys, a complete detection coverage over the full width of the arrays was not possible. In the Zeeschelde and in Rivers Rupel, Nete and Grote Nete, the acoustic receivers are attached to chains on the river bank, held in place with a 10 – 17 kg weight and kept upright with a small buoy. In the broader parts of the rivers, receivers are placed on both river banks to cover the full river width. During the period of this study a total of 86 receivers were deployed within the study area (**Fig. 3.1**). Note that the receiver network extends further into the Belgian part of the North Sea, but is not shown in **Fig. 3.1** since we did not detect Chinese mitten crabs on that part of the network.

### 3.2.3 Tagging procedure

Adult Chinese mitten crabs were caught during their downstream migration in a fixed crab trap in the Kleine Nete River (**Fig. 3.1**, see Schoelynck et al. (2021) for details on the fixed crab trap) in three consecutive years (2020 till 2022). This trap was used because it allows efficient capture of large numbers of migrating Chinese mitten crabs. The large catch numbers allowed for selection of sufficiently large, size-matched and healthy individuals, while maintaining a balanced sex ratio. Large individuals (carapace width > 55 mm) were selected to ensure that the terminal moult was completed and that these crabs were ready to spawn. Furthermore we checked whether the crabs were ‘healthy’; i.e. active, no limbs missing and no damages to the carapace. The sex, carapace width (to 0.01 mm) and wet weight (to 0.01 g) of the individual crabs were determined (Suppl. Mat. 8.2 **Table S. 7**).

The crabs were tagged in the laboratory of the University of Antwerp. The dorsal part of the carapace was dried with paper towel and sanded superficially with a rotary tool. As such, the carapace becomes roughened, which improves attachment of the tag (Cote et al., 2019). Next, the acoustic tags were mounted externally on the crabs according to the method described by Brousseau et al. (2004) (**Fig. 3.2**). The tag was secured on the non-adhesive side of a Velcro strip (2.5 cm × 2.5 cm) using a cable tie and quick-setting cyanoacrylate (Pattex, super glue ultra gel). The other matching Velcro strip was attached with its non-adhesive side onto the dorsal carapace with cyanoacrylate. Finally the two adhesive sides of the Velcro strips were glued together with cyanoacrylate. Each tag was kept in place manually for 10 minutes. Thereafter, the crabs were placed individually in closed transparent tanks (39 × 28 × 14 cm). Each tank had a small amount of tap water that did not reach the top of the carapace, to allow the adhesives to cure. The whole tagging procedure including the drying time took about 1 h per crab. Afterwards the tanks were completely filled with tap water and

aerated with an air stone. The crabs were held in the laboratory until their release on the next day (maximum 24 hours after capture). This tagging technique was tested prior to the field study (Suppl. Mat. 8.2).



**Fig. 3.2** Adult male Chinese mitten crab with acoustic tag prior to release in November 2021.

In October 2020, eight crabs were tagged and released as a first test batch (e.g. tag retention, settings tags). During this first deployment, only female crabs were used as literature suggests that only female crabs migrate through the whole estuary to release their eggs in saline water, while male adult crabs die after mating (Panning, 1938). We used ID-2LP6 acoustic tags (6.3 × 22 mm, weight in air 1.9 g, weight in water 1.2 g, power output 137 db, frequency 69 kHz, ping frequency 90-150 sec, estimated battery life 16.3 months) from Thelma Biotel (Norway). For this first test we chose the smallest tags available with a long battery life, since it was not sure how long the migration would take. To capture the full migration route, even if tags would detach early, mitten crabs were released at different locations within the study area (Suppl. Mat. 8.2 **Table S. 7** and **Fig. 3.1**). Consequently, some individuals were released further downstream from where they were caught.

After proof of concept, the sample size was increased (following the same procedure of 2020) in the autumn of 2021 and 2022. In October and November 2021 a total of 16 crabs (equal number of males and females) were tagged. Two different tag types

were used; (1) 10 crabs were tagged with the same ID-2LP6 acoustic tags as the year before and (2) 6 crabs were tagged with V9AP-2x-BLU-1 acoustic tags (9 × 35 mm, weight in air 5.3 g, weight in water 3.0 g, power output 151 db, frequency 69 kHz, ping frequency 90-150 sec, estimated battery life 6.8 months) from InnovaSea (USA), equipped with both accelerometer and pressure sensor. The sensors allow to investigate activity patterns and the depth at which the crabs move. In November 2022, 10 crabs (equal number of males and females) were tagged with ID-HP9 acoustic tags (9 × 28 mm, weight in air 4.2 g, weight in water 2.4 g, power output 149 db, frequency 69 kHz, ping frequency 20-60 sec, estimated battery life 6.8 months) from Thelma Biotel. We decided to use this new type of tag because we had learned that all crabs of the previous years could only be tracked for less than 6 months. We minimized the probability of not detecting a crab by increasing the power output and ping frequency at the expense of battery life beyond 6 months. All crabs tagged in 2021 and 2022 were released in the Grote Nete River at a similar distance from the estuary as their catch location (Suppl. Mat. 8.2 **Table S. 7** and **Fig. 3.1**).

### **3.2.4 Data analysis**

All analyses were performed in R version 4.2.2 (R Core Team, 2022) and graphs were created using the R package *ggplot2* (Wickham, 2016). The significance level for statistical analyses was set at  $p < 0.05$ .

#### **3.2.4.1 Circadian and circatidal patterns in movement**

Migratory activity according to the diel and tidal cycle was analysed according to the method described by Silva et al. (2017). Arrival (first detection) and departure (last detection) of each crab at each receiver were used to narrow down migratory activity. In the case of selective migration, the crabs are assumed to arrive and depart during favourable migration conditions, such as specific tidal or circadian phases. If there is no preference for certain conditions, migratory activity is assumed to be continuous. Detections on the day of release were excluded from the analysis to avoid bias caused by the time of release.

To determine during which tidal phase (i.e. ebb and flood) arrivals and departures of crabs occurred, detections were linked to water level data from nearby tidal measuring stations. Tidal phase was determined based on the changes in water level. This approach is commonly used in other tracking studies (Silva et al., 2017; Verhelst et al., 2018). Water level data (mTAW, 10 min measuring frequency) was downloaded from *waterinfo.be* using the *wateRinfo* R package (Van Hoey et al., 2018) for Belgian locations and obtained from Rijkswaterstaat (*waterinfo.rws.nl*) for Dutch locations.

The time of each detection was rounded to the nearest 10 min to match the temporal resolution of the water level data. There was generally a good overlap between the locations of the acoustic receivers and tidal measuring stations (**Fig. 3.1**). In case an acoustic receiver was located between tidal measuring stations, a weighted average of the water level data was used based on the inverse distance of the two closest tidal measuring stations. Within the tidal transition zone, only one water level measuring station was available, therefore interpolation was not possible and patterns according to tidal phase could not be analysed.

Based on the date, time and geographical position of each detection, the circadian phase (i.e. dawn, day, dusk and night) was determined (suncalc R package (Thieurmel and Elmarhraoui, 2022)). Dusk was defined as the period between sunset and the end of astronomical twilight, while dawn was defined as the period between the beginning of astronomical twilight and sunrise. Due to the limited amount of observations, categories dusk and dawn were combined into one single category twilight.

In case of continuous migration, the number of arrivals or departures during each phase is expected to be proportional to the relative duration of that phase within the diel or tidal cycle (Silva et al., 2017). When crabs selectively migrate during certain phases, observed proportions will deviate from the expected proportions. We used separate Chi-squared tests (stats R package (R Core Team, 2022)) to test whether the observed proportions in arrivals and departures differed from expected proportions. For the analysis of the circadian pattern, this was further divided according to the tidal class (non-tidal, transition and tidal zone).

Because the relative duration of each circadian phase varies throughout the study period (winter to summer), we used a weighted average to calculate the relative duration of each phase and subsequently the expected proportions. Weights were determined based on the number of occurrences of each date within the dataset. Similarly, the relative duration of each tidal phase varies throughout the study area, therefore a weighted average was used with the number of occurrences of each location (acoustic receiver) in the dataset as weight.

#### 3.2.4.2 Circadian and circatidal patterns in acceleration

Circadian and tidal phase were linked to acceleration measurements according to the method described above (section 3.2.4.1). We tested whether acceleration differed between circadian and tidal phases, and sex using a linear mixed effects model ('lme' function from nlme R package (Pinheiro and Bates, 2025)). Acceleration data was log-transformed to meet the assumptions of homogeneity of variance and normality of

residuals. We created separate models for the data collected in the non-tidal, transition and tidal zone. In the model for the tidal zone (1), circadian phase, tidal phase, their interaction and sex were included as fixed factors. The models for the non-tidal and transition zone (2) included circadian phase and sex. Tidal phase was not included as a factor in the model for the transition zone due to a lack of water level data.

$$(1) \log(\textit{acceleration}) \sim \textit{circadian phase} \times \textit{tidal phase} + \textit{sex} + (1 \mid \textit{tag ID} / \textit{acoustic receiver ID})$$

$$(2) \log(\textit{acceleration}) \sim \textit{circadian phase} + \textit{sex} + (1 \mid \textit{tag ID} / \textit{acoustic receiver ID})$$

We used acoustic receiver ID nested within tag ID as random factor to account for repeated measures of each crab, and an autoregressive correlation structure ('corAR1') to account for the correlation between acceleration measurements over time for each tag ID at each acoustic receiver. Model selection was performed based on AIC values through stepwise backward selection and the model was used as input for an ANOVA ('anova' stats R package (R Core Team, 2022)) to test the significance of effects. 'Emmeans' function (emmeans R package (Lenth, 2017)) was used for pairwise comparisons within significant factors.

#### 3.2.4.3 Migration speed

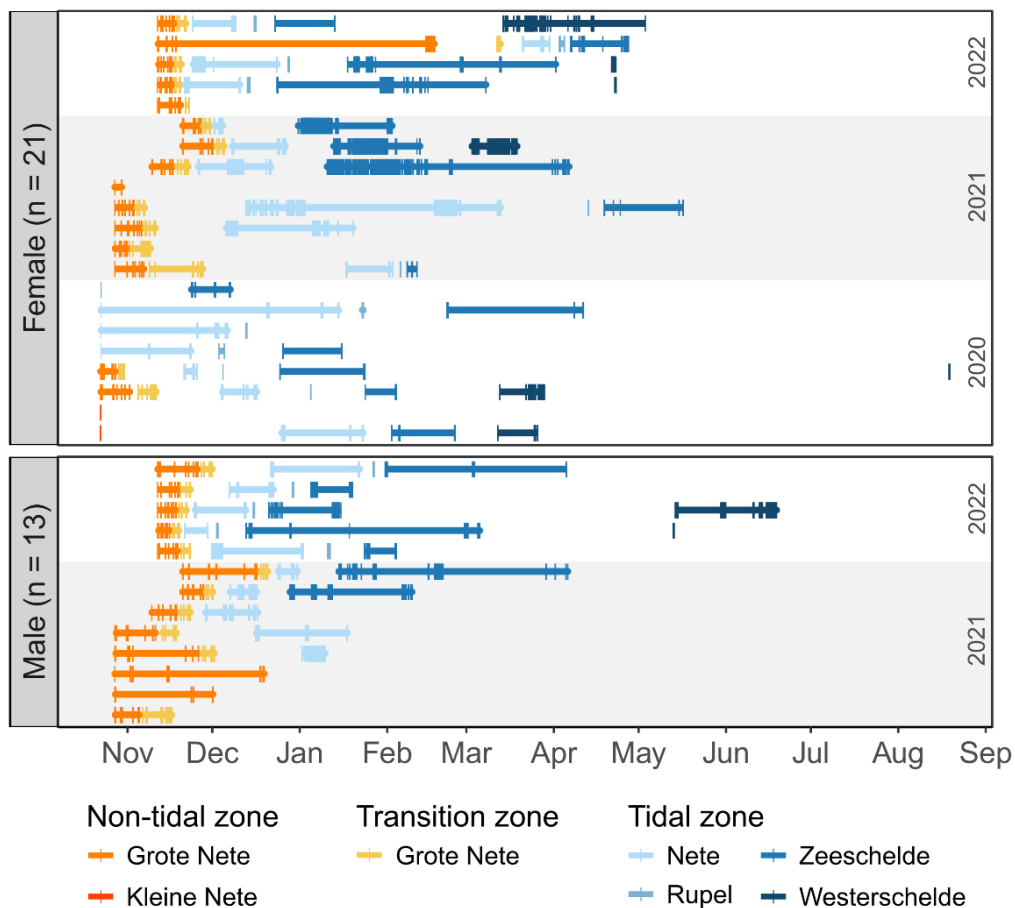
Daily migration speed (km day<sup>-1</sup>) was calculated for each crab between each pair of consecutive acoustic receivers by dividing the distance between those receivers by the time elapsed between the last detection at the first receiver and the first detection at the subsequent receiver. The effects of sex (fixed factor, i.e. male and female crabs), tidal class (fixed factor, i.e. non-tidal, transition and tidal zone) and their interaction on daily migration speed were tested using a linear mixed effects model ('lmer' function, lmerTest R package (Kuznetsova et al., 2017)). Tag ID was included as a random effect to account for repeated measurements of the same individuals, while acoustic receiver ID was included to account for spatial variability and repeated measurements at the same locations. Daily migration speed was log-transformed to meet the assumption of homogeneity of variance. The 'step' function (stats R package (R Core Team, 2022)) was used for model selection based on AIC. The model was used as input for an ANOVA ('anova' stats R package (R Core Team, 2022)). Finally, pairwise differences (with Tukey adjustment) were tested between the different tidal classes (emmeans R package (Lenth, 2017)).

### 3.3 Results

#### 3.3.1 Temporal and spatial scale of the migration

In total 34 Chinese mitten crabs were tagged, of which 33 were detected after release and included in the analysis, resulting in 90,107 detections, with on average ( $\pm$  SD)  $2,650 \pm 2,683$  detections per crab (range: 16 to 10,285 detections). All crabs showed migratory behaviour, moving downstream towards the Scheldt Estuary from their release location. Mitten crabs were tracked on average ( $\pm$  SD) over a distance of  $76 \pm 40$  km (range: 1 to 126 km) and a period of  $109 \pm 69$  days (range: 2 to 301 days) (Suppl. Mat. 8.2 8.1 **Table S. 8**).

Over the combined years of the study, most mitten crabs were tracked as far as the Scheldt Estuary (Suppl. Mat. 8.2 **Fig. S. 8**, **Fig. 3.3**), with 39 % ( $n = 13$ ) reaching Antwerp (Belgium, Zeeschelde) and 27 % ( $n = 9$ ) reaching Hansweert (the Netherlands, Westerschelde). The majority of these crabs arrived in the Scheldt Estuary during winter between mid-December and the end of January, while two crabs arrived as late as mid-April in spring (**Fig. 3.3**). The crabs arrived in the Westerschelde between the beginning of March and mid-May, with one crab being detected only in August (**Fig. 3.3**). About 18 % ( $n = 6$ ) of the crabs could only be tracked within River Grote Nete and are therefore considered to be lost before completing their migration. Additionally, 15 % ( $n = 5$ ) of the crabs reached as far as either the River Nete or River Rupel (Suppl. Mat. 8.2 **Fig. S. 8**). These rivers are generally not saline enough to support reproduction, leaving it uncertain whether these crabs completed their migration.

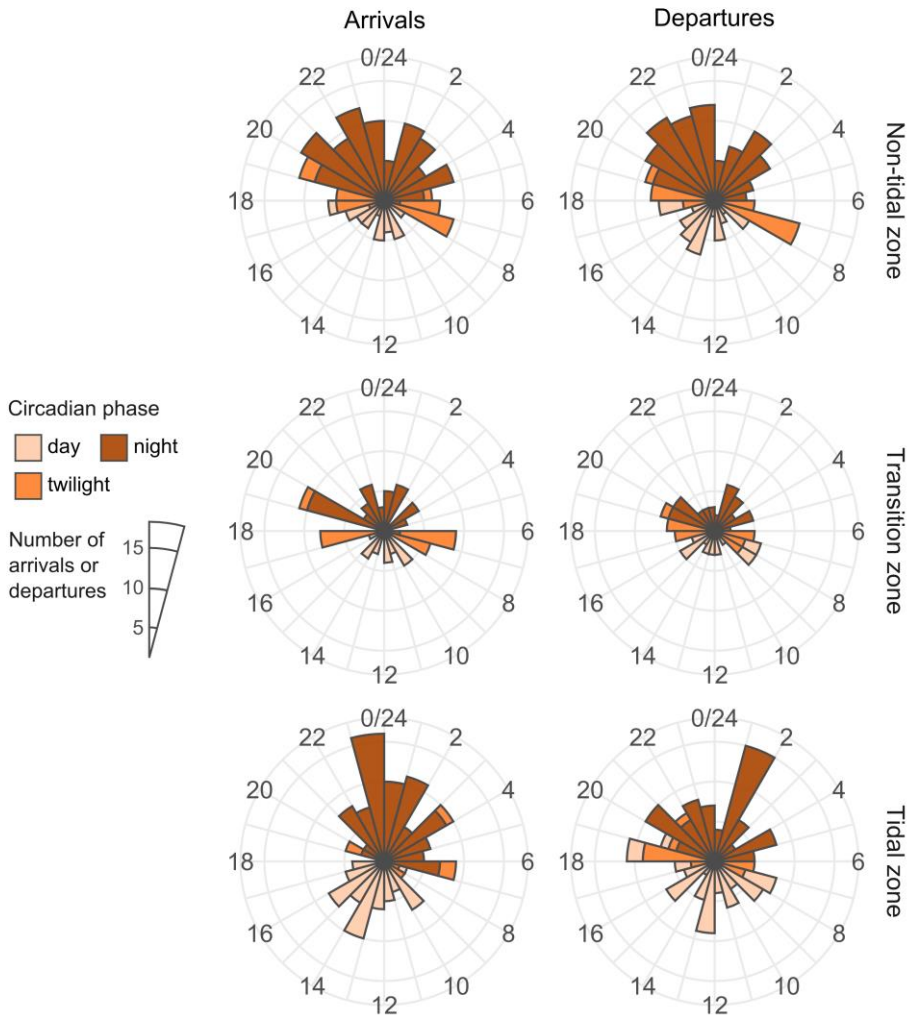


**Fig. 3.3** Individual trajectories of the 34 tagged Chinese mitten crabs between the period of October 2020 and July 2023. Crabs were tracked between 2 and 301 days. Vertical bars represent actual detections at receivers, while horizontal stretches indicate the period a crab was certainly present in a specific river. The year in which the individual crabs were released is depicted on the right.

### 3.3.2 Circadian and circatidal patterns

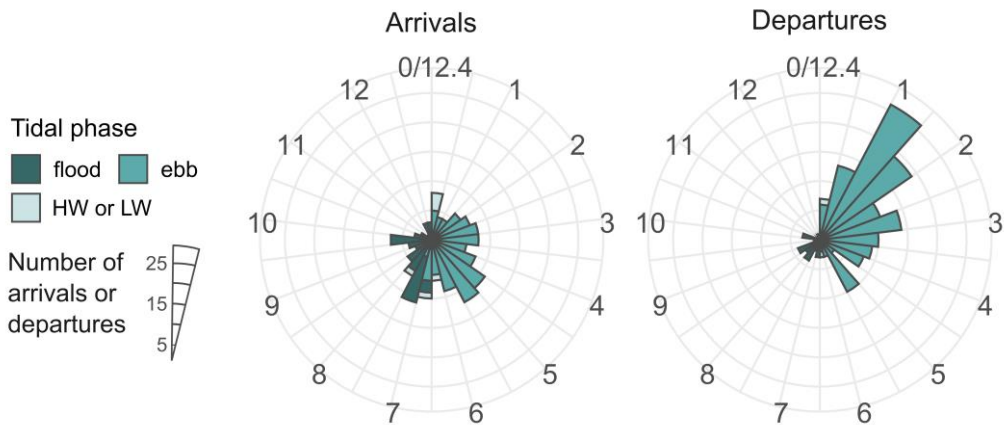
Analysis of arrival and departure of each individual crab at each acoustic receiver shows that in general Chinese mitten crabs migrated more during the darker hours of the day (**Fig. 3.4**) than expected in case of non-selective, continuous migration. In the non-tidal zone the observed detection proportions according to each circadian phase differed significantly from the expected proportions for both arrivals ( $n = 161$ ,  $\chi^2 = 22.43$ ,  $df = 2$ ,  $p < 0.001$ ) and departures ( $n = 162$ ,  $\chi^2 = 11.39$ ,  $df = 2$ ,  $p < 0.01$ ). Similarly, in the transition zone between the non-tidal and tidal zone, observed detection proportions differed significantly from the expected proportions for both arrivals ( $n =$

95,  $\chi^2 = 8.40$ ,  $df = 2$ ,  $p < 0.05$ ) and departures ( $n = 95$ ,  $\chi^2 = 7.15$ ,  $df = 2$ ,  $p < 0.05$ ), although these differences were caused by less detections during the day and more detections during twilight. In the tidal zone there was a significant difference between observed and expected proportions for arrivals ( $n = 157$ ,  $\chi^2 = 14.42$ ,  $df = 2$ ,  $p < 0.01$ ) but not for departures ( $n = 157$ ,  $\chi^2 = 2.63$ ,  $df = 2$ ,  $p = 0.27$ ).



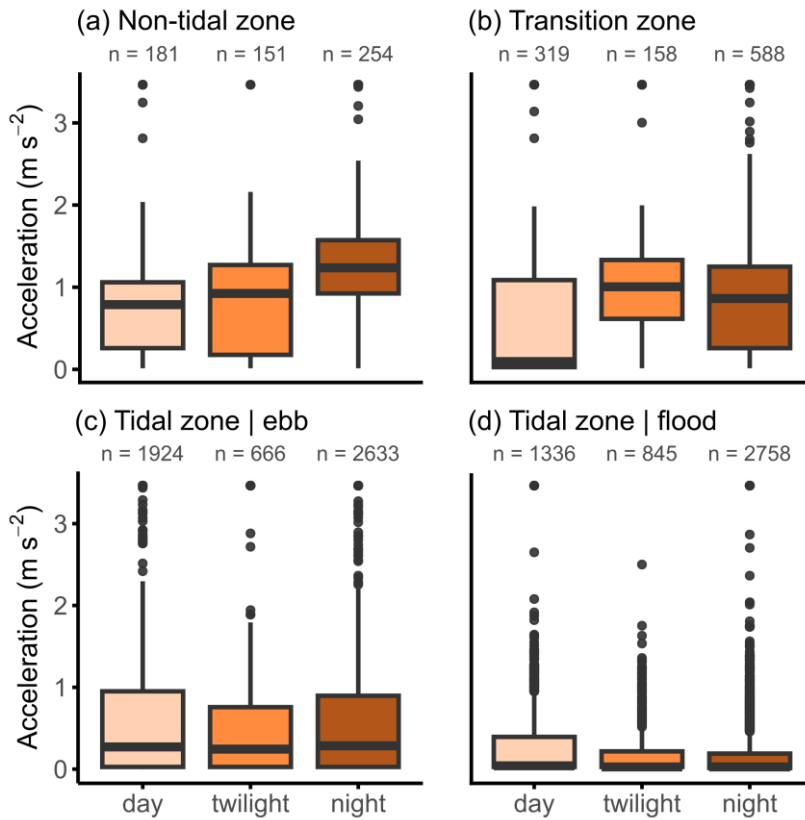
**Fig. 3.4** Distribution of both arrivals and departures ( $n = 827$ ) of crabs at acoustic receivers in the non-tidal, transition and tidal zone, according to the diel cycle. Each radial represents 1 hour. Colours visualise the time of day (circadian phase). The bars represent the number of arrivals or departures, aggregated across all receivers and crabs. The transition zone is under microtidal influence, while the tidal zone is under meso- to macrotidal influence.

Based on arrivals and departures at each receiver, we found that Chinese mitten crabs migrated more during ebb (Fig. 3.5) than expected in case of non-selective, continuous migration. The observed detection proportions differed significantly from the expected proportions for both arrivals ( $n = 151, \chi^2 = 10.04, df = 1, p < 0.01$ ) and departures ( $n = 155, \chi^2 = 60.61, df = 1, p < 0.001$ ). Arrival at an acoustic receiver occurred consistently throughout the ebb phase and extended into the early part of the flood phase. Departure occurred primarily during the first half of the ebb phase.



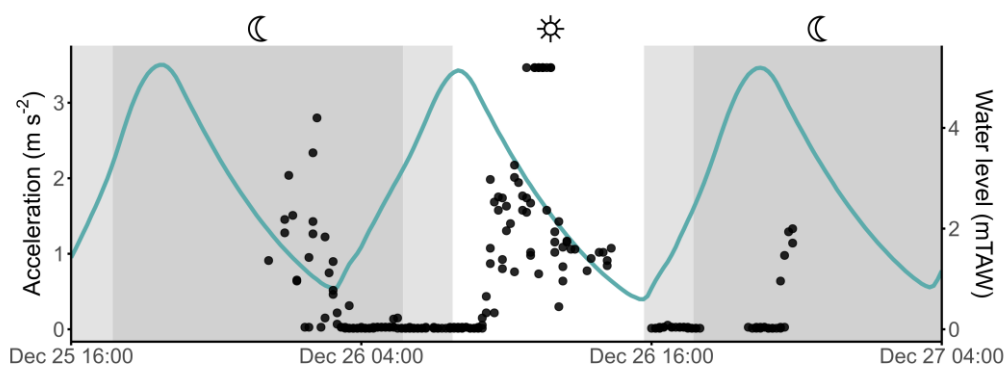
**Fig. 3.5** Distribution of both arrivals and departures ( $n = 314$ ) of crabs at acoustic receivers in the tidal zone, according to the tidal cycle. Each radial represents 0.5 hours after high water. Colours visualise the phase of the tidal cycle, with LW = low water and HW = high water. The bars represent the number of arrivals or departures, aggregated across all receivers and crabs.

In the non-tidal and transition zone, acceleration patterns were not affected by the sex of the crab and this factor was not retained in the final model. In the non-tidal zone, the model indicated a circadian pattern (Fig. 3.6) in acceleration ( $F(2,547) = 9.20, p < 0.001$ ), with a peak in activity during the night ( $1.26 \text{ m s}^{-2}, \text{Cl}_{0.95}[0.92-1.72]$ ) and reduced activity during the day ( $0.82 \text{ m s}^{-2}, \text{Cl}_{0.95}[0.55-1.24], p < 0.05$ ) and twilight ( $0.62 \text{ m s}^{-2}, \text{Cl}_{0.95}[0.42-0.93], p < 0.001$ ). Similarly, in the tidal transition zone, the model revealed a circadian pattern ( $F(2,1039) = 55.23, p < 0.001$ ), with decreased activity during daylight hours ( $0.32 \text{ m s}^{-2}, \text{Cl}_{0.95}[0.17-0.60], p < 0.001$ ) compared to night ( $1.02 \text{ m s}^{-2}, \text{Cl}_{0.95}[0.59-1.77], p < 0.001$ ) and twilight ( $1.90 \text{ m s}^{-2}, \text{Cl}_{0.95}[1.04-3.49], p < 0.001$ ).



**Fig. 3.6** Boxplots showing the acceleration of Chinese mitten crabs ( $n = 6$ ) according to circadian phase, in the non-tidal (a), transition (b) and tidal zone (c) and (d). The plots of the tidal zone are split up according to tidal phase. Number of observations per circadian phase and tidal zone is indicated on top of each boxplot. The transition zone is under microtidal influence, while the tidal zone is under meso- to macrotidal influence.

Within the tidal zone, the model did not reveal any circadian patterns (**Fig. 3.6** and **Fig. 3.7**) or differences between sexes in acceleration. In the final model only tidal phase was retained as a fixed factor. The model identified a pattern in acceleration according to the tidal cycle ( $F(1,10132) = 260.54$ ,  $p < 0.001$ ), with an increase in crab movement during ebb ( $0.25 \text{ m s}^{-2}$ ,  $\text{CI}_{0.95}[0.16-0.38]$ ),  $p < 0.001$ ) compared to flood ( $0.11 \text{ m s}^{-2}$ ,  $\text{CI}_{0.95}[0.07-0.17]$ ).



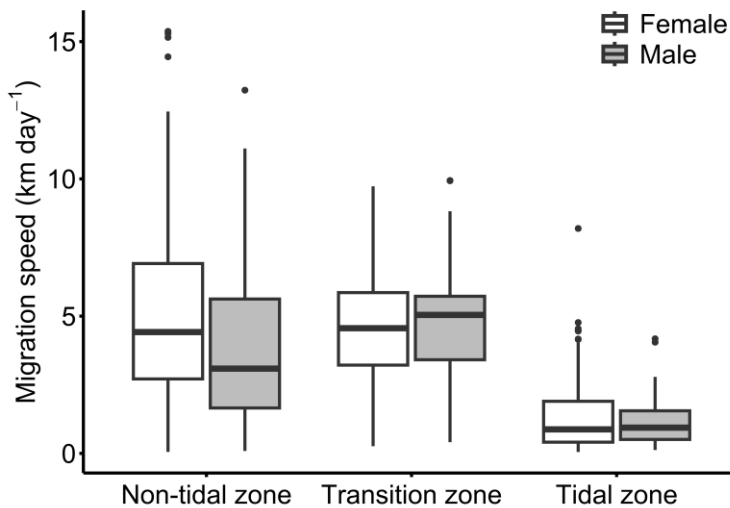
**Fig. 3.7** Example of acceleration patterns according to tidal and circadian phases within the tidal zone. The graph shows the data of one female crab (tag ID 3723, Suppl. Mat. 8.28.1 **Table S. 7**) at one acoustic receiver within the Nete River (tidal zone) from 25 to 27 December of 2021 (time in UTC). Acceleration ( $\text{m s}^{-2}$ ) measurements are shown as black dots and the blue line indicates the water level (mTAW) over time. The dark grey boxes indicate night, light grey boxes indicate twilight (i.e. dusk and dawn) and white boxes indicate day.

### 3.3.3 Depth

Within the River Grote Nete, crabs migrated at an average  $\pm$  SD depth of  $0.97 \pm 0.33$  m ( $n = 6$ , range: 0.15 to 2.26 m,  $n$  observations = 661) in the non-tidal zone and  $3.11 \pm 1.09$  m ( $n = 6$ , range: 0.45 to 6.77 m,  $n$  observations = 1,072) in the transition zone. In the tidal zone, crabs were found at an average depth of  $4.76 \pm 1.55$  m ( $n = 6$ , range: 0.30 to 8.27 m,  $n$  observations = 4,135) in the Nete River,  $12.29 \pm 5.10$  m ( $n = 5$ , range: 0.90 to 24.06 m,  $n$  observations = 5,712) in the Zeeschelde, and  $12.49 \pm 3.45$  m ( $n = 1$ , range: 2.41 to 21.96 m,  $n$  observations = 963) in the Westerschelde.

### 3.3.4 Migration speed

The average  $\pm$  SD migration speed within the non-tidal zone was  $4.65 \pm 3.51$   $\text{km day}^{-1}$ . However, large differences in daily migration speed were observed, ranging from  $0.06$   $\text{km day}^{-1}$  up to a maximum speed of  $15.37$   $\text{km day}^{-1}$  (**Fig. 3.8**). Similarly, the average  $\pm$  SD migration speed within the transition zone was  $4.71 \pm 2.26$   $\text{km day}^{-1}$  (range  $0.26$  to  $9.94$   $\text{km day}^{-1}$ ). Within the tidal rivers, the migration speed diminished and averaged  $\pm$  SD at  $1.29 \pm 1.22$   $\text{km day}^{-1}$  (range  $0.05$  to  $8.19$   $\text{km day}^{-1}$ ).



**Fig. 3.8** Boxplot showing the daily migration speed ( $\text{km day}^{-1}$ ) for female and male Chinese mitten crabs within the different tidal zones in the study area. The transition zone is under microtidal influence, while the tidal zone is under meso- to macrotidal influence.

We found no significant difference between the migration speeds of male and female crabs ( $F(1, 17.21) = 0.3584, p = 0.557$ ). The final model included tidal class as fixed factor and tag ID and acoustic receiver ID as random factors. Chinese mitten crabs migrated significantly ( $F(2, 21.137) = 19.19, p < 0.001$ ) slower within the tidal zone ( $0.84 \text{ km day}^{-1}$ ,  $\text{CI}_{0.95}[0.58-1.20]$ ) compared to the non-tidal zone ( $3.02 \text{ km day}^{-1}$ ,  $\text{CI}_{0.95}[2.04-4.48]$ ,  $p < 0.001$ ) and the transition zone ( $3.67 \text{ km day}^{-1}$ ,  $\text{CI}_{0.95}[2.19-6.11]$ ,  $p < 0.001$ ). We detected no significant difference in migration speed between the non-tidal, freshwater part of the study area and the transition zone ( $p = 0.80$ ).

### 3.4 Discussion

#### 3.4.1 Methodological considerations

This is the first study to apply acoustic telemetry to Chinese mitten crabs and is even one of the few applying this technique to elucidate long-distance decapod migrations. During this study, crabs were translocated from their capture site to different release locations, which may have caused short-term disorientation. However, the overall impact on behaviour is expected to be minimal. Chinese mitten crabs exhibit strong migratory instincts and, given the scale of their migration, they likely rely on general environmental cues, such as water temperature, flow direction and salinity gradients (Fialho et al., 2016; Kamps, 1937; Rudnick et al., 2005b), rather than site-specific cues to guide them. Moreover, similar situations occur naturally. Crabs from isolated ponds

and lakes must migrate over land to reach connected river systems (Kamps, 1937) to continue their journey to the estuary. In these cases, the crabs also enter a new river system, where they were not resident before.

Although external attachment of tags to the carapace is relatively straightforward, tagging decapods for a longer period of time can be challenging due to moulting (Florko et al., 2021). Mitten crabs undergo a final puberty moult before departing on their downstream spawning migration (Dittel and Epifanio, 2009; Kobayashi and Matsuura, 1995a; Panning, 1938) and unlike many other crab species, mitten crabs mate when the female has a hard shell (Herborg et al., 2006; Peters and Panning, 1933). The carapace width of mature adult mitten crabs can range from 30 mm to 95 mm (Panning, 1938; Rudnick et al., 2005b), but there is a large overlap in size with immature crabs (Kobayashi and Matsuura, 1995a). To minimize the chance of tag loss due to moulting, large crabs (> 55 mm) captured during the autumn migration period were selected for this study, as they were assumed to have already completed their puberty moult. Despite this precaution, one out of 34 crabs was never detected, and 11 crabs were lost within the oligohaline part of the study area. Besides tag loss due to moulting, tags could have become detached through for example abrasion, although our preliminary tag retention tests did not indicate this for the applied method in controlled conditions (Suppl. Mat. 8.2). Furthermore, crabs might have died due to predation, environmental factors or poor health condition (e.g. infection or limited fat reserve). By selecting large individuals, we also aimed to minimize the tag burden (tag-to-body mass ratio), which ranged between 0.9 and 4.9 % in this study. This is within the range that is commonly used in telemetry studies, however it is not known how tag burden affects movement rates of crabs (Florko et al., 2021; Smircich and Kelly, 2014). This should be further investigated in future studies to improve the application of acoustic telemetry on decapods. It is important to note that the selection of large animals may have introduced a size bias, potentially underrepresenting smaller adult crabs that might also participate in the downstream spawning migration.

In addition to tag loss or death, it is possible that crabs stopped migrating or got disoriented. Low migration speeds were commonly observed around and downstream of the city of Lier, where media articles and anecdotal observations report mitten crabs out of the water at night each year in autumn. This could signify that the combination of river confluences, old river arms within the city and sluices sometimes cause disorientation and migration delays, causing them to leave the water. Interestingly, at other confluences where acoustic receivers were present, crabs were never detected to wander in the wrong direction.

### 3.4.2 Place of reproduction and relation to salinity

The place of reproduction can be based on the location where male Chinese mitten crabs cease their migration (Peters, 1938). In this study, both male and female mitten crabs migrated until a similar distance from the estuarine mouth, although females were more frequently observed in the lower reaches of the estuary (i.e. Westerschelde). Generally, crabs migrated as far as the zone between Antwerp and Hansweert, located at 45 to 78 km from the estuarine mouth in the North Sea (Suppl. Mat. 8.2 **Fig. S. 8**). Although the acoustic receiver network extends further downstream of the last detections, i.e. Westerschelde and the Belgian part of the North Sea, no Chinese mitten crabs were detected in these areas. Because of this, we hypothesize that mating and spawning takes place in the zone between Antwerp and Hansweert. However, it may be possible that some crabs migrated undetected further downstream.

The zone between Antwerp and Hansweert is mesohaline and Hansweert marks the border of the polyhaline zone (Meire et al., 2005). Salinity ranged between 0.2 and 14 ‰ (mean 4 ‰) near Antwerp and 12 and 23 ‰ (mean 19 ‰) near Hansweert, during the period that the crabs were detected ([www.waterinfo.be](http://www.waterinfo.be)). In the River Elbe in Germany, a salinity of 5 – 6 ‰ was suggested to be the lower limit at which mitten crabs reproduce (i.e. mating behaviour and subsequential egg-laying), which was confirmed in a laboratory study (Peters, 1938). Although mating is possible at this concentration, egg attachment can be aberrant and higher concentrations of  $\geq 15$  ‰ are considered more optimal (Cohen and Weinstein, 2001; Peters, 1938). Most larval stages of mitten crabs require high salinities (15 – 32 ‰), but embryos and newly hatched larvae can tolerate hyposaline conditions and have optimal survival from 5 to 20 ‰ (Anger, 1991; Cohen and Weinstein, 2001; Wang et al., 2019), which corresponds well with the salinity range of the assumed spawning location in the Scheldt Estuary. However, the Scheldt River basin is rain fed, meaning that the salinity gradient in the estuary can change depending on the amount of freshwater discharge (Meire et al., 2005). We therefore hypothesise that the spawning location is dynamic and can change according to the environmental circumstances.

### 3.4.3 Timing of reproduction

In the River Grote Nete, adult mitten crabs start their downstream migration in September to late November, with a peak in October (Schoelynck et al., 2021). Our tracking data indicates that they arrive in brackish water between mid-December and late January. Presumably, these crabs go through their final puberty moult in August or September (Hymanson et al., 1999; Kobayashi and Matsuura, 1995a; Panning, 1938).

Their arrival in brackish water likely coincides with reaching sexual maturity, as the closely related Japanese mitten crab (*Eriocheir japonica*) needs four months for maturation of the ovaries after the puberty moult (Kobayashi and Matsuura, 1995a).

Once the crabs reached the assumed spawning location, they stayed around for multiple months. Generally they were detected until late April to mid-May, with two of them still being detected as late as June and August (Suppl. Mat. 8.2 **Table S. 8, Fig. 3.3**). During this time they mate and incubate their brood until larvae hatch, which may take a few months depending on environmental conditions (Panning, 1938; Rudnick et al., 2005b). Some female crabs can even have up to three successive broods (Kobayashi and Matsuura, 1995c; Peters, 1938; Rudnick et al., 2005b). After the reproduction period, adult mitten crabs become inactive and are increasingly overgrown by organisms (algae, hydroids, barnacles), indicating that they lack the energy to moult (Kobayashi and Matsuura, 1994; Panning, 1938; Peters, 1938). They die soon after the reproduction period as mature adult crabs are usually found until summer (Kobayashi and Matsuura, 1995c, 1994; Panning, 1938; Rudnick et al., 2005b), which was also the case in the present study.

Overall, these results suggest that reproduction (mating, spawning and larval release) in the Scheldt Estuary takes place from at least December to June. This fits within the known time frame for reproduction of the Chinese mitten crab worldwide: in the Elbe Estuary (Germany) reproduction takes place between October and July (Panning, 1938), in the Tagus Estuary (Portugal) at least from March until May (Anastácio et al., 2018), in the Thames Estuary (United Kingdom) from September to July (Clark, 2011), in San Francisco Bay (USA) between October and June (Rudnick et al., 2003), and in the Yangtze River (China) from October to April (Hymanson et al., 1999).

It is evident that in this study only a select part of the mitten crab population was tracked, possibly leading to an underestimation of the reproductive time frame. Crabs were collected from a single location within the river basin during a limited period, specifically from late October to late November. Notably, these crabs were captured approximately 140 km upstream from the river mouth, while mitten crabs can also inhabit more downstream areas (Rudnick et al., 2005a). Crabs that migrate earlier or reside in lower river sections may reach the estuary sooner than those monitored in this study. Conversely, it is also possible that these crabs exhibit slower migration speeds or take more breaks, resulting in similar arrival times in the estuary. Future research could further explore potential variability in migration patterns between downstream and upstream populations.

It has been suggested that some mitten crabs do not die after spawning and migrate back to upstream foraging areas (Adema, 1991; Kamps, 1937). This hypothesis was based on the observation of a small number of crabs with barnacles attached to their carapace in inland waters (Adema, 1991; Kamps, 1937). Furthermore, Peters (1938) proposed that at least some female crabs have the potential to spawn during a second spawning period based on histological evidence. Within the present study, we found no evidence for adult mitten crabs migrating back after reproduction. Moreover, no crabs were detected moving upstream throughout the whole study area and study period.

#### **3.4.4 Patterns in migratory behaviour**

We demonstrate for the first time that mitten crabs can distinguish ebb and flood tide, as our results indicate that they selectively use the ebb tide to migrate down the estuary to reach their spawning habitat. This behaviour was earlier hypothesized by Peters and Panning (1933), who suggested that mitten crabs bury themselves in the sediment during flood. While circatidal rhythms in activity are common among brachyuran crabs, typically influencing small scale movements like seeking shelter and foraging (Hewitt et al., 2023; Holsman et al., 2006; Hunter and Naylor, 1993; Luppi et al., 2013; Warman et al., 1993), there are only a few documented cases of the selective use of tides for long-distance migration. For example, adult female blue crabs (*Callinectes sapidus*) use ebb-tide transport to move to spawning locations and flood-tide transport to move back into the estuary after larval release (Forward et al., 2003; Tankersley et al., 1998). Similarly, the movement of the giant mud crab (*Scylla serrata*) is suggested to be synchronised with the tide, with female spawning migration occurring during new and full moon (Hewitt et al., 2022). It is unknown how Chinese mitten crabs are able to discriminate between tides and navigate in the correct direction. This ability is likely driven by endogenous controls (e.g. circatidal or circalunidian rhythm) or exogenous cues (e.g. salinity, current velocity, hydrostatic pressure or olfactory stimuli), or a combination of both (Darnell et al., 2012; Luschi et al., 2013). As a relatively slow-moving species, the use of STST can be highly advantageous for these crabs, as it significantly reduces the energetical cost of migration, particularly in areas with high flow velocities (Forward and Tankersley, 2001).

As evidenced by our tracking and acceleration data, Chinese mitten crabs migrate mainly at night, presumably to minimize predation risks. This nocturnal migratory behaviour has also been described for adult mitten crabs in San Francisco Bay (USA) (Rudnick et al., 2003) and for juvenile mitten crabs in the Thames Estuary (UK) (Gilbey

et al., 2008). However, there is a clear change in migratory behaviour when mitten crabs enter rivers with strong tidal influence, as activity became less synchronised with circadian phases. This is in contrast to, for instance, adult female blue crabs, which primarily use nightly flood or ebb tides to move in or out the estuary during their spawning period (Forward and Tankersley, 2001). Possibly mitten crabs predominantly act on the tidal migration cue over the diel migration cue once they are exposed to tidal currents. Their behaviour could also be influenced by increased river depth and turbidity, which reduce light levels within the tidal rivers and Scheldt Estuary compared to other rivers in the study area (Abrahams and Kattenfeld, 1997; Alberts-hubatsch, 2015; Meire et al., 2005).

Although Chinese mitten crabs occasionally moved to shallow areas, they primarily migrated along the maximum depths of each river, which increased along their trajectory. For instance, in River Grote Nete, where the average depth is  $0.94 \pm 0.37$  m and the maximum depth is 2.41 m (pers. comm. VMM), the crabs were typically found at an average depth of  $0.97 \pm 0.33$  m. In the Scheldt Estuary, where reproduction takes place, they were detected at an average depth of  $12.29 \pm 5.10$  m, while mean depth ranges between 10 and 20 m in this part of the estuary (Meire et al., 2005). This finding is consistent with earlier reports of Chinese mitten crabs residing in the deeper parts of estuaries during winter and spring (Rudnick et al., 2003) and ovigerous females being collected at depths of 10–15 m in the Elbe Estuary, Germany (Anger, 1991). During their migration the crabs might benefit from higher current velocities in deeper parts of the rivers compared to the shallow riverbanks. Furthermore, it is common among crustaceans to move to deeper waters during the colder months (Hines et al., 1995; Zarrella-Smith et al., 2022). While the Scheldt Estuary is well-mixed, deeper waters may still offer a more stable refuge, particularly in terms of temperature, compared to the more variable and exposed shallow areas.

### **3.4.5 Migration speed and activity**

Panning (1938) derived the first migration speeds for adult mitten crabs in the 1930s, marking more than 1,500 crabs in Germany during an extensive mark-recapture study. He documented migration speeds up to 8 and 12 km day<sup>-1</sup> over distances of 257 to 368 km. In our study, we found that in freshwater systems with unidirectional currents, mitten crabs can reach even higher speeds of more than 15 km per day. However, generally the migration speed was lower in these rivers, averaging around 4.65 km day<sup>-1</sup>. Similar migration speeds in freshwater rivers have been documented in other studies. Mark-recapture studies recorded migration speeds between 0.22 and 2.78 km day<sup>-1</sup> in the nearby River Kleine Nete (Belgium) (Schoelynck et al., 2021), and

between 0.3 and 5 km day<sup>-1</sup> (average 1.25 km day<sup>-1</sup>) in freshwater channels in the Netherlands (Kamps, 1937). Additionally, an experimental study found an average speed of 6.4 km day<sup>-1</sup> in a freshwater canal without current (Fialho et al., 2016). Differences in reported migration speeds may not only reflect population and environmental variability but could also be influenced by differences in study methodologies, such as the duration between catch checks and the spatial scale over which migration speed is measured.

Generally mitten crabs moved quickly down unidirectional freshwater rivers without interruption, which is similar to the behaviour of Japanese mitten crabs (Kobayashi, 1999). Within the transition zone to tidal rivers, where the crabs experienced limited tidal influence, the migratory behaviour and migration speed remained largely the same as in the non-tidal part. However, in the freshwater tidal parts of the study area (Rivers Nete and Rupel), a few female crabs seemed to exhibit delayed migration, staying for several months in the same area before eventually continuing moving downstream to brackish areas. Possibly these female crabs gather before moving further downstream, similar to staging behaviour of freshwater fish. Generally, in meso- and macrotidal rivers Chinese mitten crabs noticeably slowed down, with a mean migration speed of 1.29 km day<sup>-1</sup>. This could in part be explained by the use of STST. While this behaviour is energy efficient, it increases residence time and decreases overall migration speed because the crabs have to wait for the favourable tidal phase (Silva et al., 2017). Additionally, there is great variability in current velocity throughout the ebbing phase, compared to the relatively stable current velocity in non-tidal rivers. Furthermore, once the mitten crabs reach suitable habitat for reproduction, migration slows down or stops, and crabs likely shift to more resident behaviour, e.g. foraging and burying in the sediment (Kobayashi and Matsuura, 1994; Peters, 1938). For example, a tagging study on edible crabs (*Cancer pagurus*) noticed a dramatic decline in activity when females were carrying eggs (Hunter et al., 2013).

### **3.4.6 Conclusion**

This study reveals key insights into the movement behaviour of the Chinese mitten crab during its downstream spawning migration. Using acoustic telemetry, we observed that mitten crabs move faster in non-tidal rivers compared to tidal rivers. In non-tidal rivers they migrate primarily during the night. In tidal rivers, migration was closely linked to the tidal cycle, with crabs taking advantage of the ebb tide to move downstream. This represents a new example of an animal using selective tidal stream transport and demonstrates that this behaviour is not only important for the larval stages of brachyuran crabs but also for adults. These findings suggest that Chinese

mitten crabs adjust their movement behaviour to maximise fitness and conserve energy for spawning.

This study focused on the ecology and movement behaviour of the species. The insights of this study could also support more effective management strategies for Chinese mitten crabs. In their non-native range, removal programs may improve efficiency by targeting reproductive adults during their downstream migration, which occurs primarily at night, and by concentrating efforts in key locations where crabs take advantage of strong tidal or unidirectional currents. Conversely, in their native range, this knowledge could be applied to facilitate migration and support the continuation of their life cycle.

### **3.5 Acknowledgements**

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# 4

## **Breaking it down: leaf litter processing by invasive Chinese mitten crabs and associated effects on water quality in laboratory conditions**

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## Abstract

In freshwater streams, leaf litter decomposition is a crucial ecosystem function that provides energy and nutrients. Biological invasions by non-native decapods, like the common invasive Chinese mitten crab, could potentially alter decomposition rates and therefore affect this key ecosystem function. This experimental study sheds light on the detritivorous feeding behaviour of these crabs and its consequences for water quality, including dissolved nutrient concentrations, fine particulate organic matter and biological oxygen demand. Two different crab life stages were tested and compared to a control condition without crabs. The crabs were fed with leaf litter originating from five different trees, including alder, beech, Canadian poplar, willow and large-leaved linden. Our findings revealed that juvenile crabs had minimal impact on leaf litter breakdown and nutrient concentrations. In contrast, adult crabs significantly accelerated litter breakdown rates and substantially increased dissolved nitrogen concentrations in the water column. Both juvenile and adult crabs were found to enhance the amount of fine particulate organic matter and elevate biological oxygen demand. Considerable variations across different leaf litter diets were observed, demonstrating the importance of leaf litter characteristics on the feeding behaviour of Chinese mitten crabs. These results underscore the potential of Chinese mitten crabs to expedite leaf litter processing and nutrient release in freshwater ecosystems, highlighting the significant role that invasive species can play in altering ecosystem functioning.

## 4.1 Introduction

The processing of terrestrial leaf litter in streams is a major ecosystem function that makes nutrients and energy available for aquatic communities (Swan et al., 2021). Particularly in the temperate region, this ecosystem function is largely facilitated by leaf-shredding invertebrates or ‘shredders’ (Hieber and Gessner, 2002; Jonsson and Sponseller, 2021; Wantzen et al., 2008). Freshwater ecosystems worldwide are facing diverse threats, which could profoundly change this function (Ferreira et al., 2010; Fincham et al., 2023; Marks, 2019; Pile et al., 2023). Examples of factors that have an effect are pollutants like fungicides and pesticides (Fernández et al., 2015; Schäfer et al., 2012), rising water temperatures due to climate change (Follstad Shah et al., 2017) and changing riparian vegetation (Doherty-Bone et al., 2018a; Going and Dudley, 2008). Furthermore, the ongoing introductions and spread of non-native invasive species have the potential to reshape the composition of macrozoobenthic communities and therefore affect the pivotal function that they perform.

Large decapods such as crabs, crayfish, shrimp and prawns, are globally an important part of freshwater macrozoobenthos and have been widely acknowledged as important leaf litter processors (Coughlan et al., 2010; Cowl et al., 2006; Duarte et al., 2012; Mancinelli et al., 2013). In Western and Central Europe the introduction of alien decapod species has been significant (Holdich et al., 2009). In Great Britain for example it was estimated that 88 % of the freshwater decapod biodiversity comprises non-native species (Keller et al., 2009). Most decapod species are opportunistic omnivores, the impact of their introduction on leaf litter processing can therefore be direct through their own shredding and feeding activities and indirect through their interactions (predation, competition and replacement) with native species (Doherty-Bone et al., 2018b; Dunoyer et al., 2014; Jackson et al., 2016; Moore et al., 2012).

Despite the complexity and species-specific nature of their effects, research suggests that primarily the direct effects of decapods on leaf litter decomposition are of particular importance (Mancinelli et al., 2013). Therefore, there is a need for increased focus on the detritivorous feeding behaviour of (invasive) decapods, with particular emphasis on freshwater crabs, given their current underrepresentation in this research field (Mancinelli et al., 2013; Yang et al., 2020). Studies in mangrove forests have established the ability of crabs to process leaf litter (Emmerson and McGwynne, 1992; Mfilinge and Tsuchiya, 2008; Robertson and Daniel, 1989; Schories et al., 2003) and field studies in the (neo-)tropical region have clearly demonstrated their potential as effective detritivores in streams as well (Moss, 2005; Yang et al., 2020), therefore their impact is also expected to be significant in temperate waterbodies.

The Chinese mitten crab (CMC, *Eriocheir sinensis*, H. Milne-Edwards, 1853) is an interesting case-study in this regard because of its widespread distribution. The Chinese mitten crab is a large catadromous decapod that spends most of its life in freshwater but migrates back towards brackish or salt water once in its life to reproduce. The species is native to East Asia and was first observed as alien species in Europe in 1912, in the River Aller in Germany. After this, the species spread rapidly across all coastal countries in Europe and has also invaded North America more recently (Dittel and Epifanio, 2009; and references therein). In Belgium specifically, the Chinese mitten crab was first observed in 1933 after which the population experienced strong fluctuations in size (Ewers et al., 2023). Nowadays, the species is present in all larger waterbodies and vast numbers are caught (Schoelynck et al., 2021). Because of these large numbers, concerns have been raised about their impact on native fauna and flora and on ecosystem functioning in general, as native freshwater crabs were absent in Belgium before.

Like most decapods, the Chinese mitten crab is an opportunistic omnivore and laboratory experiments have pointed out that the crabs prefer feeding on animals over plants when they are easily accessible (Rogers, 2000). By contrast, various plant materials (i.e., macrophytes, algae and detritus) generally dominate the gut content of the crabs (Czerniejewski et al., 2010; Jin et al., 2003; Rosewarne et al., 2016; Rudnick et al., 2000; Rudnick and Resh, 2005). These findings have been confirmed by stable isotope analyses of tissue of wild-caught crabs (Rosewarne et al., 2016) and crabs from mesocosm experiments (Schoelynck et al., 2020). Although there is substantial variation between studies, indicating possible differences in diet depending on region and/or life stage, detritus or leaf litter has a major share in their diet.

Existing literature on leaf litter breakdown by the Chinese mitten crab highlights that the species is able to transform leaf litter at a higher rate than native decapod species in Europe (Doherty-Bone et al., 2018a, 2018b). However, next to the biological characteristics of the species, leaf litter decomposition rates also depend on other factors like environmental characteristics like temperature (Martínez et al., 2014; Taylor and Chauvet, 2014) or leaf litter characteristics such as leaf toughness, nutrient content (especially nitrogen) and chemical defences (M. Zhang et al., 2019). The detritivorous feeding behaviour of crayfish and shrimp has been extensively studied, a meta-analysis of field research on leaf litter breakdown by these decapods revealed that the initial C:N ratio of leaf litter is in fact more important than decapod size to determine the decomposition rate (Mancinelli et al., 2013). No studies on freshwater

crabs were included in this meta-analysis, as there is a lack of research exploring how various leaf litter types influence their capacity for leaf litter processing.

The present study advances the knowledge of detritus processing by Chinese mitten crabs by assessing the effect of crab size and leaf litter diet. This was examined by measuring (1) the rate of leaf litter breakdown of distinct tree species, and (2) water quality including the production of fine particulate organic matter, dissolved nutrients and the effects on biological oxygen demand (BOD). Secondly, we investigated whether the effect on water quality is mostly related to detritivory itself or excretions by the crabs. We hypothesized that both crab sizes would accelerate leaf litter breakdown and fine particulate organic matter production. We anticipated that this process would enhance the release of dissolved nutrients as a result of egestion, excretion and increased fragmentation, leading to a higher biological oxygen demand. Additionally, we expected substantial differences between leaf litter diets, with highest breakdown rates occurring when crabs are feeding on leaf litter types with high nutritional quality.

## **4.2 Methods**

### **4.2.1 Leaf litter**

Prior to the experiment, naturally fallen leaves of five tree species were collected from the ground in autumn 2018 around the Antwerp University campus Drie Eiken (Wilrijk, Belgium). The tree species included in the experiment are alder (*Alnus glutinosa*), beech (*Fagus sylvatica*), Canadian poplar (*Populus x canadensis*), willow (*Salix cf. alba*) and large-leaved linden (*Tilia platyphyllos*). These species are common in proximity of water bodies in Belgium and differ in leaf litter characteristics such as nutrient content and leaf toughness. All leaves were oven dried for 72 h at 70°C and subsequently stored in paper bags.

To verify the differences in nutrient content of the leaves of the different tree species, a random subset of each tree was analysed. The samples ( $\pm 10$  g) were prepared by grinding the dried leaves with an Ultra Centrifugal Mill ZM 200 (Retsch, Germany), sieve size 0.5 mm. Total carbon and nitrogen content of the ground leaves were determined in duplicate using a FLASH 2000 Organic Elemental Analyser, based on Flash Dynamic Combustion (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Total phosphorus content was determined by acid destruction according to the procedure described by Walinga et al. (1989). The samples were first digested with H<sub>2</sub>SO<sub>4</sub>, HOC<sub>6</sub>H<sub>4</sub>COOH and H<sub>2</sub>O<sub>2</sub> and were then analysed on a colorimetric segmented flow

analyser (SAN++, Skalar, Breda, The Netherlands). Biogenic silica (BSi) was determined following the alkaline extraction method described by DeMaster (1981). Samples of 30 mg ground leaves were incubated in 0.5 M NaOH at 80°C for 5 hours. After filtering the samples (Chromafil® Xtra MV-45/25, Macherey-Nagel, Düren, Germany), the concentration was determined (SAN++, Skalar, Breda, The Netherlands).

#### 4.2.2 Chinese mitten crabs

For each experimental run, freshly caught Chinese mitten crabs were obtained from a crab trap on the Kleine Nete River (51°11'35" N 4°45'23" E, Flanders, Belgium). Multiple experimental runs were performed because the different size classes of the crab were caught in different seasons, during their respective migration periods. The juvenile crabs were caught in spring and the adult crabs in autumn. The crabs were selected based on size as well as overall health, i.e. visually active and no damages to carapace or limbs. Males and females were selected based on availability. Each crab was measured behind the fourth pair of lateral spines of the carapace with a digital calliper (carapace width, up to 0.01 mm) and weighted on a digital balance (wet mass, up to 0.01 g). The juvenile mitten crabs had an average carapace width of  $19.8 \pm 1.7$  mm and wet mass of  $3.0 \pm 0.7$  g, and included 21 females and 29 males. The adult mitten crabs measured on average  $56.2 \pm 5.9$  mm in width and weighted  $78.9 \pm 20.8$  g, 34 females and 16 males were used.

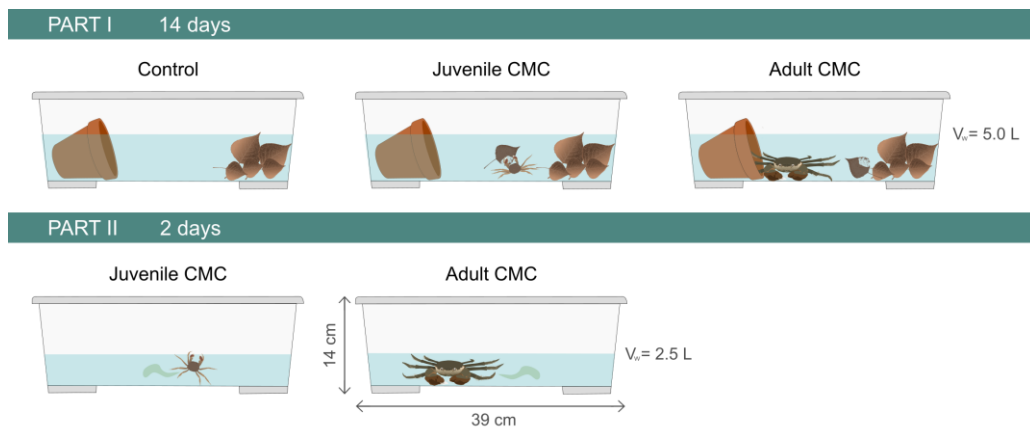
#### 4.2.3 Study design

The purpose of this study is to assess to which extent crab size and type of leaves affect (1) the rate of leaf litter breakdown, and (2) the effect on water quality related to leaf litter breakdown including dissolved nutrient concentrations, fine particulate organic matter (FPOM, < 1 mm) production and BOD.

In the first part of the experiment (**Fig. 4.1**), three treatments were tested over a period of fourteen days: (1) juvenile Chinese mitten crabs (< 2.5 cm carapace width) and leaves, (2) adult Chinese mitten crabs (> 3.5 cm carapace width) and leaves and (3) a control with only leaves but no crabs. For simplicity, these treatments will be referred to as (1) juvenile CMC, (2) adult CMC and (3) control for the remainder of the paper. Within each treatment the five tree species were examined separately, with a total of ten replicates per combination of treatment and tree species. The experiments for the juvenile CMC treatment were performed in spring, while the experiments for the control and adult treatments were performed in autumn (**Table 4.1**). Note that the same leaves were used in each experiment, irrespective of the season during which

the experiment was run, and that background values of the water used were measured in three separate tanks in every experiment. Based on the two treatments with crabs (juvenile CMC and adult CMC) and leaves, the total effect of the crabs (excretion and detritivory together) and leaching of the leaves on the production of dissolved nutrients was assessed. The separate effect of leaching alone was derived from the control treatment.

In the second part of the experiment (**Fig. 4.1, Table 4.1**), the separate effect of Chinese mitten crab excretions was measured over a period of two days. All crabs (juvenile CMC and adult CMC) used in the first part of the experiment were placed individually in tanks with fresh water and without leaves, to measure the isolated effect of excrements. Treatments will be labelled as (1) juvenile CMC and (2) adult CMC. Combining the results of both experimental parts, the individual contributions of leaching, excretions and detritivory can be disentangled.



**Fig. 4.1** Schematic overview of the experiment, with the different treatments used in each part. CMC stands for Chinese mitten crab. Leaves of five different tree species are used separately in part I to measure the effects of leaching and detritivory. A shelter (terracotta pot) was provided for the crabs in this longer experiment. In part II, no extras were added so that the sole effect of excretion could be measured.

#### 4.2.4 Experimental microcosms

For the first part of the experiment, transparent plastic tanks (39 × 28 × 14 cm) were filled with 5 L tap water. The tanks were closed off with a lid to prevent crabs from escaping and limit evaporation. Within each lid, two holes (2.0 cm diameter) were created to enable gas exchange. The water was aerated with an air stone throughout the experiment. Each tank contained one terracotta plant pot (11 cm diameter, 9 cm height) to provide shelter for the crabs. A diurnal light cycle was installed with 16 hours

of light per day. Experimental conditions were measured with a 2FD47F Multi 3430 Multiparameter Meter (WTW, Weilheim, Germany) at the start of the experiment. The water temperature was on average  $17.9 \pm 1.0^\circ\text{C}$ , pH  $8.2 \pm 0.2$  and electrical conductivity  $584 \pm 133 \mu\text{S/cm}$ .

The effects of the crabs on leaf litter breakdown were assessed over a fourteen-day period. On day one, the pre-weighted whole dried leaves (5.00 g) and one crab (with known sex, carapace width, and mass) were added to each tank. The tanks were checked every day to follow up on the health of the crabs. In the rare occasion that a crab died, the crab was immediately replaced with a new individual similar in size.

After fourteen days, water samples were taken to determine dissolved nutrient concentrations:  $\text{NO}_3\text{-N}$  (nitrate),  $\text{NO}_2\text{-N}$  (nitrite),  $\text{NH}_4\text{-N}$  (ammonium),  $\text{PO}_4\text{-P}$  (orthophosphate), dissolved silica (DSi) and the amount of dissolved organic carbon (DOC) (**Table 4.1**). The crabs were retrieved and the content of the tanks (water and leaves) were poured over a 1 mm sieve to separate the larger fragments. These fragments were gathered with tweezers, stored in paper bags and oven dried for 72 h at  $70^\circ\text{C}$ . The dried residual leaves were weighted to the nearest 0.01 g, to determine the difference in dry mass before and after the experiment. After stirring the remaining water, two water samples (1 L) were taken to determine the amount of fine particulate organic matter (FPOM, < 1 mm) and biological oxygen demand (BOD).

For the second part of the experiment, the tanks were cleaned and installed anew with fresh tap water (2.5 L) and the same crab but without leaves or shelter, to measure the effects of crab excretions separately. On day seventeen (i.e. after two more days), the same measurements (**Table 4.1**; dissolved nutrients, DSi, DOC) as described above were performed. Based on these measurements, it can be determined how much of the dissolved nutrients are originating from the excretions of the crabs, distinguishing it from the amount of dissolved nutrients that are produced as a secondary product of the detritivory activity itself. Finally, the crabs were retrieved and thereafter euthanized.

**Table 4.1** Overview of the study design, showing the two sequential parts of the experiment, the different treatments and number of replicates (n).

<b>PART I Effect detritivory and leaching</b>				
Period	Season	Tree species × treatment	n	Measured parameters
14 days	Autumn	<i>Alnus</i> × adult CMC	10	Leaf breakdown rate (g dry weight lost/day), FPOM production rate (g dry weight/day), dissolved nutrient concentrations
14 days	Spring	<i>Alnus</i> × juvenile CMC	10	
14 days	Autumn	<i>Alnus</i> × control	10	
14 days	Autumn	<i>Fagus</i> × adult CMC	10	
14 days	Spring	<i>Fagus</i> × juvenile CMC	10	

14 days	Autumn	<i>Fagus</i> × control	10	(mg/L, nitrate, nitrite, ammonium, orthophosphate, organic carbon, silica) and BOD5 as oxygen (mg/L) consumed over 5 days
14 days	Autumn	<i>Populus</i> × adult CMC	10	
14 days	Spring	<i>Populus</i> × juvenile CMC	10	
14 days	Autumn	<i>Populus</i> × control	10	
14 days	Autumn	<i>Salix</i> × adult CMC	10	
14 days	Spring	<i>Salix</i> × juvenile CMC	10	
14 days	Autumn	<i>Salix</i> × control	10	
14 days	Autumn	<i>Tilia</i> × adult CMC	10	
14 days	Spring	<i>Tilia</i> × juvenile CMC	10	
14 days	Autumn	<i>Tilia</i> × control	10	
<b>PART II Effect excretion</b>				
Period	Season	Treatment	n	Measured parameters
2 days	Autumn	Adult CMC	50	Dissolved nutrient concentrations (mg/L, nitrate, nitrite, ammonium, orthophosphate, organic carbon, silica)
2 days	Spring	Juvenile CMC	50	

#### 4.2.5 Water analyses

Prior to analysis, all water samples were filtered through Chromafil-PET-45/25 filters (Macherey-Nagel, Düren, Germany) with 0.45 µm pore size. The concentrations (mg/L) NO<sub>3</sub>-N, NO<sub>2</sub>-N, NH<sub>4</sub>-N, PO<sub>4</sub>-P and DOC were determined with a San++ Automated Wet Chemistry Analyzer (Skalar Analytical B.V., Breda, The Netherlands) through automatized colorimetry. Concentrations of DSi were measured with an ICP analyser (iCAP 6300, Thermo Fisher Scientific, Cambridge, UK).

To determine the amount of FPOM, a water sample with known volume (varied between 50 and 1000 mL depending on FPOM concentration) was filtered over pre-weighed binder free grade GF/C glass microfiber filters (Whatman, Maidstone, UK). Next, the filters were oven dried at 105°C for 7 days and weighted again. The FPOM concentration (mg/L) was determined from the weight difference of the filters. The FPOM concentration was multiplied by the end volume to derive the total amount of FPOM in each tank.

For the BOD analysis, 300 mL of sampled water (or less, diluted with dilution water), 2 mL of seed solution and 310 µl ATU (2 g/L) were transferred to a Winkler bottle and aerated until saturated with oxygen. Dilution water was prepared by adding phosphate buffer salts (pH 7.2), MgSO<sub>4</sub>, CaCl<sub>2</sub> and FeCl<sub>3</sub> to demineralised water. Seed solution was obtained by mixing the content of one capsule of PolySeed® (InterLab, The

Woodlands, Texas) in 500 mL dilution water. BOD5 was determined, meaning that samples were incubated dark for five days at 20°C. BOD was expressed as the amount of oxygen consumed (mg/L) after five days. Measurements of samples with a decrease in oxygen concentration of less than 20 % or more than 80 % were considered as unreliable and excluded from analysis.

#### **4.2.6 Data analysis**

All statistical analyses were performed in R version 1.3.1093 (R Core Team, 2022) and the significance level was set to  $p < 0.05$ . All measured concentrations of dissolved nutrients were corrected for control values in tap water prior to analyses. Aligned ranks transformation ANOVA (ART ANOVA, Wobbrock et al., 2011) was used to test the potential differences in leaf litter breakdown and associated effects between the treatments and leaf litter types. ART ANOVA (ARTool R package (Wobbrock et al., 2011)) was used as a non-parametric approach for factorial ANOVA because (1) the dependent variables consisted of ratio data, (2) the homogeneity of variances assumption was violated, and (3) the data was non-normally distributed. Leaf breakdown rate, FPOM production, the concentrations of dissolved nutrients and BOD5 were entered as continuous dependent variables. Treatment (control, juvenile crab, adult crab), leaf litter type (tree species) and their interaction were included as categorical fixed independent variables. Post-hoc pairwise comparisons (ARTool R package (Wobbrock et al., 2011)) were performed with Tukey adjustment to determine the differences among treatments, leaf litter types and their interactions. Effect sizes of each factor and their interaction were calculated as partial eta-squared.

To test whether the excretions of crabs have a significant effect on dissolved nutrients, one-sample Wilcoxon signed rank tests (stats R package (R Core Team, 2022)) were used to test if the measured concentrations per parameter and per crab size were significantly different from zero. Pearson correlation tests (or Spearman in case of non-normality) (stats R package (R Core Team, 2022)) were performed to explore the relation between leaf litter nutrient content (carbon to nitrogen ratio, total phosphorus and biogenic silica) prior to the experiment and leaf breakdown rates by the crabs, which were corrected for control breakdown rates.

## 4.3 Results

### 4.3.1 Leaf nutrient content

The total carbon content of the different types of leaf litter was rather uniform. *Salix* and *Alnus* leaf litter contained the most nitrogen, and had accordingly the lowest C:N ratio (**Table 4.2**). Furthermore, they had a similar amount of biogenic silica prior to the experiment. The lowest amounts of nitrogen were measured in *Fagus* and *Populus* leaves, which also had a very similar carbon to nitrogen ratio. The leaf litter of both trees also had higher amounts of biogenic silica compared to the other tree species. The leaf litter of *Tilia* had intermediate values for nitrogen, biogenic silica and the C:N ratio, but contained the highest amount of phosphorus.

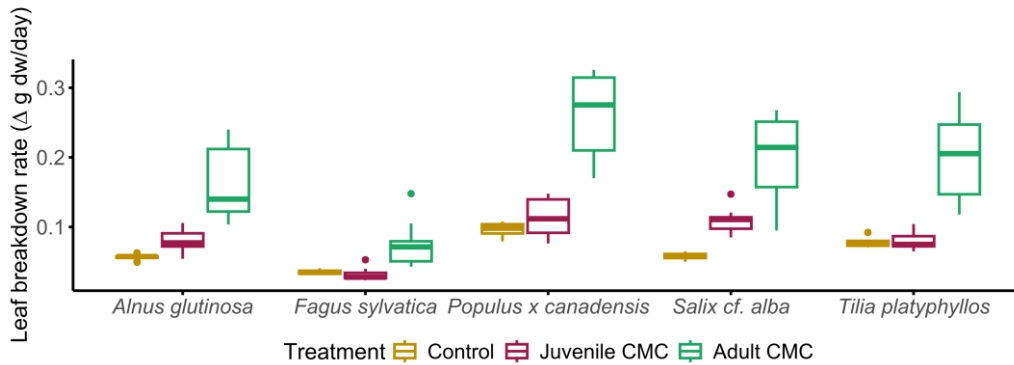
**Table 4.2** Nutrient content in leaf litter. Average ( $\pm$  SD) carbon to nitrogen ratio (C:N) and total carbon (Ctot), nitrogen (Ntot), phosphorus (Ptot), and biogenic silica (BSi) content of leaf litter per tree species prior to the experiment

Tree	C:N	Ctot (mg C/g)	Ntot (mg N/g)	Ptot (mg P/g)	BSi (mg Si/g)
<i>Alnus glutinosa</i>	24.55 $\pm$ 0.59	485.13 $\pm$ 2.06	19.76 $\pm$ 0.56	0.87	0.30
<i>Fagus sylvatica</i>	57.25 $\pm$ 0.42	471.24 $\pm$ 1.69	8.23 $\pm$ 0.09	2.27	9.10
<i>Populus x canadensis</i>	58.10 $\pm$ 0.40	422.96 $\pm$ 0.22	7.28 $\pm$ 0.05	2.94	8.55
<i>Salix cf. alba</i>	22.03 $\pm$ 0.61	448.86 $\pm$ 3.12	20.38 $\pm$ 0.70	1.39	0.57
<i>Tilia platyphyllos</i>	40.56 $\pm$ 1.57	415.42 $\pm$ 0.77	10.25 $\pm$ 0.42	3.97	3.15

### 4.3.2 Leaf breakdown

Leaf breakdown rates differed significantly between treatments and tree species (**Table 4.3**) and the interaction of the two factors had a significant effect as well. Crab presence and size (treatment) had the largest effect on leaf breakdown rate (0.76), followed by leaf litter type (0.58) and the interaction effect (0.34). For all leaf litter types, it is apparent that adult crabs processed higher quantities of leaves compared to juvenile crabs and the control treatment without crabs ( $p < 0.01$ , **Fig. 4.2**). Adult mitten crabs consumed all of the five leaf litter types offered, with average breakdown rates ranging from 0.075 to 0.260 g day<sup>-1</sup>. No significant differences were found between the leaf breakdown rates of juvenile crabs and the control treatment, except for *Alnus* and *Salix* leaves ( $p < 0.01$ ), which indicates that the mass loss of the other leaf types was largely caused by the process of leaching. Average leaf breakdown rates in tanks with juvenile crabs ranged from 0.032 to 0.114 g day<sup>-1</sup>. The greatest leaf breakdown rate was found for *Populus* leaves, irrespective of the presence of crabs. *Salix* and *Tilia* leaves

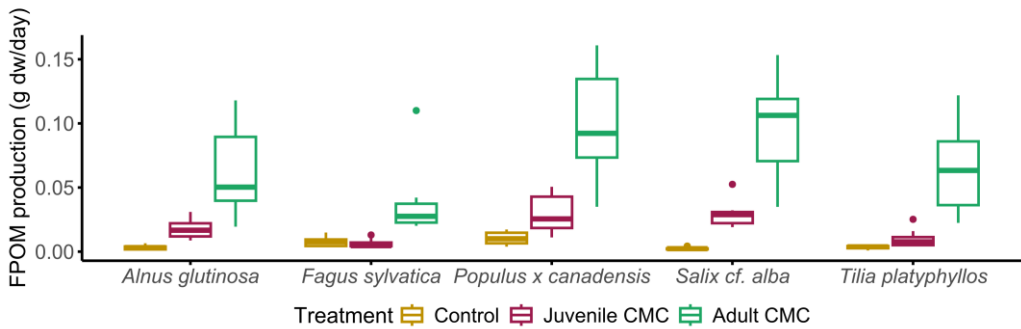
had intermediate breakdown rates and were not significantly different from each other. The leaves of *Alnus* and *Fagus* had the lowest breakdown rates.



**Fig. 4.2** Leaf breakdown rate by Chinese mitten crabs. Leaf breakdown rate is expressed as the amount of dry weight (g) removed per day. Results are visualised per treatment: control (no crab), juvenile and adult Chinese mitten crabs (CMC), for five different tree species (species shown at the bottom). Leaf weight was measured after 14 days and each boxplot represents n=10.

No significant correlations were found between the leaf breakdown rates by the Chinese mitten crab and C:N ratio ( $p = 0.35$ ,  $r = -0.33$ ,  $DF = 8$ ), total phosphorus ( $p = 0.89$ ,  $r = -0.20$ ,  $DF = 8$ ) and biogenic silica ( $p = 0.89$ ,  $\rho = -0.05$ ,  $DF = 8$ ) content of the leaf litter prior to the experiment.

ART ANOVA showed that treatment, leaf litter type and their interaction had a significant effect on the production of FPOM (**Table 4.3**). Similar to leaf breakdown rates, the treatment effect was largest (0.79), followed by the effect of leaf litter type (0.39) and the interaction between the two factors (0.31). FPOM production rates were significantly higher ( $p < 0.01$ ) in the presence of adult crabs compared to juvenile crabs and the control treatment (**Fig. 4.3**). A significant increase ( $p < 0.01$ ) in the production of FPOM was observed for juvenile crabs compared to the control treatment, except for *Fagus* leaves. Average FPOM production rates ranged from 0.037 to 0.101  $\text{g day}^{-1}$  for adult crabs and from 0.006 to 0.029  $\text{g day}^{-1}$  for juvenile crabs. The highest production of FPOM was found in tanks with *Populus* and *Salix* leaves, which did not significantly differ from each other. The production of FPOM in tanks with *Alnus* and *Tilia* leaves were significantly lower ( $p < 0.01$ ) but were not significantly different from each other. The lowest FPOM production was measured in tanks with *Fagus* leaves.



**Fig. 4.3** Fine particulate organic matter (FPOM) production. FPOM expressed as g dry weight per day, and visualised per treatment: control (no crab), juvenile and adult Chinese mitten crabs (CMC) and leaf litter type (tree species shown at the bottom). The amount of FPOM was measured after 14 days and each boxplot represents n=10.

**Table 4.3** Results of two-factor ART ANOVA; effect of treatment (control, juvenile CMC, adult CMC), tree species (Tree) and their interaction (Treatment:Tree) on response variables. Response variables include: leaf breakdown rate (g dry weight lost/day), FPOM production (g dry weight/day), dissolved nitrate, nitrite and ammonium concentration (mg N/L), dissolved total nitrogen concentration (= dissolved nitrate + nitrite + ammonium, mg N/L), dissolved orthophosphate concentration (mg P/L), dissolved silica concentration (mg Si/L), dissolved organic carbon concentration (mg C/L) and biological oxygen demand (BOD5) as oxygen (mg/L) consumed over 5 days. DF = degrees of freedom, SS = sum of squares, partial eta-sq. = partial eta-squared

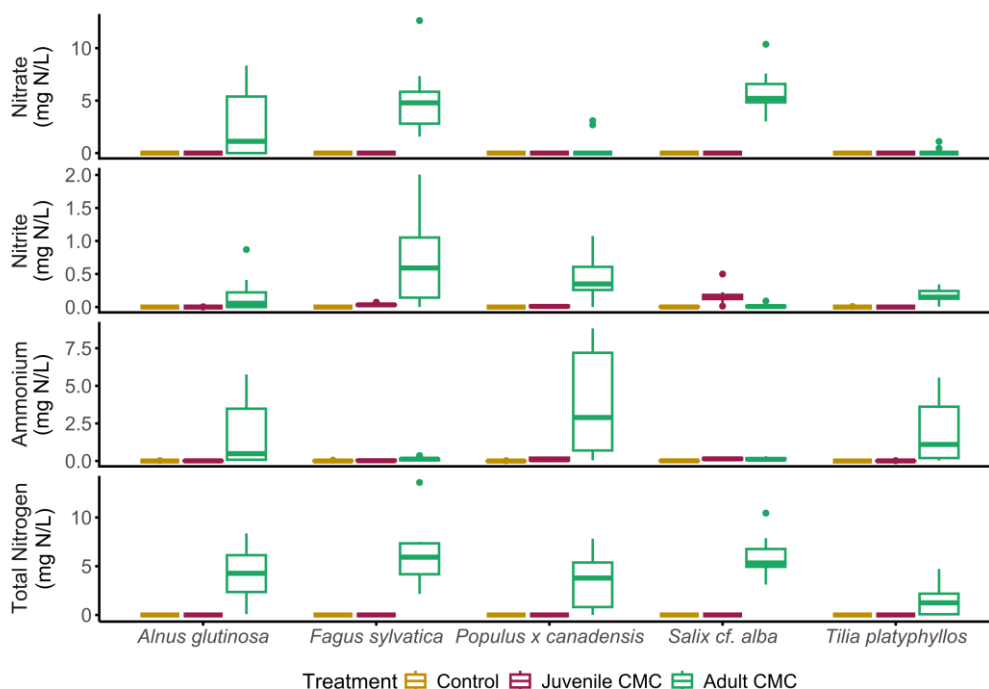
Response variable	Factor	DF	SS	F value	p value	partial eta-sq.
Leaf breakdown	Treatment	2	211650	209.97	<0.001	0.76
	Tree	4	162801	47.44	<0.001	0.58
	Treatment:Tree	8	94587	8.84	<0.001	0.34
FPOM	Treatment	2	215732	249.37	<0.001	0.79
	Tree	4	104898	21.59	<0.001	0.39
	Treatment:Tree	8	83229	7.59	<0.001	0.31
Nitrate (NO <sub>3</sub> -N)	Treatment	2	151268	299.73	<0.001	0.82
	Tree	4	133657	38.01	<0.001	0.53
	Treatment:Tree	8	157369	28.19	<0.001	0.63
Nitrite (NO <sub>2</sub> -N)	Treatment	2	148264	103.77	<0.001	0.61
	Tree	4	84408	18.34	<0.001	0.36
	Treatment:Tree	8	115888	17.90	<0.001	0.52
Ammonium (NH <sub>4</sub> -N)	Treatment	2	71216	28.32	<0.001	0.30
	Tree	4	96064	22.73	<0.001	0.40

Total nitrogen	Treatment:Tree	8	82206	11.19	<0.001	0.40
	Treatment	2	174199	1251.76	<0.001	0.95
	Tree	4	93988	21.12	<0.001	0.39
Orthophosphate (PO <sub>4</sub> -P)	Treatment:Tree	8	100762	12.67	<0.001	0.43
	Treatment	2	7928	2.04	0.134	0.03
	Tree	4	218662	131.35	<0.001	0.80
Dissolved silica (DSi)	Treatment:Tree	8	45186	3.35	0.002	0.17
	Treatment	2	95385	36.00	<0.001	0.35
	Tree	4	242962	258.16	<0.001	0.89
Dissolved organic carbon (DOC)	Treatment:Tree	8	135659	16.90	<0.001	0.50
	Treatment	2	149814	80.23	<0.001	0.54
	Tree	4	209864	108.16	<0.001	0.76
BOD5	Treatment:Tree	8	114748	12.07	<0.001	0.42
	Treatment	2	67217	77.10	<0.001	0.60
	Tree	4	47171	16.02	<0.001	0.38
	Treatment:Tree	8	38312	5.42	<0.001	0.30

### 4.3.3 Dissolved nutrients

Treatment, tree species and their interaction had a significant effect (**Table 4.3**) on the concentration of dissolved nitrate, nitrite, ammonia and total nitrogen (sum of nitrate, nitrite and ammonia). The presence of adult Chinese mitten crabs generally resulted in higher dissolved nitrogen concentrations compared to the treatments with juvenile crabs and without crabs (**Fig. 4.4**). The dissolved nitrate and total nitrogen concentrations did not change significantly in the presence of juvenile crabs compared to the control treatment. Although small, significant increases in nitrite and ammonium concentrations were found for juvenile crabs fed with *Salix*, *Populus* (nitrite and ammonium) and *Fagus* (nitrite) leaves compared to the control treatment. Dissolved nitrogen concentrations differed between tree species, however there was no leaf litter type that had consistently high or low values for all parameters (nitrate, nitrite and ammonium). The concentration of total dissolved nitrogen demonstrated that tanks with leaf litter of *Fagus* and *Salix* contained the highest concentrations, followed by *Alnus* (no significant difference with *Salix*) and *Populus*, and *Tilia*, which contained the lowest concentrations. In the case of dissolved nitrate, nitrite and total nitrogen concentrations respectively, the effect of the treatment was largest (0.82, 0.61 and 0.95), followed by the interaction effect (0.63, 0.52 and 0.43) and the effect of tree species (0.53, 0.36 and 0.39). The variability in dissolved ammonia concentrations

on the other hand was mostly explained by the effect of the tree species and interaction (both 0.40) and only to a smaller extent by the effect of the treatment (0.30).



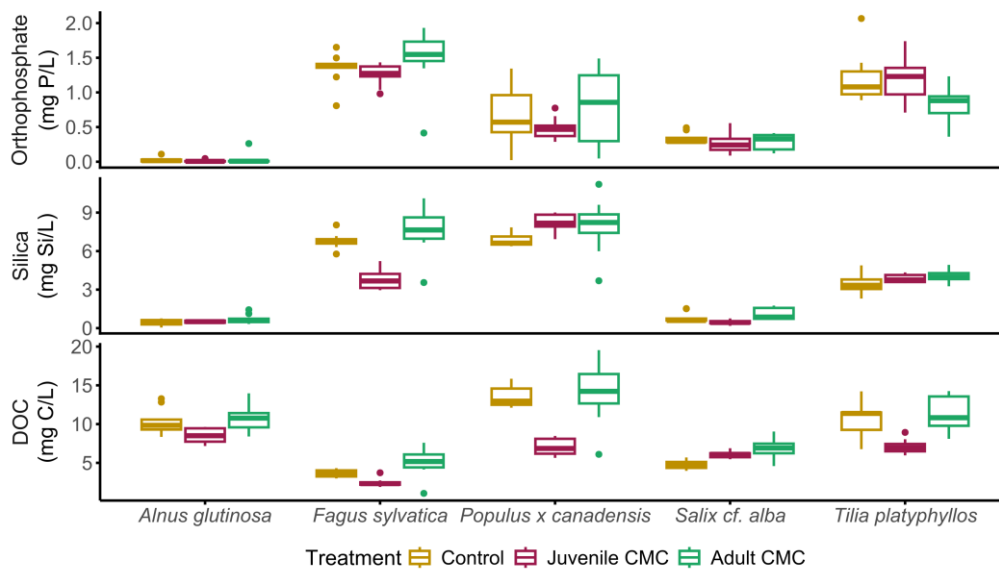
**Fig. 4.4** Concentration of dissolved nitrate, nitrite, ammonium and total nitrogen (mg N/L) measured after 14 days in 5 L water. Results (n=10) are visualised per leaf litter type (tree species shown at bottom) and per treatment: control (no crab), juvenile and adult Chinese mitten crabs (CMC).

Interestingly, the concentrations of dissolved orthophosphate did not differ significantly between treatments ( $p = 0.13$ ) but showed clear differences depending on the origin of the tree leaves (effect size = 0.80) (Table 4.3). The interaction between treatment and tree species was significant as well, although the partial eta-squared showed it was only of little importance (0.17). Significant differences in dissolved orthophosphate were found between all tree species. Highest dissolved orthophosphate values were measured for *Fagus* and *Tilia* leaves, intermediate values for *Populus* and *Salix* leaves and the lowest values for *Alnus* litter (Fig. 4.5).

Although all factors had a significant effect (Table 4.3) on dissolved silica concentrations, the effect of Chinese mitten crabs showed inconsistent patterns (Fig. 4.5). For most leaf litter types, no significant differences were found between the treatments. Exceptions to this were tanks with *Fagus* leaves, where significantly lower

dissolved silica concentrations were found in the treatment with juvenile crabs compared to the control and adult crab treatments. Dissolved silica concentrations also differed significantly between juvenile and adult crabs fed with *Salix* leaves. Effect sizes demonstrated that the strongest effect is exerted by leaf litter type (0.89). Low dissolved silica concentrations were detected for *Alnus* and *Salix*.

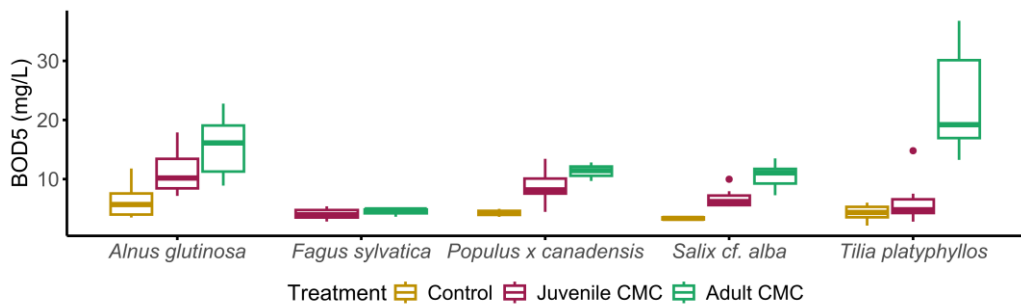
Treatment, leaf litter type and their interaction had a significant effect on dissolved organic carbon concentrations, with the largest effect (0.76) attributed to leaf litter type (**Table 4.3**). Interestingly, there was no significant difference between DOC values of the control treatment and the treatment with adult crabs, except for *Salix* leaves. For a few leaf litter types, the DOC concentrations were actually lower in the treatment with juvenile crabs compared to the control treatment and/or the treatment with adult crabs, which is somewhat counterintuitive (**Fig. 4.5**). This result was significant for *Populus* and *Tilia* leaves when comparing juvenile crab and control treatment, and for *Fagus*, *Populus* and *Tilia* leaves when comparing the treatments with juvenile and adult crabs.



**Fig. 4.5** Concentration of dissolved orthophosphate (mg P/L), silica (mg Si/L) and organic carbon (DOC, mg C/L) measured after 14 days in 5 L water. Results (n=10) are visualised per leaf litter type (tree species shown at bottom) and per treatment: control (no crab), juvenile and adult Chinese mitten crabs (CMC).

#### 4.3.4 BOD5

Oxygen consumption values (mg/L) for the control treatment of *Fagus* were below the cut-off value of 20 %, therefore these BOD5 values were not included in the analyses. Treatment, tree species and the interaction between the two had a significant effect on BOD5 values (**Table 4.3**). For all tree species, BOD5 values increased significantly with the presence of both juvenile and adult mitten crabs compared to the control treatment (**Fig. 4.6**), except for *Tilia* leaves, for which the BOD5 values were not significantly different between juvenile crab and control treatment. BOD5 values were not significantly different between the treatments with juvenile and adult crabs, except for the tanks with *Salix* and *Tilia* leaves. Partial eta-squared showed that the effect size of the treatment was largest (0.60), followed by tree species (0.38) and the interaction effect (0.30).



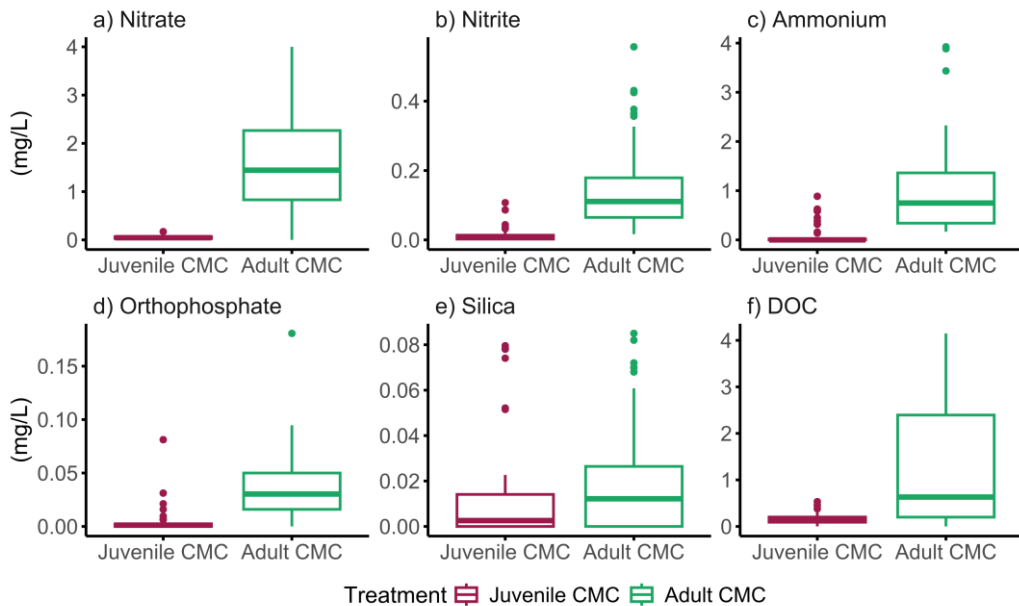
**Fig. 4.6** BOD5 (oxygen consumed in five days, in mg/L) per treatment: control (no crab), juvenile and adult Chinese mitten crabs (CMC) for five different tree species (species shown at the bottom)

#### 4.3.5 Excretion

Based on one-sample Wilcoxon tests (**Table 4.4**), the excretions of Chinese mitten crabs led to significantly higher concentrations of almost all measured parameters compared to control values in tap water. There were two exceptions; dissolved ammonium concentrations in the tanks with excretions of juvenile crabs were not significantly higher than controls ( $p = 0.59$ ) and adult mitten crabs did not have a significant effect on dissolved silica concentrations ( $p = 0.07$ ). In general the measured concentrations in tanks with juvenile crabs were close to zero, while adult crabs had a more pronounced effect (**Fig. 4.7**).

**Table 4.4** Results of one-sample Wilcoxon signed-rank tests for juvenile and adult Chinese mitten crabs (CMC) separately. Response variables include: dissolved nitrate, nitrite and ammonium concentration (mg N/L), dissolved orthophosphate concentration (mg P/L), dissolved silica concentration (mg Si/L) and dissolved organic carbon concentration (mg C/L)

	Nitrate (NO <sub>3</sub> -N)	Nitrite (NO <sub>2</sub> -N)	Ammonium (NH <sub>4</sub> -N)	Orthophosphate (PO <sub>4</sub> -P)	Silica (DSi)	DOC
Juvenile CMC	< 0.001	< 0.001	0.591	< 0.001	0.035	< 0.001
Adult CMC	< 0.001	< 0.001	< 0.001	< 0.001	0.076	< 0.001



**Fig. 4.7** Concentration of dissolved nutrients in crab excretions. Dissolved nitrate (mg N/L), nitrite (mg N/L), ammonium (mg N/L), orthophosphate (mg P/L), silica (mg Si/L) and organic carbon (DOC, mg C/L) originating from crab excretions, measured after two days in 2.5 L water in absence of leaf litter. Data were corrected for background values of tap water and are visualised per crab size: juvenile and adult Chinese mitten crabs (CMC).

#### 4.4 Discussion

We have shown that Chinese mitten crabs can act as effective detritivores, feeding on different types of terrestrial leaf litter at a high rate whilst accelerating the breakdown of coarse leaf litter into smaller fragments. This underscores their importance in the

decomposer community within freshwater ecosystems, and corresponds with what has been observed in other decapod species (Coughlan et al., 2010; Crowl et al., 2006; Duarte et al., 2012; Mancinelli et al., 2013). Additionally, adult mitten crabs increase the concentration of dissolved nitrogen (nitrate, nitrite and ammonium) (Doherty-Bone et al., 2018b). While mitten crabs can contribute to the accessibility of energy and nutrients in the aquatic system, increased nitrogen concentrations and biological oxygen demand could have a negative impact on water quality. Conversely, the effects on dissolved orthophosphate, silica and organic carbon were absent or inconclusive based on the present results. Effect sizes indicate that the variability in these parameters is more closely linked to leaf litter type.

As expected, body size of the crab and the leaf litter type strongly influence the effect of Chinese mitten crabs on leaf litter breakdown rate. For decapods in general, nutritional quality (C:N) of leaf litter and not decapod biomass has been marked as prime factor affecting leaf breakdown rates (Mancinelli et al., 2013). However, here we show that within the species, the presence and body size of the crab exerts a stronger influence on leaf litter breakdown rates than leaf litter identity. For three out of the five tested leaf litter types, juvenile crabs had a minor effect on the mass loss of coarse detritus, compared to the process of leaching. Adult mitten crabs, on the other hand, increased the breakdown rate of leaf litter significantly for all tested leaf litter types and showed a lot of individual variation. In the field, the population of mitten crabs is in number generally dominated by juvenile crabs while large, adult crabs only make up a smaller share (Schoelynck et al., 2021). However, some studies report a prevalence of adult crabs (Wójcik-Fudalewska and Normant-Saremba, 2016) in certain habitats and have emphasized the variation in distribution of different size classes (Rudnick et al., 2003). This implies that the impact in the field could be very variable according to the local population structure and density.

Juvenile Chinese mitten crabs significantly increased the breakdown of leaf litter with high nutritional quality (i.e., low C:N, *Alnus* and *Salix*), while no effect was observed when they were forced to feed on leaf litter with low nutritional quality. This is a common pattern among shredders, they are capable to discriminate among leaf litter types and preferably feed on litter with high nutritional quality (Graça, 2001; Motomori et al., 2001; Quadros et al., 2014; Rincón and Martínez, 2006; Santonja et al., 2018). However, this pattern was not observed for adult crabs and overall the nutritional composition of leaves, i.e. the carbon to nitrogen ratio and total phosphorus content, was not correlated with the breakdown rates by the crabs. This could be the result of several factors. First of all, while *Populus*, *Tilia* and *Fagus* leaves all had low nutritional

quality in this study, leaf breakdown rates by crabs were substantially different. This could partially be explained by species-specific trends in decomposing rates of the leaf litter types used (Cornelissen, 1996; Pérez-Harguindeguy et al., 2000). *Populus* and *Tilia* leaves had high breakdown rates in the absence of crabs as well, indicating that these are fast-decomposing tree species, while the opposite was true for *Fagus*, which is known to be a more recalcitrant species (Rouifed et al., 2010). Secondly, physical toughness of leaves does not only affect their decomposition rates, it also affects the palatability of leaf litter by shredders (Cornelissen, 1996; Graça, 2001). Factors that enhance leaf toughness and the presence of physical or chemical defences like tannins and lignins (Hladyz et al., 2009; Schindler and Gessner, 2009) could affect feeding rates and preferences of Chinese mitten crabs. For instance, *Fagus* leaf litter is known to have high amounts of cellulose and lignins (Hladyz et al., 2009; Jacob et al., 2010) and was barely consumed by both juvenile and adult Chinese mitten crabs in the present study. Conversely, high concentrations of biogenic silica are suggested to slow down processing rates by shredders (Schaller and Struyf, 2013), as it has been shown to wear down mandibles of herbivorous insects and reduce efficiency of food uptake (Massey and Hartley, 2009), but no such effect was observed in the present study. Given the high leaf breakdown rates by adult Chinese mitten crabs for *Populus* and *Tilia*, it appears that palatability was not a limiting factor, and crabs potentially increased their feeding rates to compensate for the low nutritional quality of the leaf litter (Flores et al., 2014).

Similar to other shredders, both juvenile and adult Chinese mitten crabs significantly increase the amount of suspended fine particulate organic matter (Fernandes et al., 2015; Fincham et al., 2023; Patrick, 2013; Santonja et al., 2018). This is the combined result of enhanced fragmentation of the coarse leaf litter and the production of faecal pellets (Halvorson et al., 2015). Although juvenile crabs showed only a small effect on mass loss of leaf litter, they do contribute to the fragmentation and thus the overall breakdown of coarse leaf litter. The conversion to FPOM is a crucial ecosystem function as it makes nutrients in leaf litter accessible to various organisms, e.g. those with a collector or filter feeding strategy, and it promotes the overall breakdown of detritus for example by increasing the surface area that microbes can interact with (Jonsson and Malmqvist, 2005; Joyce et al., 2007; Marks, 2019). Increased bacterial breakdown however, can lead to heightened oxygen consumption within the water column, which was demonstrated by a significant increase of oxygen needed by microorganisms to break down the biodegradable organic substances in the experiment (BOD<sub>5</sub>).

Next to mediation of nutrient cycling through enhanced fragmentation and faecal pellet production, excretion can also be an important pathway (Marks, 2019; Villanueva et al., 2012; Zandonà et al., 2021). Macroinvertebrates are inefficient feeders, so while they do process large quantities of leaf litter, actual assimilation of nutrients, and especially nitrogen, into their tissue is very limited (Siders et al., 2021, 2018). We have shown that adult Chinese mitten crabs enhance nutrient availability, by increasing the concentration of dissolved nitrate, nitrite and ammonium (and thus total nitrogen) through the fragmentation and feeding on leaf litter. However, this increase is primarily attributed to the excretions of the crabs, rather than being the result of shredding itself and subsequent increased leaching. Juvenile Chinese mitten crabs feeding on leaf litter resulted in dissolved nitrogen concentrations equal to or smaller than the control treatment, suggesting that the effect was not measurable or that any nitrogen removal processes surpassed production rates. Although variation in dissolved nitrogen concentrations were observed between leaf litter diets, no clear pattern emerged in relation to initial nitrogen content of the leaf types nor to consumption rates of the crabs. This aligns with earlier studies that demonstrated that excretion of nitrogen did not reflect diet or trophic position of the organism (McManamay et al., 2011; Zandonà et al., 2021). Other factors such as body stoichiometry (McManamay et al., 2011; Musin et al., 2023), body mass (Alves et al., 2010; Musin et al., 2023) or assimilation efficiency and metabolic requirements (Zandonà et al., 2021) are potentially more important.

Although the excretions of Chinese mitten crabs increase the amount of dissolved orthophosphate and silica, the overall concentrations were not affected when crabs were feeding on leaf litter. This is, in the case of dissolved orthophosphate, in contrast to an earlier and similar study by Doherty-Bone et al. (2018b). For both parameters, strong differences were found between the different leaf litter types, and as evidenced by the effect sizes, the concentrations were strongly related to leaf litter type. The nutrient content analyses prior to the experiment revealed that there is a good association between dissolved orthophosphate and silica concentrations after 14 days and total phosphorus and biogenic silica content of the leaves.

The variation in the concentration of DOC was largely caused by differences between leaf litter types and to a lesser extent by the presence of the Chinese mitten crab. Generally, leaf litter consumption by Chinese mitten crabs did not lead to higher levels of DOC, which is opposite to other macroinvertebrate studies that showed an increase in DOC following leaf litter breakdown both in laboratory conditions (Doherty-Bone et al., 2018b; Meyer and O'Hop, 1983) and in the field (Crowl et al., 2001). Interestingly,

DOC concentrations were in general lower in treatments with juvenile Chinese mitten crabs compared to the other treatments. While our current research does not offer an explicit explanation for this observation, it may be associated to differences in feeding behaviour and characteristics of the faecal pellet production between the different size classes of crabs. Recent research demonstrated that juvenile Chinese mitten crabs are able to feed through filtration (Zeng et al., 2023), which could result in uptake of DOC (Marks, 2019).

The results were obtained in a controlled, small-scale experimental environment and offer insight in the fundamental process of detritivory by the Chinese mitten crab. It is evident that the ecological system was simplified since no other trophic levels were present (e.g. phytoplankton, macrophytes, other macroinvertebrates, fish) and crabs were fed with detritus only, while in the field they also consume other food resources. However, the experiment allows to infer the average *per capita* effects for both the juvenile and adult Chinese mitten crabs, which are necessary to predict their local effect (Parker et al., 1999). While acknowledging these experimental constraints, the results allow the estimation of the upper limit of (1) leaf breakdown and FPOM production rates by Chinese mitten crab and (2) the nitrogen input by the crabs into aquatic systems where alternative food sources are scarce.

As leaf litter processing is a key ecosystem function, alteration or acceleration of leaf litter breakdown rates due to the introduction of mitten crabs carries in theory diverse implications for local aquatic ecosystems (Fincham et al., 2023; Pile et al., 2023; Santonja et al., 2018). First of all, it affects the food web, as it influences the composition, abundance and availability of food sources (course leaf litter and FPOM) for other organisms (Firmino et al., 2022; Jackson et al., 2016; MacNeil et al., 2011; Patrick, 2013). This can affect the decomposer community (bacteria, fungi and other animals) and their interactions within the system. Secondly, accelerated leaf litter breakdown and excretions of the crabs cause the more rapid release of nutrients into the water. This increases nutrient availability for plants and animals, which can increase the productivity of the ecosystem but can also cause negative effects such as algal blooms or a decrease in oxygen (Pile et al., 2023; Rabalais, 2002; Villanueva et al., 2012).

In practice, it is expected that the effects of detritivory by the Chinese mitten crab and associated effects on water quality will largely be buffered by the system, as has been shown in mesocosm experiments with increased ecological complexity (Doherty-Bone et al., 2019; Rosewarne et al., 2016). Although neither study reported increased leaf litter breakdown or FPOM with the presence of Chinese mitten crabs, Doherty-Bone et

al. (2019) did show an increase in dissolved nitrate. Limited observed effects in experiments or in the field could be explained in part by the presence of alternative food sources, algal and microbial production taking up nutrients (Doherty-Bone et al., 2018b), and the replacement of functional redundant native species (Moore et al., 2012) by the invasive species through competition and predation. Field data to support or contradict these findings from laboratory studies are lacking for the Chinese mitten crab. However, for other omnivorous decapods it has been shown that their effects on leaf litter decomposition in the invaded area can vary between no measurable effect (Moore et al., 2012) to highly increased decomposition rates (Jackson et al., 2016; Usio, 2000) depending on the local situation. Therefore, we argue that the Chinese mitten crab has the potential to enhance terrestrial leaf litter breakdown and affect water quality in aquatic systems, but the effect size is expected to be controlled by local conditions such as the presence of other food sources and the diversity and density of other macroinvertebrates.

Obtaining field data is imperative to move forward our understanding of the effects of this invasive omnivorous crab on leaf litter breakdown and nutrient cycling in aquatic systems. Field research investigating leaf breakdown rates, dissolved nutrients, oxygen concentrations, FPOM, and aquatic community composition and abundance, would give insight in real life effects of the Chinese mitten crab in its natural environment.

#### **4.5 Acknowledgements**

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# 5

## **Burrow dynamics of the Chinese mitten crab in freshwater tidal marshes: morphometrics, environmental drivers, and implications for riverbank stability**

*Heleen Keirsebelik, Elise Bultot, Ignace Pelckmans, Ken Schoutens and Jonas Schoelynck*

## Abstract

Riverbank erosion caused by the burrowing activity of the invasive Chinese mitten crab (*Eriocheir sinensis*) is often considered a major impact on the ecosystem, yet its burrowing behaviour remains poorly understood. In this study, we combined a controlled laboratory experiment with a year-long field study in a natural and a restored tidal freshwater marsh to investigate morphometric relationships, temporal dynamics, and abiotic drivers of burrow size and distribution, and how they are associated with sediment dynamics. In experimental conditions, crabs with a carapace width between 1.5 and 3.5 cm rapidly constructed burrows. We found that larger crabs constructed wider, longer, and more voluminous burrows. Field observations in the restored marsh showed that burrow density was high from late spring to autumn, following an influx of juvenile crabs in spring, which coincided with a decrease in average burrow width. In contrast, burrow density was lower in the natural marsh and showed no temporal variation. Burrow densities were highest near the inflow of the marshes and in steep riverbanks, while larger burrow openings were associated with steep slopes and sediment with a small median grain size. Our findings suggest that the largest burrow openings are found in stable areas with little sediment turnover. Interestingly, in the restored marsh, we observed that higher burrow densities in spring coincided with increased erosion rates measured over a one-year period. This correlation may reflect both a preference to burrow in environments that are more prone to erosion and a direct impact of crab burrowing itself. Overall, our findings highlight that the geomorphic impact of burrowing by mitten crabs is seasonal and site-specific. The results of this exploratory study provide a valuable baseline for future manipulative experiments aimed at untangling the mechanistic links between mitten crab burrows and erosion.

## 5.1 Introduction

Non-native invasive species are among the leading causes of biodiversity loss and ecosystem disruption worldwide (IPBES, 2023; Isbell et al., 2023; Reid et al., 2019). One mechanism through which invasive animals can alter their environment is by creating burrows, a behaviour that can significantly impact geomorphic and ecological processes, particularly along aquatic margins (Faller et al., 2016; Harvey et al., 2019, 2014; Haubrock et al., 2019). For example, the burrowing behaviour of nutria (*Myocastor coypus*), has been linked to bank collapse (Sofia et al., 2017), while invasive crayfish have been implicated in accelerating riverbank retreat (Sanders et al., 2021). Despite evidence that it could have a profound effect on the ecosystem, this impact is largely unquantified (Harvey et al., 2019). One burrowing invasive species of global concern is the Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards 1853) (Harvey et al., 2019). Originally, these crabs only inhabited the coastal regions surrounding the Yellow Sea, but nowadays the species' range covers Europe and parts of North America as well (Dittel and Epifanio, 2009; Ewers et al., 2023). The species is known to burrow both in its native and non-native range (Hymanson et al., 1999).

Shortly after their introduction in Europe, their presence in various countries became apparent by large numbers of crabs clogging fishing gear, but also by numerous burrows in riverbanks of tidally influenced waterbodies (Kamps, 1937; Peters and Panning, 1933). Early studies documented densities up to 68 burrows m<sup>-2</sup> in the Netherlands and 30 burrows m<sup>-2</sup> in Germany (Kamps, 1937; Peters and Panning, 1933). More recently, average burrow densities ranging between 14 and 20 burrows m<sup>-2</sup> were observed in San Francisco Bay, USA (Rudnick et al., 2005a). It has been hypothesised that the extensive burrowing activity of mitten crabs contributes to bank instability and increased erosion. Sediment excavation undermines the structural integrity of banks, which increases the likelihood of slumping (Davidson and De Rivera, 2010). Additionally, burrows can alter hydrological conditions by changing pore water pressure and creating pathways for seepage, reducing soil cohesion (Bendon et al., 2024; Onda and Itakura, 1997; Xiao et al., 2019). Burrow openings and the resulting microtopographic changes may alter surface flow and sediment erodibility, potentially accelerating lateral erosion (Farron et al., 2020; Harvey et al., 2019). In severe cases, these combined effects could ultimately lead to bank collapse. In Germany, high burrow densities resulted in the degradation of riverbanks in multiple locations (Panning, 1938). Rudnick et al. (2005a) established that mitten crabs can excavate 1 to 6 % of sediment per 0.5 m<sup>3</sup> and noted riverbanks sinking in. However, beyond these observations, there are no studies on the link between mitten crab burrows and

erosion and sedimentation rates. Not only could man-made infrastructure like levees be affected by burrowing activity, but it may also influence erosion rates of natural creeks and rivers. Although this impact is assumed to be significant and is repeatedly suggested in scientific literature, only a handful of studies have investigated these burrows.

While the impact is poorly understood, the morphology has been characterised in detail. Unlike most other crab species, mitten crabs burrow horizontally into riverbanks. Both the opening and the tunnel itself are oval-shaped, matching the body shape of the crabs. The crabs burrow from a few cm to 80 cm deep, but generally their burrows reach a length of less than 50 cm (Kamps, 1937; Rudnick et al., 2005a). The burrows are created at a down-sloping angle of 10 to 20 degrees, and because of this, the terminal chamber(s) often retain water during low water conditions (Panning, 1938; Rudnick et al., 2005a). Burrows can be simple, consisting of one opening and tunnel, or can be highly complex through a combination of horizontally curved tunnels, bifurcations, and interconnections (Rudnick et al., 2005a). One entrance can lead to multiple tunnels, and a single tunnel may open at several points along the bank (Rudnick et al., 2005a). In a tidal marsh, burrows were interconnected over 0.3 m<sup>2</sup> or a length of 0.7 m of riverbank (Keirsebelik et al., 2024).

The morphometric relationships between crab size and burrow dimensions remain largely unquantified, particularly with respect to burrow length and volume. It has been estimated that the width of a freshly excavated burrow is 1.2 to 1.8 times wider than the carapace width of its creator (Kamps, 1937). However, over time, burrows get inhabited by other individuals, which are generally substantially smaller than the dimensions of the entrance (Rudnick et al., 2005a). The height of the burrow ranges between 1 and 8 cm, and the width varies between 2 and 14 cm (Kamps, 1937). The crabs inhabiting them have a carapace width of 0.4 to 4.5 cm, but typically, juvenile crabs with a carapace width of about 2 cm are most common (Kamps, 1937; Rudnick et al., 2005a, 2000).

Mitten crabs create burrows to avoid predation and desiccation, and to seek shelter while moulting (Hymanson et al., 1999; Rudnick et al., 2005a). The latter was evidenced by multiple sightings of moulting crabs and the presence of empty moults during burrow excavations (Kamps, 1937). Burrow occupancy rates vary widely between sites and are likely influenced by seasonal dynamics (Kamps, 1937; Rudnick et al., 2005a, 2000). A single burrow can contain more than one crab, although the number of occupants is limited by the number of terminal chambers (Rudnick et al., 2005a). Larger crabs are thought to use these shelters for only a short period, while

juvenile crabs occupy burrows more regularly (Kamps, 1937; Rudnick et al., 2005a). In addition to temporal fluctuations in occupancy, at least one study reported seasonal variation in burrow density, with lower densities observed during winter (Rudnick et al., 2005a).

Next to temporal variability, there is spatial variability in burrow density and size. Environmental variables are important drivers of crab burrow density (Chen et al., 2022; Li et al., 2018), but for mitten crabs, the nature of these relationships is largely unresolved. Mitten crabs generally burrow within the tidal portions of rivers, with only a few reports of burrows in channels or rivers without tidal influence (Kamps, 1937; Panning, 1938). During low water, the burrows become visible, as they are primarily located between the low and high water lines (Peters and Panning, 1933; Rudnick et al., 2005a). The burrows are mainly found in steep, vertical riverbanks. Chinese mitten crabs seem to avoid burrowing in gently sloping riverbanks and places with generally high sedimentation rates (Hymanson et al., 1999; Kamps, 1937; Rudnick et al., 2005a). Chinese mitten crabs create their burrows in cohesive sediment dominated by silt and with only moderate or low levels of coarser sediment like sand and gravel, but no relation between burrow density and sediment composition has been established (Panning, 1938; Rudnick et al., 2005a). So far, no influence of vegetation cover on burrow density has been found (Rudnick et al., 2005a). Some authors suggest that the crabs prefer bare banks, while others report a preference for burrowing among the roots of riverbank vegetation, such as reeds (Hymanson et al., 1999; Kamps, 1937; Peters and Panning, 1933). The spatial distribution of burrows across environmental gradients may strongly influence their geomorphic impact.

The present study investigates the burrowing behaviour of the invasive Chinese mitten crab in freshwater tidal marshes in Flanders, Belgium. Through both a controlled laboratory experiment and a year-long field study, we explore following research questions:

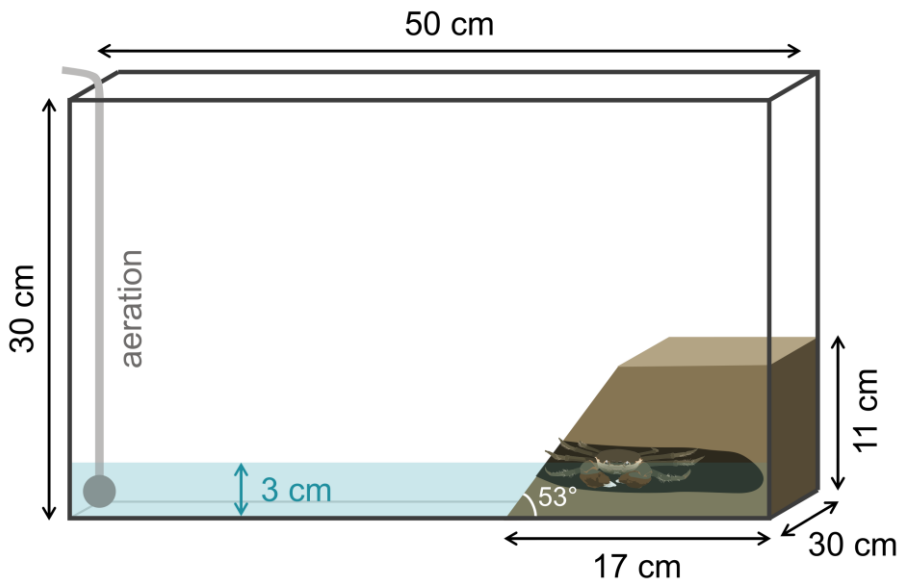
- (1) What are the morphometric relationships between Chinese mitten crab size and the dimensions of freshly excavated burrows?
- (2) How do burrow density and size vary across environmental gradients?
- (3) Is there temporal variation in burrow density and size?
- (4) Do burrow density and size reflect Chinese mitten crab abundance and size?
- (5) Are burrow characteristics associated with patterns of erosion or sedimentation in riverbanks?

## 5.2 Methods

### 5.2.1 Burrowing behaviour and morphometric relationships under experimental conditions

To address the first research question, an experimental setup was used to investigate the burrowing behaviour of mitten crabs under controlled laboratory conditions. The study consisted of three experimental runs, each involving six glass aquaria with a single crab ( $n = 18$ ). Each aquarium ( $50 \times 30 \times 30$  cm) featured an artificial bank that covered the full width of the tank ( $17 \times 30 \times 11$  cm) with an 8 cm slope at an angle of  $53^\circ$  (Fig. 5.1). The sediment used to construct the banks was collected from a historical tidal marsh and had a median grain size of  $26 \mu\text{m}$ , comprising 5 % clay, 70 % silt, and 25 % sand, with a bulk dry density of  $1.36 \text{ g cm}^{-3}$ .

The aquaria were filled with tap water to a height of 3 cm, simulating low-water conditions. The water temperature was maintained at  $18^\circ\text{C}$ . The water was aerated throughout the experiment using an air pump. A diurnal light cycle was implemented, providing 10 hours of light per day. Chinese mitten crabs for the study were collected from the tidal marsh Lippenbroek (Fig. 5.2b) no more than three days before the experiment. The size (carapace width) of each crab was measured using a digital calliper (0.01 mm accuracy), and ranged between 15.77 and 35.16 mm.



**Fig. 5.1** Schematic overview of the experimental set-up. Dimensions of the aquarium and artificial bank are indicated on the sides. The burrow and crab are included for illustrative purposes and are not drawn to scale.

A single crab was introduced into each aquarium and observed for an initial 15 minutes, during which their behaviour was noted. After three days, the crabs were retrieved, and the water was drained from the aquaria. The width of each burrow opening was measured using a feeler gauge (0.5 cm accuracy). Burrows were filled with polyurethane (PU) foam, which was allowed to dry before the casts were excavated. The full length of each burrow tunnel was measured with a measuring tape, and its volume was estimated by creating 3D models using a standard Structure-from-Motion (SfM) photogrammetry workflow in Agisoft Metashape Professional (version 2.0; Agisoft, St. Petersburg, Russia).

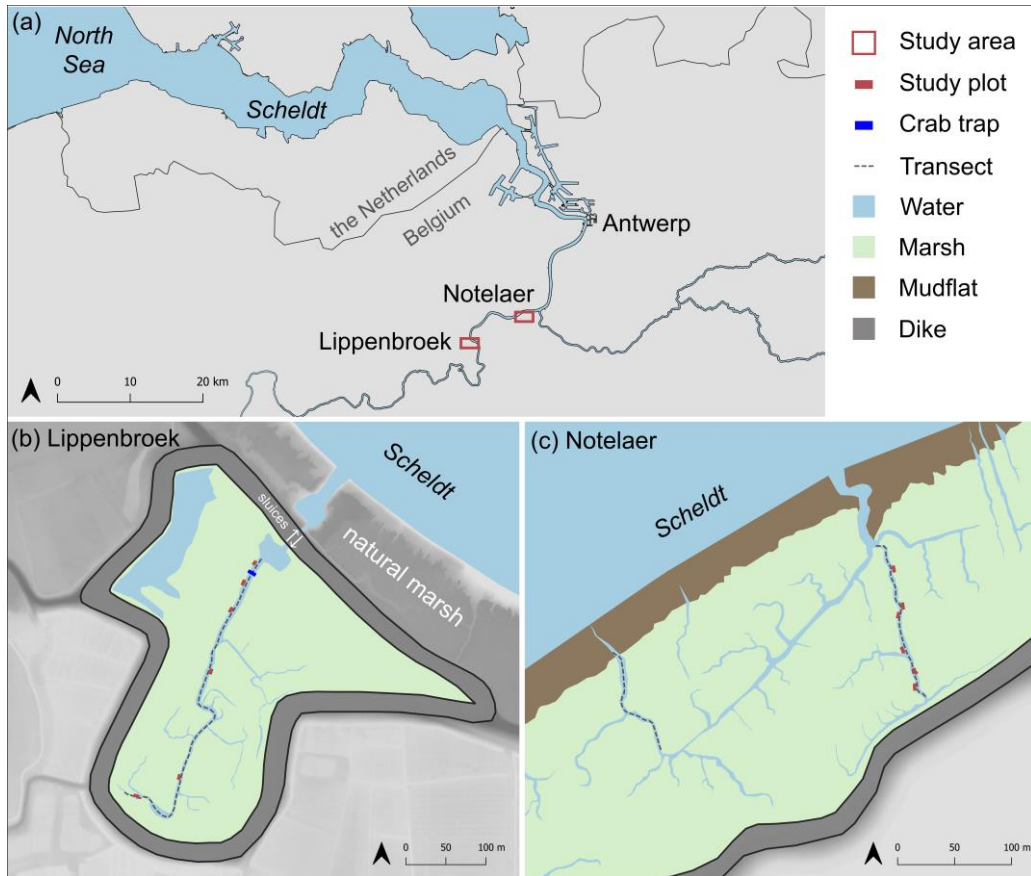
After each experimental run, the artificial banks were removed, reconstructed, and prepared for the subsequent experiment. Each crab was only used once and thereafter euthanized according to standard ethical procedures.

### 5.2.2 Study area

The fieldwork for this study was conducted in two tidal marshes along the freshwater section of the Scheldt Estuary in Belgium (**Fig. 5.2a**): Lippenbroek (51°05'11.2" N, 4°10'21.5" E), a restored tidal marsh, and Notelaer (51°07'08.3" N, 4°16'24.4" E), a natural tidal marsh.

Lippenbroek (**Fig. 5.2b**) is a former embanked agricultural polder of 10 ha that was restored to tidal marsh in 2006. The area is surrounded by a ring dyke and experiences a reduced semidiurnal tidal regime that is controlled through a separate inlet and outlet sluice (Cox et al., 2006). Through tidal exchange, a new sediment layer ( $\pm 50$  cm thick) has been deposited on top of the old soil, which had become highly compacted due to agricultural practices (Van Putte et al., 2020). The old soil layer has a bulk dry density of  $1.10 \text{ g cm}^{-3}$ , while the upper layer averages around  $0.54 \text{ g cm}^{-3}$  (Van Putte et al., 2022, 2020). The burrow surveys were performed in the main creek of the area, a former agricultural ditch that intersects both soil layers (Keirsebelik et al., 2024; Maris et al., 2020). The marsh vegetation is dominated by reed (*Phragmites australis*), interspersed with willow trees (*Salix* sp.) and wetland plants like water mint (*Mentha aquatica*), bulrush (*Typha latifolia*), and great manna grass (*Glyceria maxima*) (Maris et al., 2020).

Notelaer is a mature, natural marsh of 27 ha, bordered by an embankment (**Fig. 5.2c**) (Temmerman et al., 2003). The marsh experiences a semidiurnal tidal cycle and consists of tidally deposited sediment with a large silt fraction. Willow trees are found on high elevations, while dense reed vegetation generally dominates lower elevations (Temmerman et al., 2003).



**Fig. 5.2** Overview of the two study locations along the Scheldt Estuary (a). Panel (b) shows Lippenbroek, a restored tidal marsh enclosed by a dike; panel (c) shows Notelaer, a natural tidal marsh. Permanent study plots are indicated in red, while the dashed lines represent the study transect(s) in each area.

### 5.2.2.1 Crab data

A crab trap was installed in Lippenbroek, based on the design described by Schoelynck et al. (2021), to examine population dynamics of Chinese mitten crabs in the area (Chapter 2). From this dataset, one year of data (December 2021 – 2022) was used to assess whether temporal variation in burrow density and size in Lippenbroek reflected patterns in crab abundance and size (research question 4). There were no data available on crab abundance and size for the natural tidal marsh, Notelaer.

## 5.2.3 Spatial patterns in burrow characteristics

### 5.2.3.1 Burrow measurements and environmental characteristics

To explore spatial patterns of burrowing activity (research question 2), a comprehensive field survey was carried out in autumn in September 2023, within a one-week period (**Table 5.1**).

During low water, 1 m × 1 m vertical plots were established every 10 meters along transects in the middle of the banks on both sides of the main creeks in each tidal marsh (**Fig. 5.2b,c**; ‘Transect’). This resulted in 66 plots in Lippenbroek and 52 plots in Notelaer. All visible burrows within a plot were counted to determine burrow density. The height and width of every burrow were measured to the nearest 0.5 cm using a custom-made feeler gauge. Burrow surface area (cm<sup>2</sup>) was determined by calculating the elliptic surface area based on the height and width measurements.

At every plot, different environmental variables were recorded (**Table 5.1**). Bank angle was measured using a mobile phone application (Measure app, iPhone). A straight pole was laid across the study plot in alignment with the riverbank slope, providing a stable surface on which the phone was placed to obtain the general bank angle. The width of the creek (m) was determined by measuring the distance between the two opposing riverbanks at their highest point using a measuring tape. After all burrow measurements were completed, an undisturbed soil sample (100 cm<sup>3</sup>) was collected inside the plot using a Kopecky ring, which was used to determine sediment characteristics. The distance of each plot to the main river Scheldt (m) was determined in QGIS based on its geographical coordinates.

### 5.2.3.2 Sediment analyses

First, the grain size distribution of each sample was analysed. After homogenizing the sediment sample, a subsample of 0.7 to 1.0 g was mixed with 10 mL HCl and 15 mL H<sub>2</sub>O<sub>2</sub> to break down organic matter and iron complexes overnight. The next day, 25 mL H<sub>2</sub>O<sub>2</sub> was added and brought to a boil until the reaction was completed. The mixture was poured over a sieve (Ø 1 mm) to remove any remaining large particles like roots or rocks. Finally, the grain size distribution of the mixture was analysed using laser diffraction (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). Based on the grain size distribution, median grain size (d<sub>50</sub>; µm) and percentage of clay (< 2 µm), silt (< 63 µm) and sand (≤ 2000 µm) were determined.

Next, bulk dry density (BDD) and organic matter content (OM) of each sample were analysed. The remaining sediment from each soil sample was oven-dried in aluminium cups at 105°C to a constant weight. The total dry mass, including the subsample for grain size distribution, was calculated using the measured conversion factor between wet and dry mass. BDD ( $\text{g cm}^{-3}$ ), an indicator for the compaction of the soil, was calculated by dividing the total dry mass ( $\pm 0.01 \text{ g}$ ) by the sample volume ( $\text{cm}^3$ ).

Dried samples were then homogenised using a mortar and pestle. A 15-30 g subsample was transferred to a pre-weighted crucible, dried again for three hours at 105°C to a constant weight, after which the initial dry weight was determined up to 0.0001 g. Afterwards, the samples were combusted for four hours at 550°C in a muffle furnace, to burn the organic matter, and weighed again. The difference between the initial dry weight and combusted weight was divided by the initial dry weight to estimate the proportion of organic matter (%) in each sample (Heiri et al., 2001).

## **5.2.4 Temporal patterns in burrow characteristics and bank erosion over a one-year period**

### **5.2.4.1 Burrow measurements and environmental characteristics**

To investigate seasonal variation in burrow density and size (research question 3), as well as the relationship between burrowing activity, bank erosion, and sedimentation (research question 5), we monitored burrow characteristics and sediment dynamics over the course of one year (**Table 5.1**).

Fixed plots were installed and monitored from October 2021 to October 2022, in Lippenbroek and Notelaer. In each tidal marsh, six permanent study plots were established along the riverbank (**Fig. 5.3**). On average, plots measured 0.95 m in height and 2.60 m in width, with a surface area of approximately  $2.5 \text{ m}^2$ . The plots were distributed along the entire length of the main creeks in both marshes (**Fig. 5.2b,c**; 'Study plot' in red), and covered a range in burrow densities and environmental conditions. Each rectangular plot was delineated by five iron rods (30 cm long), marking the four corners and the centre of the plot. The rods were inserted approximately 20 cm deep, perpendicular into the riverbank. The top of each rod was coloured orange to improve visibility.



**Fig. 5.3** Example of a permanent study plot (Plot 4) at Notelaer. The plot is positioned vertically along the bank and marked by four rods inserted perpendicularly at its corners. In this image, the rods are connected with red-and-white ribbon to clearly delineate the plot boundary for burrow counting.

Burrow monitoring was carried out every two months. During each sampling event, all burrows within each plot were counted and measured following the procedure described in the previous section (5.2.3.1).

At the start of the study, in November 2021, a set of environmental variables was recorded for each study plot (**Table 5.1**). The presence or absence of vegetation within each plot was noted, as well as the type of vegetation. Bank angle, creek width, distance of plot to the main river, and sediment characteristics (BDD, OM, d50, clay silt, and sand fraction) were determined following the procedure described in previous sections (5.2.3.1 and 5.2.3.2). For the sediment analysis, three (instead of one) undisturbed sediment samples were collected just outside of each study plot, over the full vertical extent of the plot.

A differential GPS (dGPS) was used to measure the cross-sectional profile of the creek at each study plot by taking a measurement every 25 cm. From these measurements, mean and maximum creek depth (m) were derived (**Table 5.1**). Additionally, the

coordinates of each rod within each study plot were determined at the beginning and mid-study using a dGPS.

The coordinates of the rods were used to determine hydrological characteristics. Water level data (October 2021 – November 2022) from nearby monitoring stations were obtained from Waterinfo.be through the waterRinfo R package (Van Hoey et al., 2018). The elevation (mTAW) of the lowest, middle, and highest rod in each plot was used to calculate average inundation frequency and duration for those three points per plot using the Tides R package (Cox and Schepers, 2018).

#### 5.2.4.2 Structure-from-motion photogrammetry

Structure-from-motion photogrammetry was used to quantify sediment loss and accretion at each riverbank study plot. At each study location, each study plot was photographed at both the beginning and end of the one-year monitoring period to collect a set of pictures that would be used for structure-from-motion photogrammetry. In Lippenbroek, this was done at the beginning of November 2021 and the end of October 2022. In Notelaer, at the end of October 2021 and October 2022. Before photographing the plot, overhanging vegetation was trimmed, and leaves or other debris were carefully removed from the riverbank surface. Photographs were taken using a consumer-grade Olympus Tough 6 camera and a Samsung Galaxy S21 smartphone (no flash, 9:16 aspect ratio, auto HDR, autofocus). For each sampling occasion, approximately 500 overlapping images were captured from various angles to ensure comprehensive coverage of the plot.

The images were processed using a standard Structure-from-Motion (SfM) photogrammetry workflow in Agisoft Metashape Professional (version 2.0; Agisoft, St. Petersburg, Russia) to generate a 3D dense point cloud for each plot at each time point. Before photo alignment (accuracy: high), low-quality images were removed to enhance reconstruction accuracy. The tie point cloud was refined through an iterative optimization process by removing low-quality points using gradual selection and performing camera optimization. Markers were added and georeferenced using dGPS measurements to ensure accurate georeferencing. Finally, dense point clouds (accuracy: high, depth filtering: mild) were generated from depth maps derived from the optimized tie point cloud.

#### 5.2.4.3 Calculation of volume changes

The two dense point clouds of the same plot were manually aligned in CloudCompare using point pair picking. The same segmentation approach was applied to both time

points to isolate the area over which erosion would be calculated, excluding large root systems and other static features.

To measure lateral erosion, a principal component analysis (PCA) was applied to the dense point clouds. The PCA transformation reoriented the steep vertical riverbank, allowing a top-down perspective as if an observer were standing in the creek and looking at the riverbank. The second dense point cloud of the same plot was rotated according to the PCA transformation of the first dense point cloud to standardize its orientation. The reoriented point clouds were then rasterized into digital terrain models (DTMs) at a 0.025 m resolution, using the median elevation within each grid cell to reduce noise. Sediment erosion and sedimentation were quantified by calculating the elevation difference between the two DTMs. The results were visualized using elevation maps and erosion difference plots (Suppl. Mat. 8.3 **Fig. S. 12**, **Fig. S. 13**).

Volume changes were calculated by multiplying the height difference of each grid cell by its surface area ( $0.025 \times 0.025$  m). The sum of all positive values represents sedimentation (material accumulation), while the sum of all negative values represents erosion (material loss). These values were divided by the total surface area of the plot to express sedimentation and erosion as volume change per square meter ( $\text{dm}^3 \text{m}^{-2}$ ).

In addition, two overall volume change metrics were calculated per plot (**Table 5.1**). Net volume change, calculated as the total sum of positive and negative values, represents both the direction and magnitude of the overall change per plot. The absolute volume change, on the other hand, is the total sum of the absolute values and reflects the total sediment turnover, serving as a proxy for how dynamic each study plot is.

**Table 5.1** Overview of the two field campaigns conducted to investigate spatial and temporal variation in Chinese mitten crab burrow characteristics and associated sediment dynamics (sections 5.2.3 and 5.2.4). With LB = Lippenbroek, and NL = Notelaer.

Dataset	Location	Period	Monitoring frequency	Sampling plots	Metrics sampled	Research question
Spatial survey (section 5.2.3)	Lippenbroek Notelaer	September 2023	Once	66 LB, 52 NL	Burrow density and size	2
					Bank angle, creek width, distance from main river, BDD, OM, d50, clay, silt, and sand fraction	
Temporal survey (section 5.2.4)	Lippenbroek Notelaer	October 2021 until October 2022	Bi-monthly (burrow characteristics)	6 LB, 6 NL	Burrow density and size	3-4-5
			Once (environmental characteristics)		Bank angle, creek width, distance from main river, BDD, OM, d50, clay, silt, and sand fraction, mean and maximum creek depth, average inundation frequency and duration at lowest, middle and highest point of each plot	
			Beginning and end (sediment dynamics)		Erosion, sedimentation, net and absolute volume change	

## 5.2.5 Data analysis

All analyses were performed in R version 4.2.2 (R Core Team, 2022), and graphs were created using the R package `ggplot2` (Wickham, 2016). The significance level for statistical analyses was set at  $p < 0.05$ .

### 5.2.5.1 Morphometric relationships

Linear regressions (stats R package (R Core Team, 2022)) were performed between crab size (cm) and burrow width (cm) and length (cm). An exponential regression was performed between crab size (cm) and burrow volume (cm<sup>3</sup>).

### 5.2.5.2 Temporal patterns in burrow density and size

Overall differences in burrow density and burrow width between the two study sites were tested with a linear-mixed-effects model (LMM) with location as a fixed effect, and month and plot ID as random effects (lmerTest R package (Kuznetsova et al., 2017)).

To test for differences in burrow density between months, a LMM was applied, including month as a fixed effect and plot ID as a random effect, to account for repeated measurements in the same plots. Two different models were used for the two locations, Lippenbroek and Notelaer. In case of a significant effect of month, post-hoc pairwise comparisons between months were performed with Tukey-adjustment (emmeans R package (Lenth, 2017)). Similarly, temporal differences in burrow width between months were tested with a LMM per study location, including month as a fixed effect and plot ID as a random effect.

To assess the relationship between average burrow density and the number of crabs caught per month in the trap, a Pearson correlation test was conducted for the restored marsh, Lippenbroek.

### 5.2.5.3 Morphometric relationships in the field

To evaluate the accuracy of crab size estimations based on burrow measurements, following the experimentally defined morphometric relationship (section 5.3.1), we compared the distributions and means of calculated crab sizes to those of observed (measured) individuals in Lippenbroek. For each selected month, the observed size data consisted of individuals caught during that month as well as the preceding month, to ensure adequate sample sizes. Nevertheless, size comparisons were only

performed for December 2021, February 2022, and May 2022, as insufficient numbers of crabs were caught in other months to allow reliable size comparisons.

First, we applied two-sample Kolmogorov–Smirnov (KS) tests to assess whether the distributions of calculated and observed crab sizes differed within each sampling month (stats R package (R Core Team, 2022)). To examine differences between mean calculated and observed crab size across months, we conducted a two-way analysis of variance (ANOVA), including month, type (calculated vs. observed), and their interaction as factors. Pairwise comparisons between observed and calculated sizes were performed within each month using Tukey-adjusted p-values to account for multiple comparisons (emmeans R package (Lenth, 2017)).

#### 5.2.5.4 Spatial patterns in burrow density and size

The relationship between environmental variables (Suppl. Mat. 8.38.1 **Table S. 9**) and burrow density and size was analysed using separate generalized linear models (glmmTMB R package (Brooks et al., 2017)). A negative binomial distribution (log link) was applied for burrow density, while a Gaussian distribution (identity link) was used for burrow size (width and surface area). Several environmental variables were highly collinear, such as d50 and the clay, silt, and sand fractions, as well as OM and BDD. Therefore, only one variable from each group was included, with the selection based on the strongest correlation with the dependent variable identified during exploratory analysis. The model for burrow density included bank angle, distance from the main river, BDD, d50, and location (i.e., study sites) as fixed factors. The models for burrow size (width and surface area) included bank angle, distance from the main river, OM, d50, and location as fixed factors. Model selection was conducted based on AIC values through stepwise backward selection (stats R package (R Core Team, 2022)).

#### 5.2.5.5 Link between riverbank sediment dynamics and burrow density and size

Based on the field measurements described in section 5.2.4.1, several variables related to burrow density and size were compiled for each study plot. Burrow density (number per m<sup>2</sup>), recorded at the beginning (October 2021), middle (May 2022), and end (October 2022) of the study period, was included. In addition, the annual average burrow density per plot was calculated (over the seven sampling occasions), along with the changes in burrow density from the beginning to both mid- and end-study. Mid-study values and changes from the beginning to mid-study were included, as this period showed the most pronounced changes in burrow characteristics (see section 5.3.2).

Similarly, the mean surface area of individual burrows ( $\text{cm}^2$ ) and the total burrow surface area per plot (i.e., the sum of all burrow surface areas) were recorded at each of the three time points. For each plot, average values (over the seven sampling occasions) were calculated for both mean and total burrow surface area. Furthermore, changes in total burrow surface area from the beginning to both mid- and end-study were included as well.

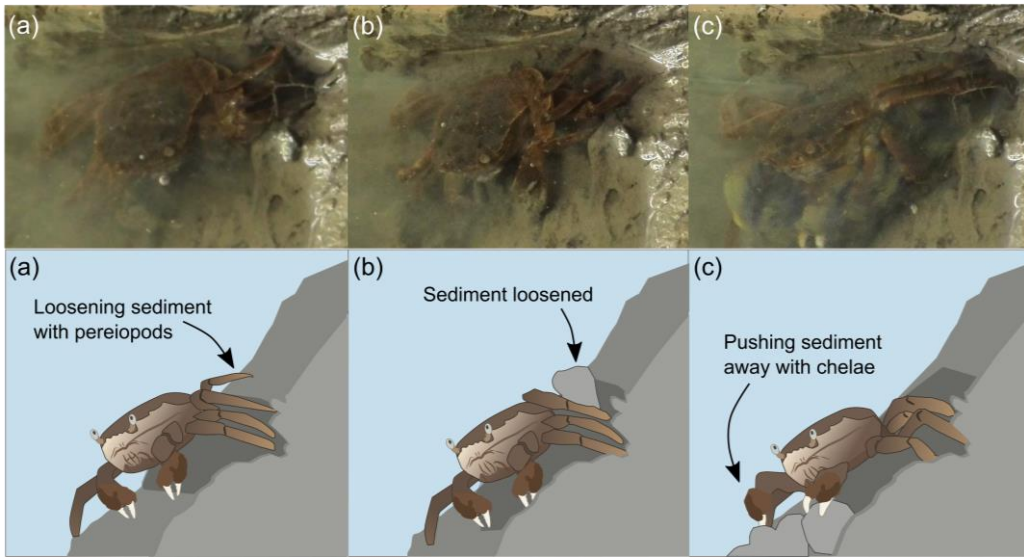
To explore the potential association between the largest burrows and erosion, the average burrow width and surface area of the top 25 % largest burrows per plot were calculated for each sampling occasion. The averages of these values (over the seven sampling occasions) per plot were also included, as they may reveal patterns not captured by overall burrow size averages.

To explore how these burrow characteristics and environmental variables (Suppl. Mat. 8.38.1 **Table S. 10**) relate to sediment dynamics (i.e., volume changes over time), correlation tests were performed (stats R package (R Core Team, 2022)). Separate correlations were tested between sedimentation, erosion, absolute, and net total volume change (all expressed in  $\text{dm}^3 \text{m}^{-2}$ ), and the burrow and environmental variables. Analyses were performed for both study locations combined, as well as separately for each location.

## **5.3 Results**

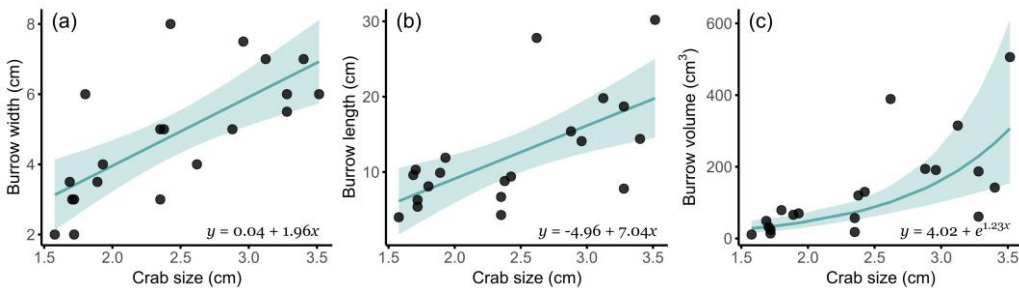
### **5.3.1 Burrowing behaviour and morphometric relationships**

All crabs except one created a burrow, and three crabs even created two burrows each. Observations revealed that the crabs burrow by repeating two distinct movements. In the first movement, the crab uses its pereopods (i.e., walking legs) to loosen the sediment beneath and behind its body (**Fig. 5.4a,b**). In the second movement, the crab uses its chelae to push the loosened sediment forward and away from underneath itself (**Fig. 5.4c**).



**Fig. 5.4** Snapshots and illustration of a burrowing Chinese mitten crab. The crab uses its pereiopods to loosen the sediment (a,b), and subsequently pushes the loosened sediment away from beneath its abdomen with its chelae (c).

Simple regressions were performed to establish morphometric relationships between crab size (cm) and burrow width (cm), burrow length (cm), and burrow volume ( $\text{cm}^3$ ). Significant linear relationships were found for both burrow width ( $F(1,18) = 19.06$ ,  $p < 0.001$ , adjusted  $R^2 = 0.49$ ) and burrow length ( $F(1,18) = 12.72$ ,  $p < 0.01$ , adjusted  $R^2 = 0.38$ ) (**Fig. 5.5a,b**). For burrow volume, a significant exponential relationship was found ( $F(1,18) = 22.64$ ,  $p < 0.001$ , adjusted  $R^2 = 0.53$ ) (**Fig. 5.5c**).



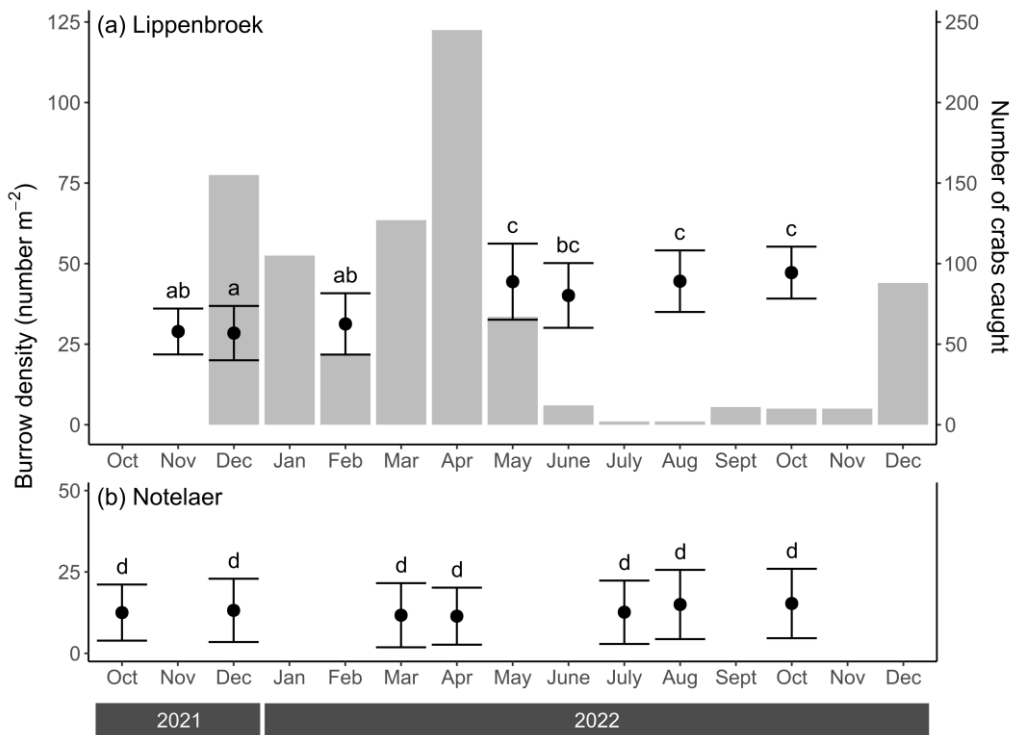
**Fig. 5.5** Simple regressions between crab size (carapace width in cm) and (a) burrow width (cm), (b) burrow length (cm), and (c) burrow volume ( $\text{cm}^3$ ) in experimental conditions. The predicted mean of each variable and its 95% confidence interval are shown in blue, while the black dots represent the measured values.

### 5.3.2 Temporal patterns in burrow density and size, and crab abundance

A total of 878 crabs were captured in Lippenbroek from December 2021 to December 2022. There was a seasonal pattern in the number of crabs caught per month at Lippenbroek, with high catch numbers from December to May (winter-spring), and low catch numbers from June to November (summer-autumn) (**Fig. 5.6a**).

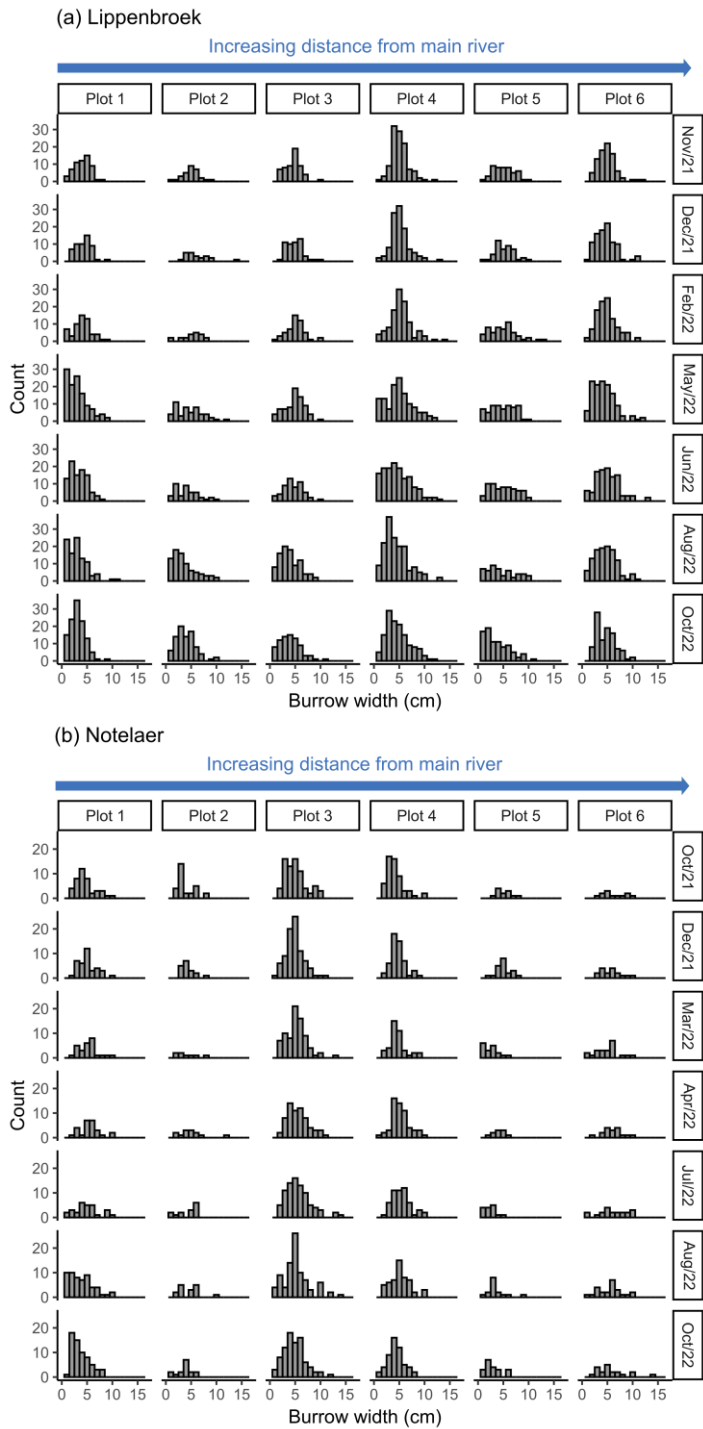
Burrow density was overall higher in Lippenbroek compared to Notelaer ( $F(1,10.38) = 9.34$ ,  $p < 0.05$ ). In Lippenbroek, burrow density varied significantly across months ( $F(6,30) = 9.67$ ,  $p < 0.001$ ). Post-hoc tests indicated significantly higher burrow densities from May through October (mean 40-47 burrows  $m^{-2}$ ), compared to the preceding winter months (mean 28-31 burrows  $m^{-2}$ ; Tukey-adjusted  $p < 0.05$ ) (**Fig. 5.6a**, Suppl. Mat. 8.3 **Fig. S. 9a**). There was no significant correlation between monthly mean burrow density and the number of crabs caught in the trap (Pearson's  $r = -0.72$ ,  $p = 0.108$ ).

In contrast, at Notelaer, burrow density did not differ significantly between months ( $F(6,30) = 2.19$ ,  $p = 0.072$ ), with monthly means ranging from 11 to 15 burrows  $m^{-2}$  (**Fig. 5.6b**, Suppl. Mat. 8.3 **Fig. S. 9b**).



**Fig. 5.6** Monthly number of crabs caught in the permanent trap at Lippenbroek (grey bars), alongside mean burrow density (black circles  $\pm$  SD) at each location, based on six plots per time point. The permanent trap was installed in December 2021. Letters above error bars indicate statistically significant ( $p < 0.05$ ) differences in burrow density between months within each location.

A total of 5,036 burrows were measured across all months and study sites. There was no significant difference in burrow width between the two marshes ( $F(1, 10.04) = 0.581$ ,  $p = 0.463$ ). At both locations, mean burrow width showed significant temporal variation across months (Lippenbroek:  $F(6, 3450) = 11.23$ ,  $p < 0.001$ ; Notelaer:  $F(6, 1564) = 3.46$ ,  $p < 0.01$ ). At Lippenbroek, burrow widths were generally smaller during late summer and autumn (August and October) compared to the preceding months (Suppl. Mat. 8.3 **Fig. S. 10a**). This pattern was especially pronounced in the first three plots of the area, located closest to the main river (River Scheldt) (**Fig. 5.7a**). At Notelaer, mean burrow width showed a slight increase in April and July, followed by a decrease in October, suggesting a modest seasonal trend (**Fig. 5.7b**, Suppl. Mat. 8.3 **Fig. S. 10b**).

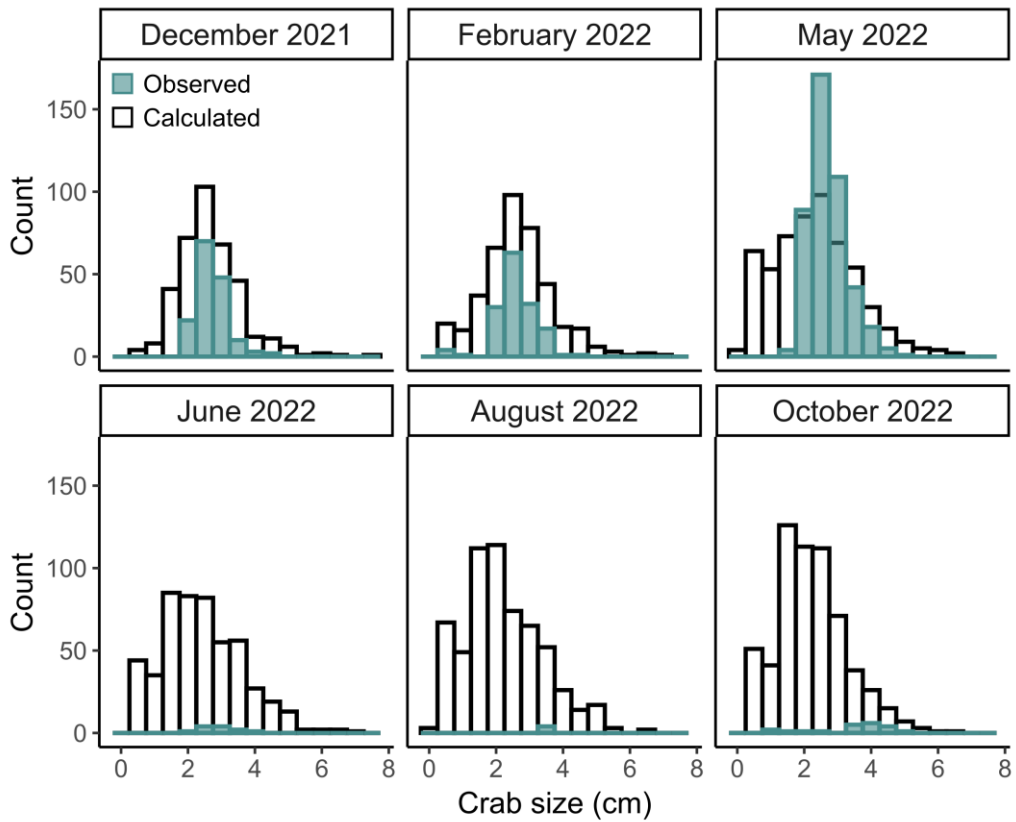


**Fig. 5.7** Size distribution of burrows per study plot per sampling occasion in (a) Lippenbroek and (b) Notelaer.

### 5.3.3 Morphometric relations in the field

To evaluate the accuracy of crab size estimations derived from burrow measurements, we compared the calculated crab sizes to the actual measured widths for December 2021, February and May 2022. Note that although burrow and crab data are available for June, August, and October 2022 (**Fig. 5.8**), statistical analyses could not be conducted due to insufficient crab data.

Across all months, calculated and observed size distributions differed significantly, as indicated by Kolmogorov–Smirnov (KS) tests (December:  $D = 0.281$ ,  $p < 0.001$ ; February:  $D = 0.248$ ,  $p < 0.001$ ; May:  $D = 0.387$ ,  $p < 0.001$ ). These differences were especially pronounced in May, where the calculated crab sizes were skewed toward smaller individuals (**Fig. 5.8**). This was reflected in a significantly lower mean calculated size compared to the observed crab size during that period ( $p < 0.001$ ). In contrast, for February and December, there were no significant differences between mean calculated and observed crab size (February:  $p = 0.993$ ; December:  $p = 0.257$ ), suggesting that the significant distributional differences detected by the KS tests in those months were likely driven by variation in distribution shape rather than central tendency.



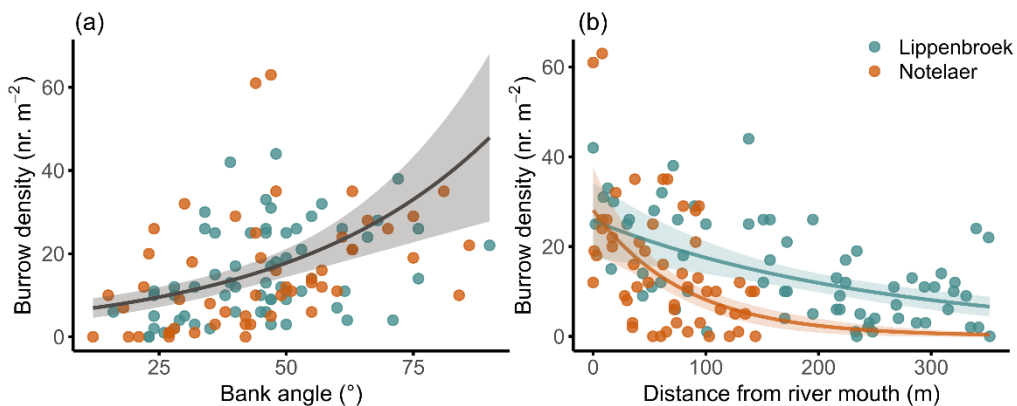
**Fig. 5.8** Monthly size distributions of Chinese mitten crabs in Lippenbroek. Blue bars represent actual observed crab sizes from individuals caught in the permanent trap, while white bars show estimated sizes calculated from burrow width measurements.

### 5.3.4 Spatial patterns in burrow density and size

The final model for burrow density included bank angle, distance from the main river, location and the interaction between location and distance from the main river as fixed factors (**Table 5.2**). The model suggests a positive relationship between burrow density and bank angle (**Fig. 5.9a**), indicating that a steeper slope is associated with higher burrow counts. Furthermore, burrow density declines with increasing distance from the main river, with the rate of decline varying between locations, as evidenced by the significant interaction between location and distance from the river mouth (**Table 5.2**, **Fig. 5.9b**).

**Table 5.2** Output of GLM (negative binomial distribution, log link) for the variable burrow density (nr. m<sup>-2</sup>) after stepwise backward selection. For the intercept, selected variables and their interactions, the estimate, standard error (SE), Z value and p value are given.

Term	Estimate	SE	Z value	p value
Intercept	2.138	0.259	8.271	< 0.001
Bank angle	0.025	0.004	5.742	< 0.001
Location Notelaer	0.086	0.240	0.36	0.719
Distance from river mouth	-0.004	0.001	-4.748	< 0.001
Location Notelaer:Distance from river	-0.009	0.002	-3.444	< 0.001

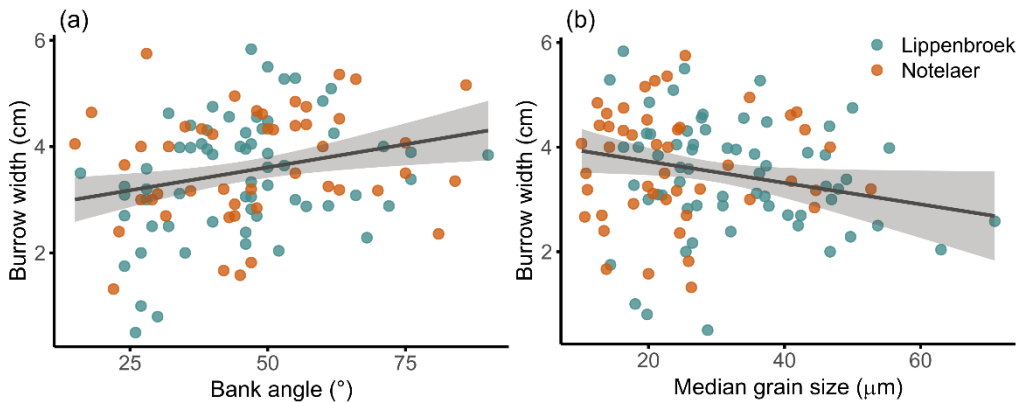


**Fig. 5.9** Relation between burrow density (number of burrows m<sup>-2</sup>) and (a) bank angle (°), and (b) distance from river mouth (m). The predicted mean of each variable and its 95 % confidence interval are shown as a line and ribbon in grey (two locations combined) or per location separately (orange and blue). The dots represent the actual values measured in the field. Blue indicates location Lippenbroek, a restored tidal marsh, and orange location Notelaer, a natural tidal marsh.

After stepwise backward selection based on AIC, the final model for burrow width included bank angle, median grain size (d<sub>50</sub>), and organic matter content (OM) as fixed factors (**Table 5.3**). The model reveals a positive relationship between burrow width and bank angle, indicating that steeper slopes are associated with wider burrows (**Fig. 5.10a**). Additionally, burrow width decreases with increasing median grain size (**Fig. 5.10b**). While the model suggests a negative relationship between burrow width and organic matter content (and thus a positive relationship with BDD), this term was not statistically significant (**Table 5.3**). The final model for burrow opening surface area included only bank angle as a fixed factor (**Table 5.4**, Suppl. Mat. 8.3 **Fig. S. 11**). Steeper slopes are associated with larger burrow openings, which partly confirms the results of the model for burrow width.

**Table 5.3** Output of GLM (gaussian distribution, identity link) for the variable burrow width (cm) after stepwise backward selection. For the intercept and selected variables, the estimate, standard error (SE), Z value and p value are given.

Term	Estimate	SE	Z value	p value
Intercept	4.141	0.714	5.797	< 0.001
Bank angle	0.017	0.006	2.83	< 0.01
d50	-0.020	0.010	-2.028	< 0.05
OM	-10.477	5.775	-1.814	0.070



**Fig. 5.10** Relation between burrow width (cm) and (a) bank angle (°), and (b) median grain size ( $\mu\text{m}$ ). The predicted mean of each variable and its 95 % confidence interval are shown as a line and ribbon in grey (two locations combined). The dots represent the actual values measured in the field. Blue indicates location Lippebroek, a restored tidal marsh, and orange location Notelaer, a natural tidal marsh.

**Table 5.4** Output of GLM (Gaussian distribution, identity link) for the variable burrow opening surface area ( $\text{cm}^2$ ) after stepwise backward selection. For the intercept and selected variables, the estimate, standard error (SE), Z value, and p value are given.

Term	Estimate	SE	Z value	p value
Intercept	4.574	1.008	4.538	< 0.001
Bank angle	0.052	0.021	2.505	< 0.05

### 5.3.5 Link between riverbank sediment dynamics and burrow density and size

Absolute volume change, a measurement of the magnitude of the volume change and a proxy for how dynamic the area is, was on average  $23 \pm 6 \text{ dm}^3 \text{ m}^{-2}$  (range: 16-32  $\text{dm}^3 \text{ m}^{-2}$ , **Table 5.5**) in the restored tidal marsh Lippenbroek. In the natural tidal marsh Notelaer, absolute volume change was typically lower, however, two study plots experienced substantial erosion events, leading to a higher mean and much greater variability ( $25 \pm 21 \text{ dm}^3 \text{ m}^{-2}$ , range: 8-57  $\text{dm}^3 \text{ m}^{-2}$ ; Suppl. Mat. 8.3 **Fig. S. 13**). Net volume change, which reflects the overall gain or loss of sediment, indicated net erosion in three study plots at Lippenbroek, while the remaining three plots showed net accretion (sedimentation) over the one-year period (Suppl. Mat. 8.3 **Fig. S. 12**). In contrast, in Notelaer, four plots experienced net sediment loss, while two showed minor sediment accumulation (**Table 5.5**, Suppl. Mat. 8.3 **Fig. S. 13**).

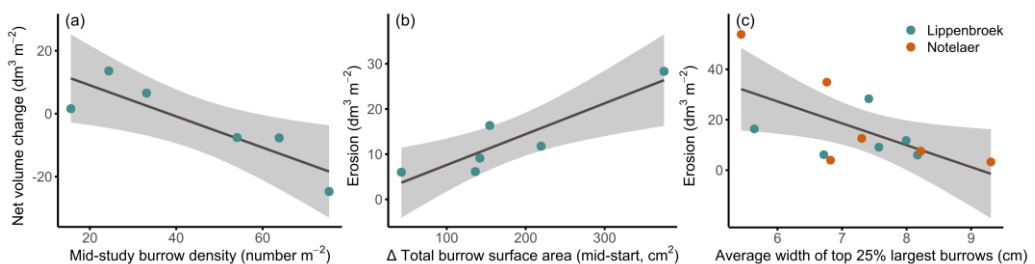
**Table 5.5** Erosion and sedimentation volumes ( $\text{dm}^3 \text{ m}^{-2}$ ), and total absolute and net volume changes ( $\text{dm}^3 \text{ m}^{-2}$ ) per study plot in the restored tidal marsh, Lippenbroek, and natural tidal marsh, Notelaer. Negative net volume change indicates overall sediment loss, while positive values indicate net sediment accretion.

		Erosion ( $\text{dm}^3 \text{ m}^{-2}$ )	Sedimentation ( $\text{dm}^3 \text{ m}^{-2}$ )	Absolute volume change ( $\text{dm}^3 \text{ m}^{-2}$ )	Net volume change ( $\text{dm}^3 \text{ m}^{-2}$ )
Lippenbroek	Plot 1	16.35	8.66	25.02	-7.69
	Plot 2	9.13	10.71	19.84	1.58
	Plot 3	6.14	19.73	25.87	13.59
	Plot 4	11.77	4.17	15.94	-7.59
	Plot 5	6.01	12.56	18.57	6.55
	Plot 6	28.33	3.61	31.94	-24.72
Notelaer	Plot 1	12.61	7.37	19.98	-5.24
	Plot 2	34.89	9.28	44.17	-25.61
	Plot 3	7.52	3.13	10.65	-4.38
	Plot 4	3.97	4.01	7.98	0.04
	Plot 5	53.86	3.38	57.24	-50.48
	Plot 6	3.28	4.46	7.75	1.18

The volume of eroded sediment ( $\text{dm}^3 \text{ m}^{-2}$ ) was negatively correlated with the average width of the top 25 % largest burrows across both study sites ( $r = -0.621$ ,  $p < 0.05$ ; **Fig. 5.11c**). In Lippenbroek, erosion showed positive correlations with the increase in burrow density ( $r = 0.837$ ,  $p < 0.05$ ) and total burrow surface area ( $r = 0.901$ ,  $p < 0.05$ ; **Fig. 5.11b**) between the beginning and mid-study, as well as with burrow density mid-

study ( $r = 0.856, p < 0.05$ ). In contrast, there were no significant correlations between erosion in Notelaer and the tested environmental and burrow characteristics.

Multiple significant correlations were detected between sedimentation ( $\text{dm}^3 \text{m}^{-2}$ ) and environmental characteristics, but not with burrow characteristics. When data from both sites were combined, sedimentation was positively correlated with creek width ( $r = 0.598, p < 0.05$ ). At Lippenbroek, a negative correlation was observed with riverbank angle ( $r = 0.91, p < 0.05$ ). In Notelaer, sedimentation was positively correlated with bulk dry density ( $r = 0.952, p < 0.01$ ), median grain size ( $r = 0.922, p < 0.01$ ) and sand fraction ( $r = 0.871, p < 0.05$ ), and negatively correlated with silt fraction ( $r = -0.886, p < 0.05$ ) and organic matter content ( $r = -0.812, p < 0.05$ ).



**Fig. 5.11** Significant correlations for study area Lippenbroek between (a) net volume change and the burrow density mid-study, and (b) total eroded volume and the change in total burrow surface area between beginning and mid-study. The third panel (c) shows the correlation between eroded volume and the average width of the top 25 % largest burrows across both locations.

Similar to erosion, the absolute volume change ( $\text{dm}^3 \text{m}^{-2}$ ) was negatively correlated with the average width and surface area of the top 25 % largest burrows ( $r = -0.691, p < 0.05$ ;  $r = 0.642, p < 0.05$ ). Additionally, there was a negative correlation with the annual average surface area of individual burrows ( $r = -0.646, p < 0.05$ ) and with average burrow surface area mid-study ( $r = -0.6, p < 0.05$ ). No significant correlations with environmental or burrow characteristics were detected at Lippenbroek, while in Notelaer there was a negative correlation between absolute volume change and total burrow surface area mid-study ( $r = -0.816, p < 0.05$ ).

Net volume change ( $\text{dm}^3 \text{m}^{-2}$ ) did not show significant correlations with any of the tested environmental and burrow characteristics when both study locations were analysed together. However, in Lippenbroek, it was negatively correlated with mid-study burrow density ( $r = -0.87, p < 0.05$ ; **Fig. 5.11a**), the change in total burrow surface area from the beginning to mid-study ( $r = -0.867, p < 0.05$ ), and the annual average

burrow density ( $r = -0.84, p < 0.05$ ). For Notelaer, only a positive correlation with bank angle was found ( $r = 0.837, p < 0.05$ ), and no significant correlations were found with burrow characteristics.

## 5.4 Discussion

### 5.4.1 Temporal and environmental variation in burrow characteristics

The results of this study revealed that in the restored tidal marsh Lippenbroek, increased erosion was strongly associated with changes in burrow characteristics during the first half of the monitoring period (i.e., winter and spring), particularly with the increase in total burrow surface area. Similarly, net volume change also showed that areas experiencing an overall loss in sediment were correlated to similar burrow-related parameters, including the annual average burrow density. In contrast, none of the measured environmental variables were significantly correlated with erosion. These patterns may reflect a dual mechanism: on the one hand, Chinese mitten crabs may prefer environmental conditions that are naturally more susceptible to erosion; on the other hand, their bioturbation activity, especially during winter and early spring, may actively enhance erosion. We argue that both are likely.

Erosion was not correlated with any of the measured environmental characteristics. However, it is possible that other unmeasured factors, such as surface roughness, presence of roots, bank curvature, soil water content, or flow velocities, affect both erosion and burrow density (Chen et al., 2022; Davidson and De Rivera, 2010; Grabowski et al., 2011; Wang et al., 2009; Zhao et al., 2022). In both study areas, our field data revealed that burrow densities were highest in steeper riverbanks and near the tidal area's entrance. Previous studies on Chinese mitten crabs have also suggested a weak positive relationship between burrow density and bank angle, although they have been observed burrowing on flat surfaces as well (Hymanson et al., 1999; Rudnick et al., 2005a; Veldhuizen, 2001). Burrow densities were consistently higher near the main river Scheldt, which was at the creek mouth in the case of the natural tidal area, and near the inflow in the restored tidal area. This pattern may indicate density-dependent dynamics, where crabs start burrowing as soon as they enter the area and only move further when the population increases, or a preference for specific environmental characteristics, or a combination of both. A similar spatial pattern was observed by Li et al. (2018) for the semi-terrestrial crab *Helice tientsinensis* in Chinese salt marshes, where burrow densities were highest in the lower marsh zone near the sea.

However, given the lack of significant correlations with environmental variables and the exceptionally high burrow densities observed in Lippenbroek, the second explanation, i.e., bioturbation-driven erosion, is also plausible. Previous studies on crustaceans have demonstrated that burrowing activity can accelerate erosion in riverbanks and marshes by promoting lateral erosion and increasing the likelihood of bank collapse (Davidson and De Rivera, 2010; Escapa et al., 2007; Sanders et al., 2021). Moreover, studies on invasive crayfish have shown that they not only prefer steep, cohesive riverbanks that are already prone to erosion, but also actively contribute to bank steepening over time through their burrowing activity (Faller et al., 2016; Sanders et al., 2021). For mitten crabs, burrow densities exceeding 30 burrows  $m^{-2}$  are considered damaging, and the burrow densities recorded in Lippenbroek rank among the highest reported in literature (Kamps, 1937; Peters and Panning, 1933; Rudnick et al., 2005a; Veldhuizen, 2001). The excavated volume alone already leads to an estimated 3% sediment loss per 0.5  $m^3$  of riverbank (Rudnick et al., 2005a). The creation of an extensive burrow network could affect the hydrology of the riverbank and undermine its stability, making it more susceptible to slumping (Harvey et al., 2019). Additional factors, such as increased surface roughness from burrow openings (Fig. 5.12) and altered microtopography around these openings, can further enhance lateral erosion (Farron et al., 2020; Harvey et al., 2019).



**Fig. 5.12** High burrow density in the main creek of the restored tidal marsh Lippenbroek. Burrow openings are associated with irregularities in the topography of the riverbank.

The impact of burrowing may be particularly pronounced in winter and spring, as a result of increased rainfall and the associated higher water discharge in the Scheldt River (Meire et al., 2005; Olaniyan, 2009). Similar patterns were observed in San Francisco Bay, where bank erosion intensified following rain events in spring (Rudnick et al., 2005a). Furthermore, burrow density increased most notably between winter and late spring in Lippenbroek, potentially amplifying erosion during this period due to heightened burrowing activity. Burrow density remained relatively stable throughout autumn and winter, but increased markedly in spring, after which it remained elevated through summer and early autumn. This trend was particularly pronounced in study plots situated near the inflow of the area. Interestingly, the rise in burrow density coincided with a decline in mean burrow width, suggesting the formation of new, smaller burrows created by juvenile crabs. It has been suggested that small crabs often reuse existing burrows rather than constructing new ones (Kamps, 1937; Rudnick et al., 2005a). This might indicate that in the present study, existing burrows were insufficient to house all crabs, thereby inducing new burrowing activity by juvenile crabs.

Burrow density is commonly used as a proxy for estimating crab abundance in ecological studies, though the accuracy can vary (Costa et al., 2021; de Oliveira et al., 2016; Schlacher et al., 2016). In our study, no significant correlation was found between burrow density and the number of crabs caught, this is likely attributable to the fact that most of the captured crabs were migrating. Juvenile mitten crabs typically migrate upstream in spring from lower sections of the estuary (Panning, 1938; Rudnick et al., 2005b; Schoelynck et al., 2021). Therefore, the high catch numbers in this period reflect a new influx of crabs entering the marsh when burrow density was still low. From late spring to early autumn, trap catches were low, suggesting crabs remained resident in the area, which corresponds with the observed peak in burrow density. In autumn and winter, adult mitten crabs typically migrate downstream towards brackish and marine environments to reproduce (Chapter 2; Keirsebelik et al., 2025; Panning, 1938; Rudnick et al., 2005b). Accordingly, the crabs caught in this period are likely leaving the marsh, resulting in a lower burrow density in winter and early spring. However, a substantial proportion of the crabs captured during these months were too small to reproduce. Similarly, Gilbey et al. (2008) observed lower abundances of juvenile Chinese mitten crabs during winter in the upper tidal region of the Thames. Juvenile crabs likely leave these areas to move further upstream to a more favourable habitat to continue their development, or to move to deeper waters to pass through winter (Gilbey et al., 2008; Rudnick et al., 2000).

Our observations align with earlier research in San Francisco Bay, where burrow densities and occupancy rates were highly variable across locations and seasons (Rudnick et al., 2005a). However, generally, the lowest burrow densities were found during winter and spring, while occupancy rates peaked in spring and summer (Rudnick et al., 2005a). Low crab burrow density in winter is a fairly common seasonal pattern across species and habitats, and has been linked to the effect of temperature on the burrowing activity of crabs (Chen et al., 2022; Egawa et al., 2021; Rosa and Borzone, 2008; Wei et al., 2024). In San Francisco Bay, reduced burrow densities during these seasons were attributed to an increased frequency of high water events that may wash away burrows (Rudnick et al., 2005a). While similar processes may be at play in the present study, burrow density did not significantly decline during winter and spring. Therefore, the observed seasonal pattern likely reflects reduced crab abundance and burrowing activity during colder months. Because occupancy rates of crab burrows are highly variable and often site-specific, burrow density cannot be used to derive absolute numbers of Chinese mitten crabs (Rudnick et al., 2005a; Schlacher et al., 2016). However, our results suggest that it does reflect relative, seasonal changes in crab abundance.

Interestingly, the relationship between burrow characteristics and erosion observed in the restored tidal marsh Lippenbroek was not found in the natural tidal marsh Notelaer. In Lippenbroek, erosion was closely associated with temporal changes in burrow density and total surface area. In contrast, these parameters remained relatively stable in Notelaer, with consistently low burrow densities and no pronounced seasonal trends. Nevertheless, some indications of new burrowing activity were observed at the plot level. For example, one plot experienced a slump during winter, and while burrow density recovered, burrow width declined, potentially reflecting the creation of new, smaller burrows. The lack of seasonal increase in burrow density was unexpected, especially because this marsh is located closer to the Scheldt Estuary, where juvenile Chinese mitten crabs likely start their upstream migration (Chapter 3; Keirsebelik et al., 2025). Based on this geographic position, it was expected that juvenile crabs would colonise Notelaer first, before reaching Lippenbroek. Possibly, the combination of the larger creek size and sluices at Lippenbroek creates a more attractive current for the upstream migrating crabs, which lures them into the area. However, the environmental preferences of juvenile migrating crabs are overall poorly understood (Panning, 1938; Rudnick et al., 2005b). Additionally, environmental conditions in Lippenbroek may be more favourable for crabs to remain resident and burrow, leading to higher burrow densities. Below the newly tidal deposited sediment layer, the soil in Lippenbroek is cohesive and highly

compacted, as a result of its historical use for agriculture (Stoorvogel et al., 2024; Van Putte et al., 2020). Therefore, it may be more suitable for burrowing and support higher burrow densities than Notelaer. Although this was not measured, visual observations suggest that the burrows are indeed concentrated in the compacted soil, below the newly deposited layer that is interspersed by plant roots (**Fig. 5.12**) (Van Putte et al., 2020). The overall low burrow density and temporal stability in burrow characteristics in Notelaer suggest that the influence of burrowing activity on sediment dynamics may be more limited at this site. Additionally, two of the six study plots in Notelaer experienced relatively large slumps during the monitoring period, which could have masked overall patterns in sedimentation and erosion.

In contrast to Lippenbroek, significant correlations were found between sedimentation and environmental parameters for Notelaer, such as unexpected positive correlations with bulk dry density and median grain size. However, given the narrow range in bulk dry density and organic matter content (Suppl. Mat. 8.38.1 **Table S. 10**), these correlations might not reflect ecologically meaningful variation. Similarly, correlations with median grain size, sand, and silt fractions likely represent the nature of the deposited material itself rather than true spatial patterns in sedimentation.

The combined data of both tidal areas indicated that larger absolute volume changes, i.e., greater overall sediment turnover, were associated with smaller burrows. This pattern was evident in both the average width and surface area of burrows, which tended to be smaller in more dynamic, erosion-prone locations. In line with this interpretation, burrows were generally wider in steep banks in areas with a small median grain size, a pattern that was also observed in other crab species inhabiting creek environments (Wang et al., 2009). This may reflect that it is easier to excavate larger burrows in fine, cohesive sediments. Alternatively, it could indicate that burrows tend to last longer in these environments. In case of the latter, these burrows can gradually become larger through repeated use and modification by crabs, as well as exposure to water flow, which can lead to erosion and widening of the burrow opening. These findings suggest that larger burrows may not be linked to increased erosion as expected, but rather to more stable environments where they can persist and be reworked over time.

**Table 5.6** Overview of burrow characteristics (width, height, length, volume, and density) and associated crab sizes of the Chinese mitten crab as reported in the literature. For each location, average values or ranges of average values are shown where available; total ranges of observed values within each study are indicated in square brackets.

Location	Country	Year	Width (cm)	Height (cm)	Length (cm)	Volume (cm <sup>3</sup> )	Density (nr. m <sup>-2</sup> )	Crab size (cm)	Source
San Francisco Bay	USA	1995-2002	4.2 – 4.4		31 – 44 [few cm – 50 <]	484 – 681 [199 – 1994]	2 – 39	1.4 – 2.1 [0.4 – 4.5]	Rudnick et al., 2000, 2005; Veldhuizen, 2001
	Germany	1932	[2 – 12]		[20 – 80]		[0 – 30 <]	[1.1 – 3.8]	Peters and Panning, 1933
Kommerzijlster Rijt	the Netherlands	1935-1936	[2 – 14]	[1 – 8]	[19 – 83]		[0 – 68]		Kamps, 1937
Scheidt Estuary	Belgium	2021-2023	3.5 – 5.1 [0.5 – 17]	1.6 – 2.0 [0.5 – 9]			13 – 38 [0 – 75]	2.7 [0.4 – 5.2]	Current study; Keirsebelk et al., 2024
Laboratory	Belgium	2023	4.8 [2.0 – 8.0]		12 [4.0 – 30.2]	133 [11 – 506]		2.4 [1.6 – 3.5]	Current study

### 5.4.2 Burrowing behaviour and morphometric relationships

Our findings confirm that Chinese mitten crabs with a carapace width of 15 to 35 mm exhibit burrowing behaviour. All except one crab (34 mm carapace width) constructed a burrow under laboratory conditions, indicating a strong incentive to burrow when alternative shelters are absent during low water conditions. Most individuals began digging almost immediately after introduction to the aquaria, and given the short duration of the experiment, this suggests that Chinese mitten crabs are capable of rapidly constructing burrows in the field, which may benefit their survival. Our direct observations of the sequential burrowing movements align with earlier findings by Kamps (1937), who did similar observations in aquaria and noted fresh dactylus imprints (terminal segment of pereopods) on the ceilings of newly excavated burrows in the field.

As expected, burrows were consistently wider than the carapace widths of their inhabitants. In our laboratory conditions, the mean ratio of carapace width to burrow width was 1:1.96, which is higher than the range of 1.2 to 1.8 reported by Kamps (1937), who found an average ratio of 1:1.4. Rudnick et al. (2005a) did not explicitly report this ratio; however, data from their field investigations (i.e., average carapace width:average burrow width; 2.1:4.4, 1.4:4.2, 2.3:4.3) suggest comparable ratios to the present study.

Additionally, there is a linear increase in burrow length and an exponential increase in the excavated volume of sediment as crab size increases. While this pattern may seem intuitive, interestingly, this is not the case for all crab species. In mangroves, for example, juvenile crabs have been observed to create deeper and longer tunnels than their larger conspecifics (Min and Kathiresan, 2021). Additionally, our results suggest there is high variability among individuals. Despite a limited sample size, this study is the first to quantitatively establish a relationship between crab size and both burrow length and excavated volume for this species.

It is important to note that the constraints of the laboratory setup (i.e., aquarium size and time to burrow) may have limited burrowing behaviour, particularly for larger crabs. In our experiments, burrow lengths ranged from 4 to 30 cm and excavated volumes from 11 to 500 cm<sup>3</sup> (**Table 5.6**). Rudnick et al. (2005a) reported burrows ranging from a few centimetres up to 50 cm in length, with averages between 31 and 44 cm and volumes between 199 and 1994 cm<sup>3</sup> (average 484 to 681 cm<sup>3</sup>, **Table 5.6**). Earlier work described even longer burrows, ranging from 19 to 83 cm, though these studies focused only on burrows with a minimum diameter of 2 cm (Kamps, 1937;

Peters and Panning, 1933). These findings suggest that, under natural conditions, crabs may extend or modify burrows over time, potentially making them deeper and more complex. This explanation is supported by field observations in San Francisco Bay in 1995 and 1999, which recorded initially low burrow densities characterised by simple tunnels, with an average depth of 20 cm, that gradually became deeper and more complex over time and with increasing density (Rudnick et al., 2005a, 2000; Veldhuizen, 2001). So, while the established relationships reflect freshly excavated burrows in the field, they may underestimate the length and volume of burrows that have persisted for a long time.

In December and February, the mean crab size estimated from burrow width measurements closely matched the average size of crabs caught. However, while the central tendency aligned, the overall size distributions differed. Small crabs were underrepresented in the catches, likely due to the limitations of the trapping method used, which was not as effective for individuals with a carapace width below 1 cm in the tidal marsh Lippenbroek (H. Keirsebelik, unpublished results). However, later monitoring in the area confirmed the presence of these small crabs in the marsh (Chapter 2). Large crabs were also more numerous in the estimations compared to the actual catch, though likely for different reasons. Burrow measurements suggested the presence of crabs up to 7 cm in size, and while this is theoretically possible, such large individuals have never been observed in the area. Over three years of monitoring, the largest recorded crab measured only 5 cm (Chapter 2; **Table 5.6**). Moreover, previous studies have reported that Chinese mitten crabs with a maximum carapace width of 4 to 4.5 cm were associated with burrows (Peters and Panning, 1933; Rudnick et al., 2005b, 2000). This suggests that these large burrows may not reflect actual crab size but instead result from erosion of the burrow entrance or repeated modification by multiple individuals over time (Rudnick et al., 2005a). The mismatch observed in May, both in terms of average size and size distribution, likely reflects the same mismatch as a result of migrating crabs, as previously discussed in relation to burrow density. Collecting crab data through burrow excavation would yield more accurate results, but this method leads to substantial disturbance to the creek systems, and was therefore not used in the present study.

Overall, these findings suggest that, while keeping in mind that the size and number of large crabs may be overestimated, burrow width generally serves as a reliable proxy for the size of Chinese mitten crabs in the area. This is consistent with broader evidence that morphometric relationships between crabs and their burrows are generally more

accurate than those between burrow counts and crab density, though they are often species- and site-specific (Schlacher et al., 2016).

### **5.4.3 Conclusion**

This study provides new insights into the burrowing behaviour of the invasive Chinese mitten crab and its potential role in shaping sediment dynamics within tidal marshes. Burrow characteristics showed considerable spatial and temporal variability. We found that burrow width is generally a good proxy for crab size, while shifts in burrow density reflected relative changes in crab abundance. The high observed burrow densities and the general absence of undisturbed riverbank sections highlight the intensity of bioturbation by this species. In areas where average burrow densities exceeded 30 burrows  $\text{m}^{-2}$ , increased erosion was observed in association with higher burrow densities. However, controlled experiments remain essential to confirm a causal relationship between burrowing activity and sediment loss.

The ecological implications of such intense bioturbation are still poorly understood. Future research should therefore investigate how the burrowing activity of mitten crabs influences key ecosystem functions in freshwater tidal marshes, such as hydrology, nutrient cycling, and carbon storage. Understanding these processes is critical for predicting long-term ecosystem responses to invasive species and for informing management and restoration strategies in dynamic tidal environments.

### **5.5 Acknowledgements**

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# 6

## ***Hepatospora eriocheir* infection affects the fitness of adult Chinese mitten crabs in their non-native range**

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## Abstract

For many invasive crustaceans, including the Chinese mitten crab (*Eriocheir sinensis*), the prevalence and impact of parasites in their introduced range remain largely unexplored. The microsporidium *Hepatospora eriocheir* was recently detected in mitten crabs across Europe, yet little is known about its physiological effects on its host. In this study, we experimentally investigated the influence of *H. eriocheir* infection on physiological biomarkers in adult mitten crabs collected from rivers in Germany and Belgium. We assessed metabolic rates (routine and maximum metabolic rate, aerobic scope), haemolymph glucose and lactate levels, and lipid content in the hepatopancreas. Infection prevalence reached 27 % in adult Chinese mitten crabs from the Scheldt population (Belgium) and 38 % in individuals from the Elbe population (Germany). Infected individuals showed a significantly reduced maximum metabolic rate and aerobic scope, while the routine metabolic rate remained unaffected. Glucose levels were lower in infected crabs both at rest and post-exercise, which indicates that energy reserves have dropped, whereas lactate levels showed no significant difference. The lipid content in the hepatopancreas was also reduced, likely due to the direct exploitation of the host's resources by the parasite and the damage caused to the hepatopancreatic tissue. Together, these patterns indicate an impaired energy metabolism, which may hamper energetically demanding activities such as migration and reproduction. The Chinese mitten crab is a catadromous species, and as the examined crabs were at the life stage when they commence a long-distance migration for their single reproductive event, infection by *H. eriocheir* may compromise their ability to complete this migration and successfully reproduce.

## 6.1 Introduction

Parasite escape, which is part of the enemy release hypothesis, is considered one of the key mechanisms through which invasive species can thrive in their non-native range (Blakeslee et al., 2013). Thanks to the absence or reduced diversity and abundance of pathogens, invasive species can allocate more energy to growth and reproduction (Bojko et al., 2021; Torchin et al., 2001). However, for most invasive crustaceans, data on parasite presence and their potential impacts in the introduced range remain scarce (Bojko et al., 2021). In the case of the Chinese mitten crab (*Eriocheir sinensis*, H. Milne-Edwards, 1853), various studies have reported the presence of the parasitic microsporidium *Hepatospora eriocheir* (Wang and Chen, 2007) in its introduced range (Normant-Saremba et al., 2024; Stachnik et al., 2025; Stentiford et al., 2011). However, the effects of the parasite on host fitness are poorly understood.

Microsporidia are a diverse group of obligate intracellular parasites, closely related to fungi, known to infect a wide range of animal hosts across different environments. They are increasingly recognised as important pathogens of aquatic invertebrates (Stentiford and Dunn, 2014). Currently, *H. eriocheir* is the only microsporidium known to infect the Chinese mitten crab. The Chinese mitten crab is native to East Asia, where it is an economically important species, particularly in China (Cheng et al., 2018). Recent numbers estimate that it is the third most cultivated crustacean in global aquaculture (FAO, 2022). At the same time, it is also a well-known invasive species, with vast established populations in many European rivers and estuaries (Dittel and Epifanio, 2009; Ewers et al., 2023).

The microsporidium *H. eriocheir* was originally described based on the analysis of diseased Chinese mitten crabs collected from aquaculture ponds in China between 1999 and 2001 (Wang and Chen, 2007). Interest in *H. eriocheir* further increased following outbreaks of hepatopancreatic necrosis disease (HPND) in Chinese mitten crab aquaculture since 2015, especially in Jiangsu Province, China (Cao et al., 2024; Ding et al., 2016; Hou et al., 2023, 2022). HPND reduces the growth rate and increases mortality in farmed Chinese mitten crabs (Ding et al., 2016). Although these outbreaks were previously linked to *H. eriocheir* infections, other studies suggest that the microsporidium is unlikely to be the sole or primary cause (Huang et al., 2021; Pan et al., 2017; Shen et al., 2017, 2021).

In the non-native range of the Chinese mitten crab in Europe, *H. eriocheir* was first detected in 2003 in specimens collected from the Thames Estuary, the United

Kingdom, where infection prevalence reached up to 70 % (Stentiford et al., 2011). More recently, its presence has been confirmed in mitten crabs from the Vistula Lagoon, located on the Baltic Sea coast of Poland, as well as in the Elbe River in Germany and the Kleine Nete River in Belgium (Stachnik et al., 2025). Infection prevalence varies geographically, with 52 % of the crabs infected in Poland, 44 % in Germany, and 33 % in Belgium (Normant-Saremba et al., 2024; Stachnik et al., 2025).

It has been hypothesised that the infection is spread through spores in the faeces of infected crabs, which are then ingested by other non-infected individuals (Stentiford and Dunn, 2014). There are no external signs of disease; the pathogen is reported to be highly tissue-specific, infecting primarily the cytoplasm of the epithelial cells of the hepatopancreas (Ding, 2021; Ding et al., 2016; Stentiford et al., 2011). Nevertheless, low parasitic loads can be detected in other organs of individuals heavily infected with the parasite (Ding et al., 2017). Typically, the hepatopancreas of infected crabs becomes pale or nearly white, in contrast to the bright yellow-orange colour in healthy crabs (Ding, 2021; Ding et al., 2017).

The consequences of infection with *H. eriocheir* have been investigated thoroughly in the native range of the Chinese mitten crab. Over time, the parasite severely damages the hepatopancreas tissue and can affect key physiological processes, including lipid and energy metabolism, and the gut microbiome of its host (Cao et al., 2024; Ding, 2021; Ding et al., 2018; Hou et al., 2023). Infection significantly alters the hepatopancreatic lipid profile, and causes, amongst others, a sharp decrease in triglycerides and diglycerides, which serve as major energy reserves (Ding, 2021). Mitochondria of host cells have been observed to cluster around the parasite, possibly facilitating direct energy exploitation (Ding, 2021; Ding et al., 2018). Transcriptome sequencing supports this, as infection alters ATP-binding proteins and metabolism genes, suggesting that the parasite hijacks the energy metabolism of its host (Hou et al., 2022). These findings suggest that *H. eriocheir* infection may impair host energy supply and physiological performance, which likely affects the overall fitness, however, physiological measurements are lacking.

Commonly used physiological biomarkers in crustaceans include metabolic rates, haemolymph glucose and lactate levels, and lipid reserves (e.g. Houlihan et al., 1984; Thibodeaux et al., 2009). The routine metabolic rate (RMR) reflects the oxygen consumption of an organism at rest, while the maximum metabolic rate (MMR) represents the peak in oxygen use during intense activity. The difference between these two is the aerobic scope (AS), which represents the available aerobic capacity to support energy-demanding functions beyond basal maintenance, such as movement,

response to environmental stress, and reproduction (Sokolova et al., 2012). Glucose serves as a major energy source and reflects the immediate availability of metabolic fuel for activity (Jimenez and Kinsey, 2015). Lactate, on the other hand, is the end product of anaerobic glycolysis in crustaceans and accumulates when oxygen is limiting, e.g. during high-intensity activity or hypoxia (Henry et al., 1994; Zou et al., 1996). Together, these metabolites provide insight into the organism's ability to meet energetic demands. Finally, lipid reserves in the hepatopancreas reflect long-term energy storage and are of high importance for reproduction and overcoming periods of food scarcity (Jimenez and Kinsey, 2015; Plaistow et al., 2001; Sokolova et al., 2012).

Given the documented physiological effects of *H. eriocheir* in aquaculture within the Chinese mitten crab's native range, similar impacts may be expected in introduced populations. This study aims to examine the impact of *H. eriocheir* infection on key physiological biomarkers and to assess whether similar effects occur in Chinese mitten crabs within their non-native range. Wild adult mitten crabs were collected from two rivers in their introduced range (Germany and Belgium), to assess whether the observed effects were consistent across different parts of the non-native range. We evaluated the effect of infection on metabolic rates (RMR, MMR, and AS) and haemolymph metabolism (glucose and lactate levels) at rest and post-exercise, and tissue lipid content in the hepatopancreas. Based on findings from the species' native range, we hypothesized that infected crabs would show elevated metabolic rates and haemolymph metabolite levels at rest, but reduced values post-exercise, compared to controls. Furthermore, we expected infection to deplete energy reserves, reflected by lower lipid stores in the hepatopancreas.

## **6.2 Materials and methods**

### **6.2.1 Chinese mitten crab collection**

To capture potential regional variation in physiological responses, we sampled in October 2023 fifty wild Chinese mitten crabs from areas with well-established mitten crab populations: Belgium (Scheldt River basin) and Germany (Elbe River basin). To minimise physiological size-related effects, specimens of similar size were selected as far as availability permitted, while also ensuring an approximately balanced sex ratio. All crabs were intact or missing no more than one walking leg. In Belgium, crabs were obtained from a specialised trap for mitten crabs on the Kleine Nete River in Grobbendonk (51°11'35.3" N, 4°45'23.1" E) (see Schoelynck et al., 2021). In Germany, specimens were obtained from a fisherman, who caught them using fyke nets in the Kiel Canal near Rade (54°20'33.3" N, 9°44'59.7" E).

All crabs were transported to the ECOSPHERE laboratory at the University of Antwerp (Campus Groenenborger) in cooling boxes lined with river water-soaked towels to maintain a moist environment. Upon arrival, standard morphometric measurements were taken: carapace width (measured behind the fourth lateral spine) was recorded to the nearest 0.01 mm using a digital calliper, body mass was measured to the nearest gram with a digital balance, and sex was determined based on pleon morphology. Each crab was marked on the carapace using a permanent marker for individual identification.

Crabs originating from Belgium included 26 females and 23 males, with an average carapace width of  $57.2 \pm 5.9$  mm (range: 46.4 – 74.1 mm) and an average mass of  $84 \pm 27$  g (range: 43 – 177 g). From Germany, 21 female and 29 male crabs were used, with an average width of  $59.0 \pm 6.0$  mm (range: 51.4 – 77.8 mm) and an average mass of  $105 \pm 39$  g (range: 52 – 237 g).

For the duration of the study, crabs were housed individually in transparent plastic tanks (39 × 28 × 14 cm) containing 7 L of tap water. The tanks were placed in a climate-controlled chamber set to 16°C, reflecting ambient conditions at the time of capture. Continuous aeration was provided, and a 10-hour light cycle per day was maintained. Each tank was equipped with a terracotta plant pot (11 cm diameter, 9 cm height), which served as a shelter. The crabs were left to acclimatise for at least 24 hours before the experiments began, and were fasted throughout this period.

At the end of the study, all crabs were euthanised by freezing.

### **6.2.2 Respirometry**

Respiration experiments were conducted in a climate-controlled chamber maintained at 16°C, mimicking the ambient water temperature in the two river systems. Crabs were placed in cylindrical plastic respirometry chambers (19.0 cm diameter, 13.5 cm height) with a total volume of 3.25 L. To prevent oxygen exchange with the surrounding environment, each of the five chambers was sealed with a tightly fitting lid, through which oxygen probes (WTW Oxi 3310 SET 1, Xylem Analytics Germany GmbH) were inserted in an airtight manner. Magnetic stirring plates (IKA Combimag RCT/RHT, IKA-Werke GmbH, Germany) were used to ensure continuous water circulation, with a 1 cm stir bar positioned at the base of each chamber. A metal grid was placed 2 cm above the stir bar to prevent disturbance to and from the crab.

Before introducing the crabs, each chamber was filled with aerated tap water, ensuring no air bubbles remained before sealing. Background oxygen consumption of the water

was recorded continuously for five minutes at a rate of one measurement per minute. Following this, a single crab was introduced and acclimatised for 10 minutes. The oxygen consumption was then measured over the subsequent 20 minutes. The routine metabolic rate (RMR) was calculated based on the average oxygen decline rate throughout this 20-minute measurement period.

To evaluate post-exercise oxygen consumption, crabs were subjected to an exhaustion trial in which they were repeatedly flipped onto their backs on a solid surface outside water (Booth and McMahon, 1985). The trial continued until the individual could no longer right itself and remained immobile for 10 seconds, even after being placed back on its legs. The number of turns required to reach exhaustion was recorded.

Immediately afterwards, the crab was returned to the respirometry chamber, and oxygen consumption was monitored for an additional 60 minutes. The maximum metabolic rate (MMR), representing the peak in oxygen uptake capacity, was determined by identifying the steepest decline in oxygen concentration over a one-minute interval (Opinion et al., 2020).

After the trial, crabs were returned to their holding tanks, and background respiration was reassessed through a blank run.

Aerobic scope (AS), a measure of the crab's capacity for aerobic activity, was calculated following the approach outlined by Opinion et al. (2020). AS was determined by subtracting the mass-specific RMR from MMR, with both metabolic rates ( $MO_2$ ,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) calculated using the following equation:

$$MO_2 = \frac{-1 \times (m_{crab} - m_c) \times V}{M}$$

With  $m_x$  representing the change in oxygen concentration ( $\Delta \text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ ) during the measurement period (with  $m_{crab}$  corresponding to the respirometer containing the crab and  $m_c$  to the control respiration rate of an empty respirometer),  $V$  is the respirometer volume corrected for the volume of the crab (L), and  $M$  is the body mass of the crab (kg).

### 6.2.3 Haemolymph extraction and analysis

Following the respiration measurements at rest and post-exercise, haemolymph samples (0.1-0.2 mL) were collected using a 1 mL syringe (Soft-Ject-F) equipped with a Sterican needle (0.6 mm diameter, 25 mm length, B. Braun Melsungen AG,

Germany). The haemolymph was drawn from the coxa of the third walking leg on the left side after rest and on the right side post-exercise. The collected haemolymph was immediately transferred to 2.0 mL tubes (Greiner Bio-One, Finckenhausen, Germany) and stored at -20°C until analysis.

Glucose and lactate concentrations in the haemolymph at rest and post-exercise were quantified using UV-based assay kits: Enzytec™ Liquid D-Glucose and Enzytec™ Liquid L-Lactic Acid (R-Biopharm AG, Darmstadt, Germany). Standard curves were generated from dilution series of Enzytec™ Liquid Multi-Sugar Standard High (E8445) for glucose and Enzytec™ Liquid Multi-Acid Standard High (E8465) for lactate. Absorbance was measured at 340 nm using an automated spectrophotometer (Spark® Multimode Microplate Reader, Tecan, Männedorf, Switzerland).

#### **6.2.4 Molecular detection of *Hepatospora eriocheir* infection**

After sacrifice, a pancreatic tissue sample (1 × 1 cm) was collected from each individual using a sterile scalpel and transferred to a tube with 80 % ethanol (1/3 ratio) and stored in a refrigerator until analysis. The collected tissues were lysed, and DNA was isolated using the Qiaamp DNA Mini kit (Qiagen, Hilden, Germany). Sequences of the small subunit ribosomal RNA (SSU rRNA) gene were obtained using the MF1/MR1 primer set according to the method described by Tourtip et al. (2009). PCR products were purified with QIAquick PCR kit (Qiagen, Hilden, Germany) and Sanger sequenced in both directions through Genomed laboratories (Poland) sequencing service. The resulting sequences were edited with Geneious R7 software (<https://www.geneious.com>) and aligned with the Muscle function of MEGA X software. Aligned sequences were uploaded to Basic Local Alignment Search Tool (BLAST) to compare them against existing databases and identify similar sequences (Sayers et al., 2025). The similarity of the nucleotide sequences and maximum-likelihood analyses were performed in the MEGA X (Kumar et al., 2018).

#### **6.2.5 Lipid content**

A tissue sample (1 × 1 cm) of the hepatopancreas was collected using a sterile scalpel and placed into a 1.5 mL tube. Samples were stored at -20°C until lipid analysis.

Lipid content was determined gravimetrically, following the method described by Xu et al. (2015). After homogenisation, all samples were freeze-dried. A subsample of 0.06-0.89 g dry weight was transferred to a 15 mL polypropylene tube containing 5 mL of hexane:acetone (3:1, v/v) mixture. The closed tube was vortexed for 1 minute, followed

by ultrasonication for 20 minutes. This was followed by a second vortexing step of 30 seconds and centrifugation for 3 minutes.

From the resulting supernatant, 1 mL was pipetted into a pre-weighted metal tray and dried at 110°C for 1 hour. Lipid content was calculated based on the weight (0.0001 g precision) after drying and is expressed as a percentage of wet weight.

### 6.2.6 Statistical analyses

All analyses were performed in R version 4.2.2 (R Core Team, 2022), and graphs were created using the R package *ggplot2* (Wickham, 2016). The significance level for statistical analyses was set at  $p < 0.05$ .

A generalised linear model (GLM) with a negative binomial distribution and a log link function (MASS R package (Venables and Ripley, 2002)) was used to analyse the effect of country (i.e. Belgium and Germany) and infection status (i.e. infected and non-infected) on the number of turns a crab could recover from. Country, infection status, their interaction, sex, and body mass (kg) were included as fixed effects in the model.

Three separate generalised linear mixed models (GLMMs) with a Gamma distribution and a log link function (*lme4* R package (Bates et al., 2015)) were used to evaluate the effect of *H. eriocheir* infection on routine metabolic rate (RMR), maximum metabolic rate (MMR), and aerobic scope (AS). Country, infection status, their interaction, sex, and body mass were included as fixed effects, while oxygen probe ID was included as a random effect.

Two separate GLMMs with a Gamma distribution and a log link function were applied to assess the effect of *H. eriocheir* infection on glucose and lactate levels in the haemolymph at rest and post-exercise. Country, infection status, physiological state (i.e. rest and post-exercise), their interactions (infection status × country, and infection status × physiological state), sex, and body mass were included as fixed effects. Crab ID was included as a random effect to account for repeated measures of the same individual.

A GLM with a beta distribution and a logit link function (*betareg* R package (Grün et al., 2012)) was used to investigate the effect of *H. eriocheir* and country on the total lipid content of the hepatopancreas tissue. Country, infection status, their interaction, sex, and body mass were included as fixed effects.

Stepwise backward selection based on the Akaike information criterion (AIC) was used to determine whether variables should be retained in each model. Because country

and infection status were the main variables of interest, they were retained in all models to allow hypothesis testing. Post-hoc pairwise comparisons with Tukey-adjustment were performed for each significant categorical predictor (emmeans R package (Lenth, 2017)).

Finally, the correlations among the eight measured biometric parameters (number of turns, routine metabolic rate, maximum metabolic rate, aerobic scope, glucose at rest and post-exercise, lactate at rest and post-exercise, and lipid content of the hepatopancreatic tissue) were examined using Spearman rank correlation analysis (stats R package (R Core Team, 2022)).

### 6.3 Results

#### 6.3.1 Molecular detection of *Hepatospora eriocheir* infection

A total of 99 Chinese mitten crabs were analysed. In the German population, 19 out of 50 individuals (38 %) were infected with *H. eriocheir*, compared to 13 out of 49 individuals (27 %) in the Belgian population (Fig. 6.1). According to BLAST analysis, the 18S microsporidian sequences from infected crabs in Germany and Belgium were 99–100 % similar to each other (blastn; coverage = 99.5 %; evalue = 0). These sequences were also 99–100 % similar to 18S sequences obtained in previous studies of infected mitten crabs from Poland, Germany, and Belgium. Maximum likelihood phylogenetic analysis placed all *H. eriocheir* isolates on a single branch, with 100 % bootstrap support (data not shown).

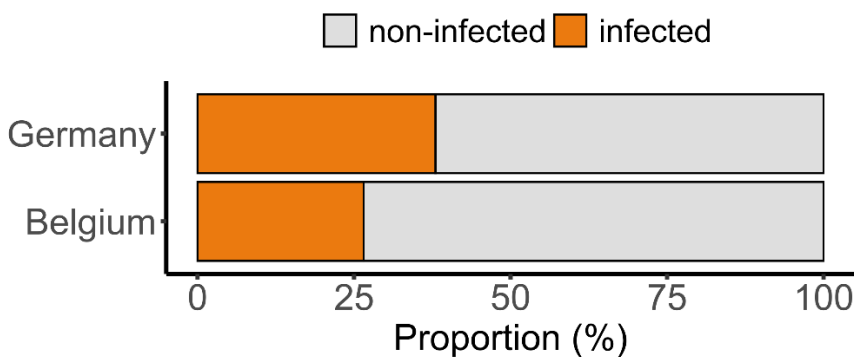


Fig. 6.1 Proportion (%) of infected (orange) and non-infected (grey) crabs per country.

### 6.3.2 Exercise

On average ( $\pm$  SD), non-infected crabs completed  $41 \pm 18$  turns (range: 6–118), while infected crabs completed  $36 \pm 15$  turns (range: 8–68) before becoming immobile. The interaction between infection status and country was not retained in the final model. Infection status had no statistically significant effect on the number of turns completed (non-infected: estimate = 0.153,  $z = 1.72$ ,  $p = 0.085$ ), nor was there a significant effect of country of origin (Germany: estimate = 0.121,  $z = 1.40$ ,  $p = 0.162$ ). However, the model did indicate a significant effect of sex (male: estimate = -0.183,  $z = -2.05$ ,  $p < 0.05$ ), with females (mean = 42,  $CI_{0.95}[37 - 47]$ ) completing more turns than males (mean = 35,  $CI_{0.95}[31 - 39]$ ). In addition, body mass had a small but significant negative effect on the number of turns (estimate = -0.00286,  $z = -2.13$ ,  $p < 0.05$ ).

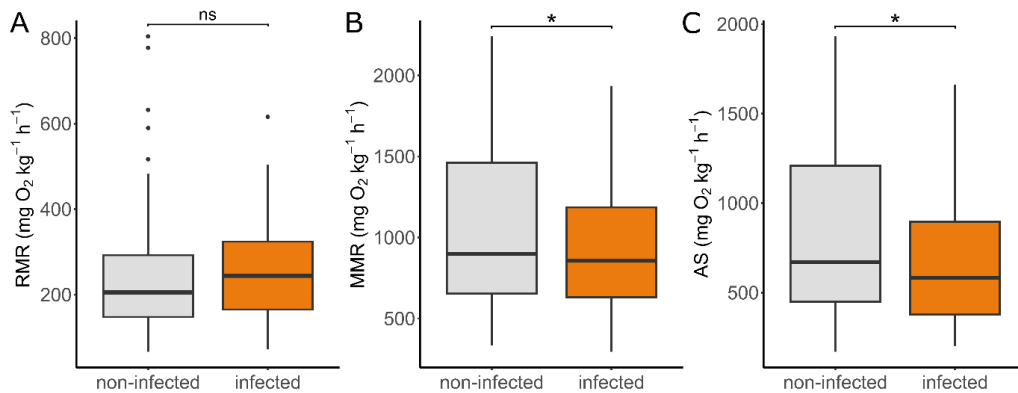
### 6.3.3 Respirometry

The interaction between infection status and country, as well as the effect of sex, were not retained in the final models for RMR, MMR and AS.

The model did not indicate a significant effect of infection status (non-infected: estimate = -0.068,  $z = -0.79$ ,  $p = 0.433$ ) or country (Germany: estimate = 0.008,  $z = 0.08$ ,  $p = 0.925$ ) on RMR (**Fig. 6.2A**). However, RMR decreases significantly with increasing body mass (estimate = -8.855,  $z = -8.55$ ,  $p < 0.001$ ).

*H. eriocheir* infection had a small but significant effect on MMR (**Fig. 6.2B**), with infected crabs demonstrating lower MMR (mean =  $892 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ,  $CI_{0.95}[697 - 1141]$ ) compared to non-infected crabs (mean =  $1059 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ,  $CI_{0.95}[846 - 1325]$ ; estimate = 0.172,  $z = 2.02$ ,  $p = 0.043$ ). Country had no significant effect on MMR (Germany: estimate = 0.094,  $z = 1.14$ ,  $p = 0.253$ ), whereas MMR decreased significantly with increasing body mass (estimate = -7.475,  $z = -7.07$ ,  $p < 0.001$ ).

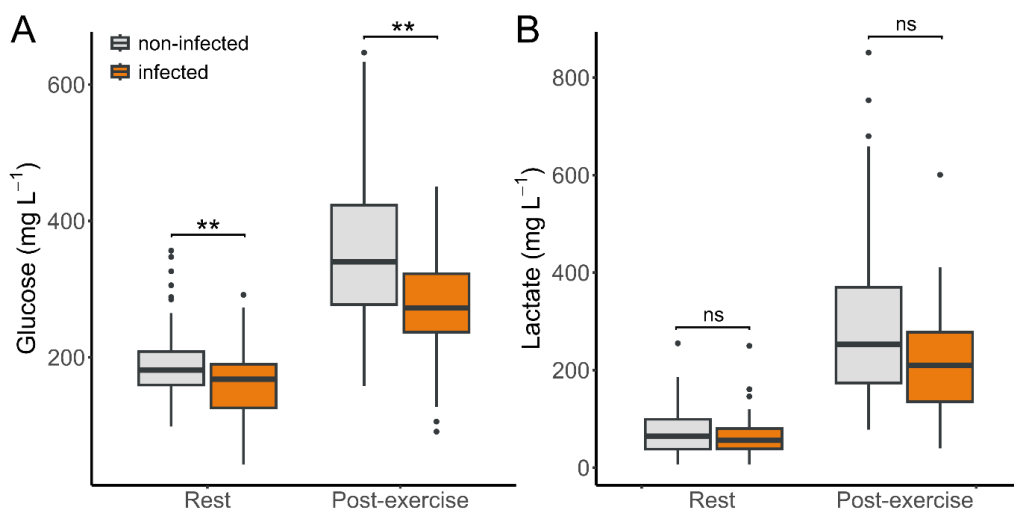
Similarly, infected crabs showed significantly lower AS (mean =  $634 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ,  $CI_{0.95}[497 - 810]$ ) compared to non-infected crabs (mean =  $808 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ,  $CI_{0.95}[661 - 988]$ ; estimate = 0.242,  $z = 2.11$ ,  $p = 0.035$ ) (**Fig. 6.2C**). Country had no significant effect on AS (Germany: estimate = 0.114,  $z = 1.03$ ,  $p = 0.305$ ), while AS decreased significantly with increasing body mass (estimate = -6.907,  $z = -4.80$ ,  $p < 0.001$ ).



**Fig. 6.2** The effect of *H. eriocheir* infection on (A) routine metabolic rate (SMR,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ), (B) maximum metabolic rate (MMR,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ), and (C) aerobic scope (AS,  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) of adult Chinese mitten crabs. Data of infected crabs ( $n = 28$ ) are shown in orange, and non-infected crabs ( $n = 62$ ) in grey. The midline represents the median, the box shows the interquartile range (IQR; 25<sup>th</sup> to 75<sup>th</sup> percentile), and the whisker lines extend to the smallest and largest values within  $1.5 \times \text{IQR}$  from the quartiles. Significance is indicated above the boxplots: ‘ns’ = not significant, ‘\*’ =  $p < 0.05$ .

#### 6.3.4 Glucose and lactate

The interactions between infection status and country, and between physiological state and infection status, as well as the main effects of sex and body mass, were not retained in the final models for glucose and lactate. Haemolymph glucose levels were significantly higher after exercise (mean =  $303 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[278 - 331]$ ; estimate =  $0.604$ ,  $z = 18.77$ ,  $p < 0.001$ ) compared to rest (mean =  $166 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[152 - 181]$ ). The model revealed that *H. eriocheir* infection significantly affected glucose levels (**Fig. 6.3A**), with infected crabs showing lower haemolymph glucose levels (mean =  $200 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[175 - 229]$ ) than non-infected crabs (mean =  $250 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[228 - 275]$ ; estimate =  $0.223$ ,  $z = 2.67$ ,  $p < 0.01$ ). Country of origin had no significant effect on glucose levels (Germany: estimate =  $0.025$ ,  $z = 0.32$ ,  $p = 0.750$ ).

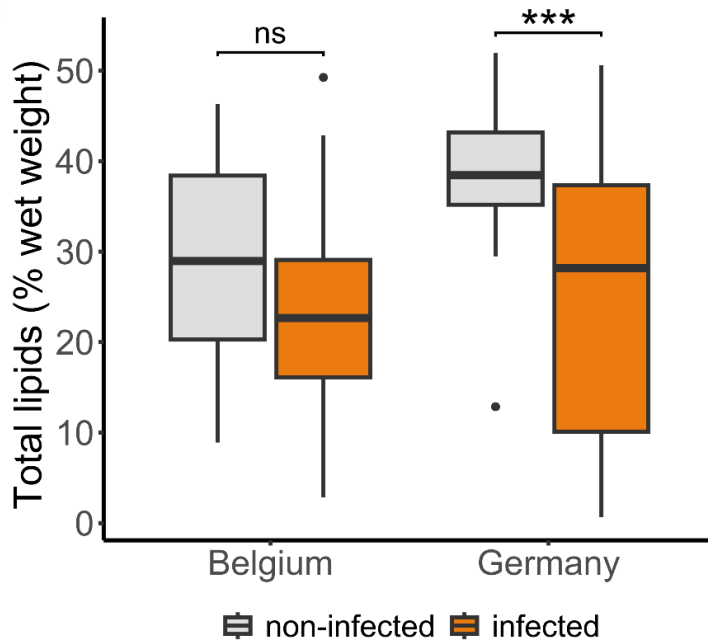


**Fig. 6.3** The effect of *H. eriocheir* infection on haemolymph (A) glucose and (B) lactate levels ( $\text{mg L}^{-1}$ ) of adult Chinese mitten crabs at rest and post-exercise. Data of infected crabs ( $n = 32$ ) are shown in orange, and non-infected crabs ( $n = 65$ ) in grey. The midline represents the median, the box shows the interquartile range (IQR; 25<sup>th</sup> to 75<sup>th</sup> percentile), and the whisker lines extend to the smallest and largest values within  $1.5 \times \text{IQR}$  from the quartiles. Significance is indicated above the boxplots: ‘ns’ = not significant, ‘\*\*’ =  $p < 0.01$ .

Similarly, haemolymph lactate levels significantly increased post-exercise (mean =  $222.8 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[193 - 257]$ ; estimate = 1.316,  $z = 22.69$ ,  $p < 0.001$ ) compared to rest (mean =  $59.8 \text{ mg L}^{-1}$ ,  $\text{CI}_{0.95}[52 - 69]$ ). However, *H. eriocheir* infection (non-infected: estimate = 0.218,  $z = 1.62$ ,  $p = 0.106$ ) and country (Germany: estimate = 0.168,  $z = 1.33$ ,  $p = 0.185$ ) did not significantly influence lactate concentrations (**Fig. 6.3B**).

### 6.3.5 Total lipids

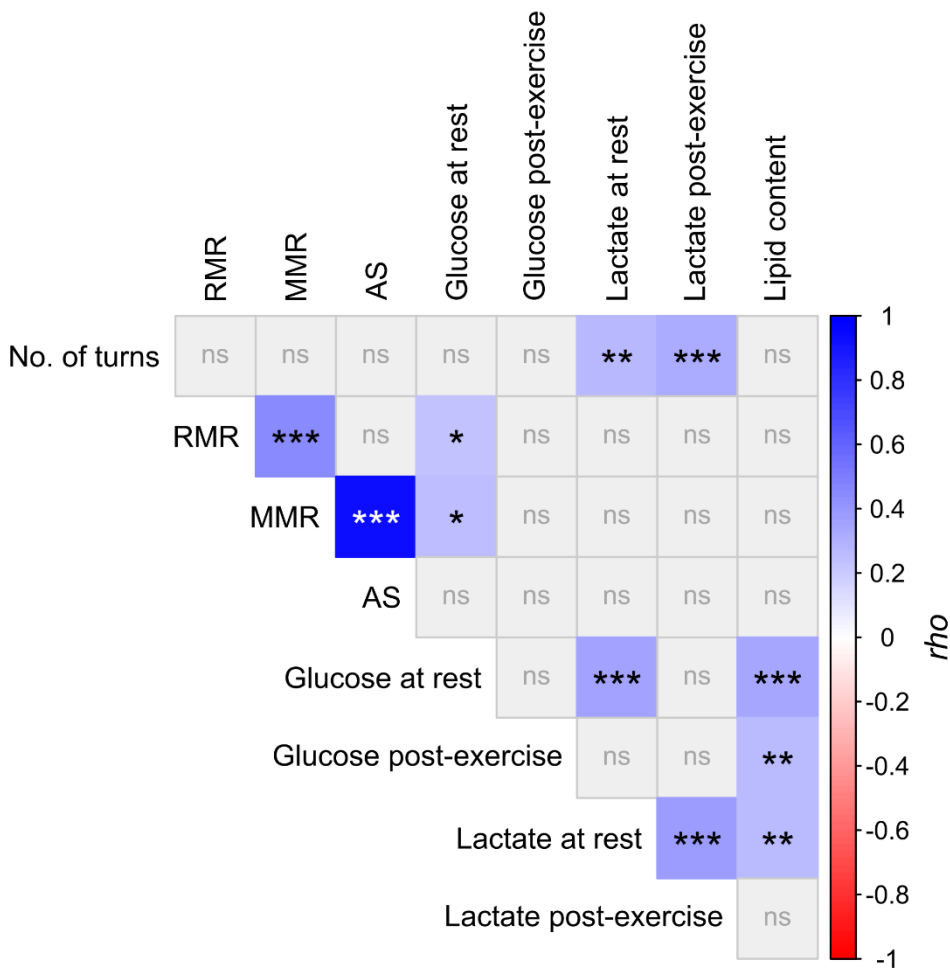
The effects of sex and body mass were not retained in the final model. The model indicated a significant interaction between country and infection status on the lipid content of the hepatopancreas tissue (Germany:non-infected: estimate = 0.596,  $z = 2.08$ ,  $p < 0.05$ ). Lipid content was significantly lower in infected crabs (mean = 0.208,  $\text{CI}_{0.95}[0.156 - 0.259]$ ; estimate = -0.182,  $z = -4.99$ ,  $p < 0.001$ ) compared to non-infected crabs (mean = 0.390,  $\text{CI}_{0.95}[0.340 - 0.440]$ ) in Germany; however, there was no significant difference between infected (mean = 0.237,  $\text{CI}_{0.95}[0.171 - 0.304]$ ; estimate = -0.057,  $z = -1.42$ ,  $p = 0.487$ ) and non-infected crabs (mean = 0.294,  $\text{CI}_{0.95}[0.252 - 0.337]$ ) in Belgium (**Fig. 6.4**).



**Fig. 6.4** The effect of *H. eriocheir* infection on lipid content (%) of the hepatopancreas tissue of adult Chinese mitten crabs captured in Belgium and Germany. Data of infected crabs (Belgium  $n = 12$ ; Germany  $n = 18$ ) are shown in orange, and non-infected crabs (Belgium  $n = 35$ , Germany  $n = 30$ ) in grey. The midline represents the median, the box shows the interquartile range (IQR; 25<sup>th</sup> to 75<sup>th</sup> percentile), and the whisker lines extend to the smallest and largest values within  $1.5 \times$  IQR from the quartiles. Significance is indicated above the boxplots: 'ns' = not significant, '\*\*\*' =  $p < 0.001$ .

### 6.3.6 Correlations among biometric parameters

Spearman's rank correlation analysis (**Fig. 6.5**) revealed a moderate positive correlation between RMR and MMR ( $\rho = 0.45$ ), as well as a strong positive correlation between MMR and AS ( $\rho = 0.93$ ). Additionally, both RMR and MMR were weakly positively correlated with haemolymph glucose levels at rest ( $\rho = 0.23$  and  $0.25$ , respectively). Haemolymph glucose levels (at rest and post-exercise), as well as lactate levels at rest, showed weak positive correlations with lipid content in the hepatopancreas ( $\rho = 0.35$ ,  $0.27$ , and  $0.27$ ). Both resting ( $\rho = 0.28$ ) and post-exercise lactate levels ( $\rho = 0.33$ ) are weakly positively correlated with the number of turns a crab could complete. Finally, lactate levels at rest were positively correlated with both glucose levels at rest ( $\rho = 0.35$ ) and lactate levels post-exercise ( $\rho = 0.38$ ).



**Fig. 6.5** Heatmap showing the results of the Spearman’s rank correlation analysis between the measured biomarkers, with RMR = routine metabolic rate, MMR = maximum metabolic rate, and AS = aerobic scope. The colours of the grid represent the strength and direction of the correlation coefficient ( $\rho$ ) in case of a significant correlation. Significance is indicated in each cell: ‘ns’ = not significant, ‘\*’ =  $p < 0.05$ , ‘\*\*’ =  $p < 0.01$ , ‘\*\*\*’ =  $p < 0.001$ .

## 6.4 Discussion

This study demonstrates that the infection by the microsporidian pathogen *Hepatospora eriocheir* can compromise the physiological condition and performance of adult Chinese mitten crabs in their non-native range. Specifically, infected crabs showed significantly reduced maximum metabolic rates, aerobic scope, resting and

post-exercise haemolymph glucose levels, and hepatopancreatic lipid content compared to uninfected individuals.

Typically, parasitic infections induce an immune response that raises basal metabolic demands, which is often reflected in elevated oxygen consumption as well as increased glucose and lactate levels in the haemolymph at rest (Binning et al., 2017; Burnett and Burnett, 2015; Eddy et al., 2007; H. Wu et al., 2017). This pattern was also expected for *H. eriocheir* infection in the Chinese mitten crab, as previous studies in the native range have reported increased immune-related activity and altered oxidative metabolism in the hepatopancreatic tissue following infection (Ding et al., 2018; Hou et al., 2023, 2022). However, in the present study, there were no significant differences in metabolic rate or lactate levels at rest between infected and uninfected individuals, and resting glucose levels were reduced in infected specimens. Nonetheless, similar reductions in glucose levels have previously been reported in other crustaceans in response to pathogens, particularly during advanced stages of infection (Stentiford et al., 2001; Vivarès and Cuq, 1981; H. Wu et al., 2017). Therefore, the observed reduction or lack of increase may reflect stage-dependent effects of the parasite. *H. eriocheir*, like all microsporidia, is an obligate intracellular parasite that lacks mitochondria and has evolved various strategies to manipulate and exploit the energy production of its host (Stentiford and Dunn, 2014). For other microsporidians, studies have shown that during early infection, the pathogen enhances specific host metabolic processes to support its proliferation (L. Zhang et al., 2023a). However, in later infection stages, key metabolic processes, including glycolysis and lipid metabolism, are altered or downregulated, even though the pathogen still exploits the energy of the host (Holt et al., 2013; Wu et al., 2022; L. Zhang et al., 2023b, 2023a). At the same time, several immune responses return to baseline levels (L. Zhang et al., 2023a). This metabolic suppression may reflect a strategy of the parasite to prevent rapid exhaustion of the host and enable chronic infection, or an adaptive response of the host to conserve energy when resources are declining (Wu et al., 2022; L. Zhang et al., 2023b). Nevertheless, prolonged infection leads to a depletion of energy reserves, as indicated by the lower glucose levels, and destruction of the hepatopancreas (Ding, 2021; Ding et al., 2018), the primary lipid and glycogen storage in crustaceans, ultimately starving the host. This was reflected in the reduced lipid reserves in the hepatopancreas, particularly in the German population of Chinese mitten crabs.

Physical activity in crabs, such as swimming, walking or self-righting, elevates the metabolic energy demand and leads to an increase in oxygen consumption, and glucose and lactate levels in the haemolymph (Adamczewska and Morris, 1994; Henry

et al., 1994; Thibodeaux et al., 2009). In the present study, both infected and uninfected crabs exhibited similar patterns of increased oxygen consumption, and glucose and lactate elevation in response to exercise, indicating that their acute physiological response to activity remained unaffected. Although a decrease in lactate levels post-exercise was observed in infected crabs compared to uninfected crabs, this difference was not statistically significant. This may indicate that the impact of *H. eriocheir* on the anaerobic carbohydrate metabolism is limited. However, the overall glucose levels measured in infected crabs post-exercise were significantly lower, indicating less energy is available for activity. This pattern is similar to chronically stressed organisms, where sustained energy demands gradually exhaust available resources (Opinion, 2025). In addition, there was a decrease in both MMR and AS, which were strongly correlated. The decline in MMR suggests that the pathogen may impair oxygen uptake and transportation in the haemolymph, though the mechanism behind this requires further investigation. In crustaceans infected with bacteria, haemocytes have been shown to aggregate around the pathogens, forming clusters that can obstruct channels in the gills, and consequently hamper gas exchange, ultimately leading to reduced metabolic rates (Burnett et al., 2006; Scholnick et al., 2006). However, another possible explanation is that infected crabs, due to depleted energy reserves such as glucose, may fatigue earlier and therefore stop the activity before reaching peak metabolic rates. In the exercise trial, infected crabs completed on average fewer turns than uninfected crabs, but this difference was not statistically significant. Interestingly, male and heavier crabs completed fewer turns, which is likely attributable to the increased mass of their chelae, particularly prominent in males (Czerniejewski and Wawrzyniak, 2006; Prestholdt et al., 2018). Finally, all mass-specific metabolic rates in this study showed a negative relationship with body mass, a pattern that is well-documented in brachyuran crabs (Griffen and Sipos, 2018).

Collectively, these findings indicate that the infection may impair the physiological performance of adult Chinese mitten crabs. The decrease in MMR observed in infected crabs corresponded to a 22 % reduction in the AS, which represents the available capacity for aerobic metabolism beyond basic maintenance (Sokolova et al., 2012). Combined with reduced glucose levels, this indicates a diminished ability to support energetically demanding activities such as predator evasion, agonistic interactions, foraging, migrating, mating or brooding (Jimenez and Kinsey, 2015; Sokolova et al., 2012). Reduced lipid content of the hepatopancreatic tissue can have further consequences, as lipids play an important role in reproduction, e.g. oocyte maturation and early larval development, and surviving periods of low food availability (Jimenez and Kinsey, 2015; Sánchez-Paz et al., 2006). The Chinese mitten crab is a catadromous

species and the studied crabs were completing their spawning migration. During this stage, energetic demands are likely at their peak, therefore infection by *H. eriocheir* may substantially reduce their physiological resilience and fitness. For one, the Chinese mitten crab typically migrates over long distances, often covering more than 100 kilometres over the span of several months (Chapter 3; Keirsebelik et al., 2025; Panning, 1938). Additionally, this migration crosses steep gradients in environmental conditions such as salinity (Chapter 3; Keirsebelik et al., 2025; Normant et al., 2012). These factors make it an energy-demanding journey, which they only undertake once in their lives (Panning, 1938). Limited energy reserves may prevent them from completing their migration. Mitten crabs usually arrive in brackish water during winter and stay in place until larval release in spring (Chapter 2 and 3; Keirsebelik et al., 2025; Rudnick et al., 2005b). Infected crabs that do reach downstream areas are left with even less energy to cope with winter conditions and to invest in reproduction and brooding. Chinese mitten crabs only reproduce once in their life, therefore this could ultimately impact their lifetime fecundity.

The effect of the infection on the examined biomarkers did generally not differ significantly between the two non-native populations, although it tended to be more pronounced in the German population, as reflected by the greater reduction in hepatopancreatic lipid content. Infection prevalence ranged from 27 % in Belgium to 38 % in Germany. This pattern is consistent with observations from the previous year, where prevalence was also lower in Belgium (33 %) than in Germany (44 %) in similarly sized crabs (Normant-Saremba et al., 2024; Stachnik et al., 2025). In other non-native populations, higher prevalences have been reported, i.e. 52 % in Poland and up to 70 % in the UK (Normant-Saremba et al., 2024; Stachnik et al., 2025; Stentiford et al., 2011). Infection prevalence likely fluctuates over time, and might be influenced by host age and population density (Stentiford and Dunn, 2014). Data on *H. eriocheir* infection prevalence in wild Chinese mitten crabs within their native range are lacking. Therefore, it is unclear whether the observed prevalence in the non-native range is high or low by comparison.

#### **6.4.1 Conclusion**

We conclude that the microsporidium *H. eriocheir* is not merely present in introduced Chinese mitten crab populations, but impacts the physiological performance of its host, revealing a vulnerability in this otherwise resilient invasive species. Future studies could explore the pathogen's impact in greater detail, including its effects on fecundity, the change in impact during different infection stages, and differences in impact between juveniles and adults, as well as between sexes.

## **6.5 Acknowledgements**

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# 7

## General discussion

## 7.1 Synthesis

Non-native species are a significant global threat to native biodiversity, particularly in freshwater ecosystems (Dudgeon et al., 2006; IPBES, 2023; Reid et al., 2019). The Chinese mitten crab is an example of a highly successful aquatic invader, having established populations across multiple regions worldwide (Ewers et al., 2023). It is recognised as one of the worst invasive species globally and is listed as a species of 'Union concern' by the European Union (European Commission, 2014; Luque et al., 2014). Nevertheless, despite its invasion success and long invasion history, much of its ecology, behaviour and impact in non-native regions remains unknown. In this thesis, we investigated the ecology of the Chinese mitten crab in Belgium, where the species has persisted for nearly 100 years (Lestage, 1935).

We have shown that the species is highly abundant and widely distributed in northern Belgium, in the region of Flanders (**Chapter 2**). Our study elucidated key aspects of its life history. The estimated life span of the Chinese mitten crab is three to six years. The crabs reproduce between October and the end of May, and megalopa settlement likely occurs in two periods: in autumn and from spring to summer (**Chapter 2**). Juvenile mitten crabs typically migrate upstream between February and June, with a peak from March until May. At some locations, there might be upstream movement year-round. Adult Chinese mitten crabs commence migrating downstream from August to December (**Chapter 2**). Through acoustic telemetry, we demonstrated adaptive movement behaviour during this spawning migration (**Chapter 3**). Adult mitten crabs migrate primarily at night and make use of selective tidal stream transport in tidal rivers to conserve energy (**Chapter 3**). Movement rates were highly variable between habitats, averaging at  $4.65 \text{ km day}^{-1}$  in non-tidal environments and  $1.29 \text{ km day}^{-1}$  in tidal rivers, but could reach up to  $15 \text{ km day}^{-1}$  (**Chapter 3**). Moreover, Chinese mitten crabs show clinal variation in size and age at maturity along the longitudinal gradient of the river basin, again highlighting the plasticity in the life history of this species (**Chapter 2**).

Both juvenile and adult Chinese mitten crabs were shown to be effective detritivores, which can feed on diverse types of terrestrial leaf litter (**Chapter 4**). Their feeding behaviour increased the production of fine particulate organic matter, dissolved nitrogen concentrations, and the biological oxygen demand (**Chapter 4**). Through this behaviour, Chinese mitten crabs have the potential to accelerate leaf litter breakdown and nutrient cycling in freshwater habitats (**Chapter 4**). In freshwater tidal marshes along the Scheldt Estuary, the species extensively burrows in banks of the creek system (**Chapter 5**). The dimensions of these burrows positively scale with crab size.

Burrow densities varied between the two studied marshes and showed temporal variation at one site. Typically, higher burrow densities were associated with steep banks and locations close to the inlet of the area, while larger burrows typically occurred on steep slopes, in sediments with a small grain size, and generally little sediment turnover. High burrow densities ( $\geq 30$  burrows  $\text{m}^{-2}$ ) in the restored tidal marsh were associated with riverbank erosion (**Chapter 5**).

Finally, we documented a high prevalence of the microsporidian parasite *Hepatospora eriocheir* in non-native populations of the Chinese mitten crab in both Belgium and Germany (**Chapter 6**). The intracellular pathogen had adverse effects on the hosts' physiological condition, through a reduction of the aerobic scope and depletion of the energy reserves, which was evidenced by lower haemolymph glucose levels and lipid content of the hepatopancreas. This suggests a reduced capability of infected crabs for energetically demanding activities (**Chapter 6**).

Collectively, these findings contribute to a deeper understanding of the species' ecology in its non-native range and shed light on the biological traits underpinning its invasion success and ecological impact in Belgium. These topics and recommendations for management are further discussed in the following sections of this chapter.

## **7.2 Factors underlying the invasion success of the Chinese mitten crab**

The process of biological invasion is commonly described as a series of sequential stages: transport, introduction, establishment, and spread (Chapter 1; Blackburn et al., 2011). In the following discussion, we examine for each invasion stage the key biological traits and anthropogenic factors that have enabled the Chinese mitten crab to successfully establish and maintain persistent populations across diverse regions worldwide, including Flanders, Belgium. Finally, we discuss the potential limitations to their invasion success in Belgium.

### **7.2.1 Transport and introduction**

The Chinese mitten crab is a secondary freshwater crab, meaning it spends most of its life in freshwater but needs brackish or marine environments to complete its life cycle, in contrast to primary freshwater crabs that fulfil their entire life cycle in freshwater (Kawai and Cumberlidge, 2016; Yeo et al., 2008). While this catadromous life cycle provides an opportunity for management (Chapter 2; Schoelynck et al., 2021) it is also a key factor in the species' success as a widespread and abundant invader.

Many major international harbours worldwide are located in river estuaries (Makarova et al., 2023), and these areas often overlap with the breeding habitat of the Chinese mitten crab. This is also the case in Belgium, where mitten crabs likely reproduce in the Scheldt Estuary near the port of Antwerp, as suggested in **Chapters 2** and **3**. The port of Antwerp-Bruges is the second largest in Europe in terms of cargo volume (PortEconomics, 2025), making it an important centre for international shipping and a potential gateway for introductions. Furthermore, there have been numerous reports of ovigerous females in the Yser Estuary (**Chapter 2**) near the marina of Nieuwpoort. Mitten crabs have a high fecundity, with one brood containing between 20,000 and 1 million eggs (Anastácio et al., 2018; Hymanson et al., 1999; Kobayashi, 2001; Peters, 1938). From these eggs, similarly high numbers of pelagic larvae are released in brackish or marine water (Anger, 1991; Kim and Hwang, 1995). This high prevalence of larvae near ports greatly increases the likelihood of larvae being taken up in ballast water, facilitating both long-distance spread and secondary introductions (Novoa et al., 2020; Wasson et al., 2001).

However, the mere uptake of larvae is not sufficient for successful introduction. Environmental conditions (e.g. salinity, temperature) during transport and at the point of introduction can be highly variable and may significantly limit the survival and establishment of the invader (Ellis and Macisaac, 2009). As a result, propagule pressure and thus invasion risk tend to decline with increasing transport duration (Briski et al., 2014). Species that are successfully dispersed via ballast water are typically benthic, euryhaline organisms with high phenotypic plasticity (Ricciardi, 2006). The Chinese mitten crab demonstrates such plasticity in its larval stages. Although each larval stage has optimal temperature and salinity ranges for development, most stages can tolerate a broad range of salinities, especially when temperatures are favourable (Anger, 1991; Wang et al., 2019). For example, the first zoeal stage (Zoea I) can survive for several days in salinities from 5 to 35 ‰, while the final megalopa stage can moult successfully in a salinity range of 1 to 40 ‰ (Wang et al., 2019). Additionally, under suboptimal conditions, extra larval stages have been observed, suggesting developmental plasticity in response to environmental stress (Anger, 1991). Both larval survival and development rates will likely be enhanced by rising sea surface temperatures as a result of ongoing climate change (Ewers et al., 2023).

The species' establishment in Northwestern Europe was almost certainly a result of transport of larvae in ballast water or (juvenile) crabs in solid ballast (Herborg et al., 2003; Panning, 1938; Peters and Panning, 1933). Given the intensity of shipping

between East Asia and Europe during that period, it is likely that there were repeated introductions (Peters and Panning, 1933). In addition, ballast water likely played a major role in the species' subsequent spread across Europe (Herborg et al., 2007b, 2003). However, thanks to the implementation of ballast water regulations aimed at decreasing the introduction and spread of invasive species (Campara et al., 2019; IMO, 2004), the significance of ballast water as an introduction pathway is expected to decline. Moreover, other anthropogenic drivers and recent developments have increased the probability of alternative introduction pathways (Dittel and Epifanio, 2009; Ewers et al., 2023). The Chinese mitten crab is currently the third most cultivated crustacean in global aquaculture and has significant cultural and culinary importance in China (Cheng et al., 2018; FAO, 2022). The commercialisation of the species at both local and global scales likely contributes to its spread through both intentional and accidental introductions (Cohen and Carlton, 1997). For instance, in January 2020, authorities intercepted a shipment of 3,700 live mitten crabs that were illegally transported from Hong Kong (China) to the port of Cincinnati, Ohio (USA) (SeafoodSource, 2020). A more local example occurred in 2023 in the city of Ghent (Flanders), where live mitten crabs were illegally sold at a food market and later escaped from the vendor's stall (HLN, 2023). In another incident in 2021, bystanders prevented a man from deliberately releasing Chinese mitten crabs in a freshwater lake in northern Antwerp (Moerkerke, 2025).

*We conclude that the catadromous life cycle of the Chinese mitten crab, characterised by reproduction in brackish and marine environments, combined with its reproductive mode (high fecundity and pelagic larvae) and broad environmental tolerance of larval stages, are key biological traits that have facilitated its global spread. In addition, anthropogenic factors, including increased globalisation, the commercial and cultural significance of the species, and ongoing climate change, likely further amplify its invasive success.*

### **7.2.2 Establishment**

While many species are introduced into new regions through human-mediated pathways, only a subset succeeds in establishing and becoming invasive (Chapter 1; Blackburn et al., 2011). Empirical evidence strongly supports the notion that propagule pressure, i.e. the number of individuals per introduction event and the frequency of introduction events, plays a key role in the successful establishment of non-native invasive species (García-Berthou et al., 2005; Jeschke and Heger, 2018). In the case of the Chinese mitten crab, temporal patterns in its abundance within non-native regions suggest that propagule pressure was consistently high throughout its invasion history

(Ewers et al., 2023). Furthermore, the species has to be able to survive and reproduce, which depends on both environmental conditions and its biological characteristics (Leung and Mandrak, 2007; van Kuijk et al., 2021). This thesis highlights a suite of biological traits that underscore the ecological plasticity of the Chinese mitten crab, enabling the survival, establishment, and persistence of non-native populations.

Successful establishment first requires survival after introduction, which is facilitated by broad environmental tolerance, flexible feeding behaviour, effective predator avoidance, and general phenotypic plasticity (i.e., ‘plasticity hypothesis’) (Hänfling et al., 2011; Jeschke and Heger, 2018; van Kuijk et al., 2021; Weis, 2010). In **Chapters 2, 3 and 5**, we have shown that the Chinese mitten crab demonstrates broad environmental tolerance, occupying a wide range of habitats along the entire longitudinal river gradient. The species has been recorded in upstream freshwater systems such as shallow rivers, ponds, and lakes, as well as in man-made canals, dynamic estuarine environments, and coastal marine areas. It is worth noting that an environmental mismatch, particularly the lack of connectivity between freshwater and marine habitats, may be one of the reasons behind the failed establishment of the Chinese mitten crab in Japan (Zhang et al., 2020).

Acoustic telemetry data presented in **Chapter 3** revealed that the Chinese mitten crab shows adaptive behavioural strategies during their migration through different environments. In shallow freshwater rivers, individuals predominantly migrate at night, consistent with predator avoidance behaviour (Ibbotson et al., 2011; Šmejkal et al., 2018). In deeper, more turbid rivers, they switched from predator avoidance behaviour to an energy-conserving strategy by using selective tidal stream transport (Chapter 3; Keirsebelik et al., 2025). Moreover, experimental and field observations in **Chapter 5** showed that mitten crabs across a broad size range are capable of rapidly constructing burrows. This behaviour likely enhances survival by reducing predation risk and preventing desiccation during low tide in dynamic tidal habitats (Kamps, 1937; Peters and Panning, 1933; Rudnick et al., 2005a). It is particularly important for small juvenile crabs, which rely on such strategies until they grow large enough to actively migrate against the current toward upstream non-tidal freshwater environments (Panning, 1938).

Like most decapods, the Chinese mitten crab is an opportunistic omnivore that can feed on a large variety of food sources, as outlined in the literature review in **Chapter 1**. Regional differences highlight their ecological plasticity to feed and thrive in different environments (Rudnick and Resh, 2005; Wójcik-Fudalewska et al., 2019). Additionally, experiments have shown that they are competitive feeders, feeding at higher rates on

various food sources compared to native and non-native crayfish species (Doherty-Bone et al., 2018b; Rosewarne et al., 2016). In **Chapter 4**, both juvenile and adult mitten crabs showed that they can survive on terrestrial leaf litter, some of which was of poor nutritional quality. Collectively, these adaptive behavioural strategies highlight the species' capacity to respond flexibly to a broad range of conditions.

Secondly, if the introduced individuals survive, they have to establish self-sustaining populations. The reproductive mode of the Chinese mitten crab shows a combination of *r*- and *K*-selected traits (Hänfling et al., 2011; Kalinina, 2015). On the one hand, the species exhibits high fecundity, producing large numbers of pelagic larvae with limited parental care. The females do brood the eggs for several weeks up to a couple of months, offering protection during early development (Kobayashi and Matsuura, 1995c; Peters, 1938). Although larval mortality is high and population growth is strongly influenced by environmental conditions during these stages (Blumenshine et al., 2012; Z. Zhang et al., 2019d), this strategy facilitates rapid population growth, dispersal, colonisation of new habitats, and adaptive responses to dynamic environments (McMahon, 2002). On the other hand, mitten crabs only reproduce once and postpone reproduction until the last moment, after residing for a few years in freshwater (**Chapter 2**). Time to maturity varies between regions, and has been suggested to take longer in non-native populations (2-6 years) in comparison to native populations (typically 1-3 years) (Hymanson et al., 1999; Kamps, 1937; Panning, 1938; Rudnick et al., 2005b). In **Chapter 2**, we have shown that the Chinese mitten crab shows flexibility in its life history, with downstream populations maturing more rapidly and reproducing at an earlier age than upstream populations. This adaptive life history enables reproduction among individuals of different cohorts and ensures that different parts of the population reproduce each year (Hymanson et al., 1999).

*The Chinese mitten crab can tolerate and thrive in diverse habitats. The species demonstrates behaviour that enhances its survival, including flexible feeding behaviour, predator avoidance, and energy-conserving behaviour, and can adapt these behaviours in response to environmental conditions. In addition, the species' high fecundity and flexible life history facilitate rapid population growth.*

### **7.2.3 Spread**

Further spread of the species in its introduced range is achieved by passive and active dispersal. Passive transport of pelagic larvae via coastal currents has likely contributed to the spread of the Chinese mitten crab between adjacent estuaries (Herborg et al., 2005). However, the larval biology of the species in its non-native range

remains largely unexplored. It has been suggested that mitten crab larvae are largely retained within estuaries or near the coast (Anger, 1991; Hanson and Sytsma, 2008). Furthermore, the duration of larval development and the direction of the prevailing currents further constrain the geographical extent of passive dispersal (Herborg et al., 2005, 2003). As previously mentioned, human-mediated secondary spread of larvae likely played an important role (Drotz et al., 2010; Herborg et al., 2007b, 2005, 2003).

The Chinese mitten crab shows a remarkable capacity for active dispersal. Analysis of historic records indicated an average upstream range expansion of 562 km year<sup>-1</sup> between 1928 and 1938 in Northern Europe (Herborg et al., 2003). As demonstrated in **Chapters 2 and 3**, individuals are commonly observed several hundred kilometres inland and have, in exceptional cases, been recorded as far as 1,000 kilometres upstream (Herborg et al., 2003; Panning, 1938). Mitten crabs can migrate at rates of up to 15 km per day (Chapter 3; Keirsebelik et al., 2025). They are also able to climb vertical structures, disperse overland, and survive up to three days out of the water under humid conditions (Fialho et al., 2016; Marques et al., 2015), though research suggests barriers can limit their upstream movement (Robinson et al., 2019). Additionally, it is worth noting that anthropogenic factors, such as the creation of canals connecting rivers that would otherwise be isolated from one another, have further facilitated the active dispersal of the Chinese mitten crab and other non-native species (Boets et al., 2016; Czerniejewski et al., 2012b; Herborg et al., 2005).

*We conclude that both passive and active dispersal mechanisms have contributed to the spread of the Chinese mitten crab within its non-native range, with human-mediated transport likely playing a key role.*

#### **7.2.4 Potential constraints and vulnerabilities**

A reduction or absence of natural enemies (i.e., ‘enemy release hypothesis’), such as competitors, predators and parasites, can significantly contribute to the success of invasive species (Blakeslee et al., 2013; Torchin et al., 2003; Weis, 2011). In this subsection, we examine the importance of such enemies for the established mitten crab population in Belgium.

By migrating to freshwater habitats, Chinese mitten crabs in Northwestern Europe escape the intense competition and potential predation pressure from native (and other non-native) crab species that are more commonly found in brackish and marine environments. In North America, this pressure has been demonstrated to limit the distribution of invasive marine crabs, like the European shore crab (*Carcinus maenas*) (DeRivera et al., 2005; Hunt and Behrens Yamada, 2003; Jensen et al., 2007). In

brackish environments in Flanders, the species may co-occur with the native European shore crab and with other non-native species, including the Asian shore crab (*Hemigrapsus sanguineus*), brush-clawed shore crab (*Hemigrapsus takanoi*), and the Harris mud crab (*Rhithropanopeus harrisii*) (Adema, 1991; Gilbey et al., 2008; Maes et al., 1997). At the time of establishment in 1933, the only large decapod present in freshwater habitats in Belgium was the native noble crayfish (*Astacus astacus*) (Boets et al., 2012). However, its distribution was constrained, as the species prefers habitats with oxygen-rich waters and high water quality (Gerard, 1989). As a result, many aquatic habitats in Belgium remained unoccupied, leaving the ecological niche available for colonisation by other more tolerant species like the Chinese mitten crab. In contrast, in Japan, where the Chinese mitten crab has not established itself, its failure may be attributed to the presence of the closely related and functionally similar Japanese mitten crab (*Eriocheir japonica*), which could have limited its establishment through niche overlap or competitive exclusion (Z. Zhang et al., 2019b; Zhang et al., 2020). This aligns with the concept of ‘limiting similarity’, which proposes that the establishment success of non-native species is greater when they strongly differ from native species, and lower when they are functionally similar to native species (Jeschke and Heger, 2018).

Nowadays, seven non-native freshwater crayfish are found in Flanders (INBO, 2025a; Steen et al., 2023). Several of these species likely co-occur and compete with the Chinese mitten crab. The spinycheek crayfish (*Faxonius limosus*) and red swamp crayfish (*Procambarus clarkii*) are the most widespread species among them (Steen et al., 2023). Preliminary findings from a shelter competition experiment indicate that Chinese mitten crabs exhibit more aggressive behaviour than spinycheek crayfish and are more successful in maintaining their shelter (Cools et al., 2021). In San Francisco Bay, where both the red swamp crayfish and Chinese mitten crab are invasive, research has shown substantial overlap in gut content between these species, along with anecdotal evidence that mitten crabs may dominate red swamp crayfish when feeding at the same food sources (Rudnick et al., 2000; Rudnick and Resh, 2005). Based on the current distribution of the Chinese mitten crab in Flanders (**Chapter 2**), there is no evidence that its range has contracted with the emergence of non-native freshwater crayfish in the region in the 1970s (Boets et al., 2012; Wouters, 2002).

**Chapter 1** provided an overview of known predators of the Chinese mitten crab in its non-native range, including various waterbirds, predatory fish, and several mammal species. However, due to limited research, the extent to which these animals regularly prey on mitten crabs remains unclear. Predation appears to be largely opportunistic,

although there is some evidence of substantial predation pressure from species such as European eel (*Anguilla anguilla*), perch (*Perca fluviatilis*), and grey heron (*Ardea cinerea*) (Fladung, 2000). Limb loss in crustaceans is frequently used as a proxy for both predation pressure and aggressive interactions with conspecifics or other species (Jensen et al., 2007; Kobayashi and Matsuura, 1997). As shown in **Chapter 2**, the incidence of limb loss in Chinese mitten crabs in the Scheldt Estuary was relatively high and varied seasonally. However, it remains uncertain to what extent the observed incidence of autotomy reflects actual predation pressure, density-dependent interactions with conspecifics, or other interactions with co-occurring species.

In addition to competition and predation, reduced pathogen diversity or prevalence can allow non-native populations to thrive (Blakeslee et al., 2013). For example, in the case of the European shore crab, studies have shown that predation pressure is likely comparable between its native and introduced ranges (Torchin et al., 2001). However, a marked reduction in pathogen infection prevalence in the introduced range appears to contribute to its successful establishment (Torchin et al., 2001). While numerous pathogens have been described in cultured Chinese mitten crabs (Chapter 1; Ding, 2023), the prevalence of pathogens in wild non-native populations has not received much attention. Non-native mitten crabs are known to carry the crayfish plague (*Aphanomyces astaci*), bacterium *Vibrio parahaemolyticus*, dinoflagellate *Hematodinium* spp., and the microsporidium *Hepatospora eriocheir* (Kerr et al., 2025; Schrimpf et al., 2014; Stentiford et al., 2011; Wagley et al., 2009). In **Chapter 6**, we showed that between one in four and one in three Chinese mitten crabs in Flanders were infected with *H. eriocheir*, and we demonstrated that it has a measurable negative impact on the fitness of adult crabs.

*We conclude that although there is predation pressure from native species and competition with functionally similar native and non-native decapods, these factors do not seem to limit the distribution of the Chinese mitten crab in Belgium. The microsporidium H. eriocheir reduces the fitness of adult mitten crabs, but it is unknown whether it affects population dynamics.*

### 7.3 Impact in Flanders

The ecological and socio-economic impacts of the Chinese mitten crab have been extensively documented across its introduced range (**Chapter 1**). In this section, we assess the current knowledge on the potential negative and positive effects of this species in Belgium. Since the species is particularly abundant in Flanders but only rarely observed in Wallonia, our focus will be on Flanders.

### 7.3.1 Ecological impact

Given the high numbers of Chinese mitten crabs caught in the various traps across Flanders (**Chapter 2**), ecological impacts on native species through competition, predation, and habitat modification appear inevitable. In aquatic habitats, the impact of non-native invasive omnivorous species is typically linked to substantial declines in macrophyte abundance and diversity, and, to a lesser extent, reductions in the abundance of benthic invertebrates (Gallardo et al., 2016). Additionally, turbidity and dissolved nutrient concentrations tend to increase considerably, while the amount of organic matter decreases (Gallardo et al., 2016). The impact of the Chinese mitten crab fits well into this pattern, and has been demonstrated through multiple experimental studies, including **Chapter 4** (Doherty-Bone et al., 2018b; Rosewarne et al., 2016; Rudnick and Resh, 2005; Schoelynck et al., 2020). However, quantifying their ecological impact in natural settings remains challenging. This is largely due to their long-established and widespread presence in Flanders (**Chapter 2**), which complicates the application of before–after or impact–control study designs. However, with the installation of new crab traps throughout the region, an opportunity presents itself to investigate how different ecosystem components respond to reduced or eliminated mitten crab abundance. Preliminary results suggest a slight increase in macroinvertebrate diversity upstream of the crab trap in Merelbeke (Tamisaft, 2025). However, longer time series are necessary to confirm this trend with confidence. Finally, it is worth noting that native predatory species, including birds, fish, and mammals, likely benefit from the presence of the Chinese mitten crab, especially in spring when juvenile crabs are abundant.

Within the study areas investigated in **Chapter 5**, almost all riverbank stretches contained mitten crab burrows; only a few to no sections remained unaffected. Moreover, the observed burrow densities were among the highest reported in the literature (Kamps, 1937; Peters and Panning, 1933; Rudnick et al., 2005a). Our findings further indicate that burrow densities exceeding 30 burrows  $m^{-2}$  are associated with erosion (**Chapter 5**). Erosion due to the excavation of burrows can alter fine sediment dynamics, which has also been documented for burrowing crayfish (Harvey et al., 2014, 2011; Sanders et al., 2023), and may accelerate marsh degradation. However, it is likely that such intense bioturbation also affects other ecosystem functions. In the restored tidal marsh, preliminary field observations suggest that crab burrows may serve as preferential flow paths for porewater (Van Putte, 2023). Given the compacted, low-porosity soil in this area (Van Putte et al., 2020), these burrows could increase the interface between porewater and sediment, potentially enhancing nutrient exchange (Van Putte, 2023). This effect may contribute positively to marsh functioning. Further

research is thus needed to assess the broader ecological implications of burrowing by mitten crabs.

### **7.3.2 Socio-economic impact**

The most frequently documented socio-economic impacts include reduced or damaged catches of commercial fishermen, erosion of riverbanks and obstruction of water intake infrastructure (**Chapter 1**).

In Flanders, commercial fisheries are focused on the North Sea, while inland waters are predominantly used for recreational fishing. With approximately 60,000 registered anglers in Flanders in 2024 (INBO, 2025b), it is a highly popular hobby and an economically relevant group. In spring 2025, a public online survey was shared to investigate the impact of the Chinese mitten crab on the fishing experience of anglers (Ryckaert, 2025). Preliminary results based on 132 respondents indicate that large mitten crabs are frequently encountered at numerous fishing locations throughout the year. Respondents reported that Chinese mitten crabs are attracted to bait, thereby disturbing target fish species and causing damage to fishing gear, including bait and lines. These interactions ultimately negatively affect the overall angling experience. As a result, many anglers indicated that they have adapted their fishing techniques or avoid certain areas altogether due to the presence of mitten crabs. These findings illustrate both the economic and social impacts of this invasive species on an important stakeholder group. The majority of respondents expressed their support for collaboration between fishing clubs and water managers to tackle the presence of the mitten crab at fishing locations.

The findings on burrowing behaviour presented in this thesis were made in natural marshes. However, Chinese mitten crab burrows have also been observed in man-made levees (Pers. obs. H. Keirsebelik), though their distribution and density in these engineered structures have not yet been investigated. Additionally, concerns have been raised regarding the potential for concealed burrowing activity beneath riprap or rocks used to stabilise artificial levees. This behaviour could pose a risk to the structural integrity of such infrastructure and warrants further investigation.

The presence of mitten crabs in water intake infrastructure has been reported several times at different locations in Flanders. In the 1940s, a circulation pump at a power plant in Ghent was obstructed for several days (Mares, 1995). More recently, crabs have been observed in water intake infrastructure of a wastewater treatment plant operated by Aquafin in Ghent in 2024 and preceding years (Pers. comm. P. Van Loon). However, these incidents are periodic and rather uncommon, and overall, it appears

that their presence does not cause much hindrance (D'hondt et al., 2021; Pers. comm. P. Van Loon).

Other periodic nuisance occurs when Chinese mitten crabs leave the water during their migrations in spring or autumn (Adema, 1991). While most of the incidents, such as encountering a crab in the garden, on the porch, or street, do not usually cause concern, their overland dispersal can also lead to more serious problems (D'hondt et al., 2021). For instance, around 2016, repeated incidents were reported in which thousands of Chinese mitten crabs exited the Demer River in Aarschot, causing significant disturbance to residents, including a hotel owner (De Standaard, 2016). On some occasions, large numbers of crabs died (e.g., due to being run over by vehicles), resulting in unpleasant odours and attracting scavengers like rats (Pers. comm. J. Schoelynck).

*Experimental studies have established the potential ecological impact of the Chinese mitten crab, which is expected in the field to affect primarily macrophytes, detritus dynamics, and water quality, and to a lesser extent, benthic macroinvertebrates. Field research is necessary to quantify the impact of the Chinese mitten crab in real-life settings. As a food source, its presence may benefit several native species. The consequences of its burrowing behaviour on the ecological functioning of marshes require further research.*

*Resident crabs negatively impact the fishing experience of recreational anglers, while migrating crabs cause limited periodic nuisance in water-intake facilities and more substantial nuisance to residents when they exit the watercourse. The impact of burrows on the structural integrity of man-made levees is plausible and requires further research.*

## **7.4 Perspective on management**

### **7.4.1 Prevention of further spread and genetic exchange**

The Chinese mitten crab is widespread in Europe and colonised parts of North America; however, in certain regions, its abundance has remained consistently low and stable over a long period of time (Drotz et al., 2010; Ewers et al., 2023; Herborg et al., 2005; Rudnick et al., 2000). For instance, in the United Kingdom, there were multiple sightings of the Chinese mitten crab before it eventually became established in the 1990s (Herborg et al., 2005), likely due to repeated introduction events, i.e., high propagule pressure. Additionally, ecological niche models indicate that water temperature is a key factor limiting the current distribution of the species within its

non-native range (Z. Zhang et al., 2019a). With ongoing climate change, increasing water temperature is expected to cause a northward shift in the distribution of many aquatic species, potentially including the Chinese mitten crab (Bradley et al., 2024; Ricciardi et al., 2021). As some climatically suitable areas remain uncolonized, and regions that are currently at the margins of its non-native range may become more favourable over time, proactive measures are essential to prevent further spread and new introductions. Since the cost to manage invasive species escalates drastically over time, prevention remains the most cost-effective and critical pillar of invasive species management (Ahmed et al., 2022; IPBES, 2023). Moreover, a recent study indicates that national policies to prevent and control non-native invasive species, reduced the establishment rate of non-native species across Europe (Canelles et al., 2025). Potential management strategies to limit the spread of the Chinese mitten crab include ballast water treatment, ban on transportation of live mitten crabs, public awareness campaigns, early detection and rapid response efforts, and the installation of crab traps to prevent upstream colonisation.

However, in many countries within its non-native range, the Chinese mitten crab is considered naturalised, meaning that the species has established self-sustaining populations (Chapter 1 and 2; Dittel and Epifanio, 2009). As a result, active management is often abandoned because management is time-intensive, has a high cost, and is considered ineffective in reducing the overall population of the species (Keirsebelik et al., 2020). However, based on ongoing research, we argue that even in these countries where the species is naturalised, implementing strategies to prevent further spread, limit gene flow between populations, and avoid new introductions should be a minimal requirement for responsible management (Smith et al., 2020).

For example, a remarkably high diversity of the mitochondrial cytochrome c oxidase subunit I (COI) gene was observed in the Elbe Estuary (Germany), whereas many other river systems within the non-native range of the Chinese mitten crab show limited mitochondrial DNA (mtDNA) variation (Hayer et al., 2019). Therefore, new introductions either from the native range or via secondary dispersal within the non-native range could introduce novel genetic material into established populations (Ewers et al., 2023; Ironside et al., 2025). Increased genetic diversity may enhance the fitness, adaptability, and resilience of invasive populations, potentially accelerating population growth or improving survival under changing environmental conditions (Bradley et al., 2024; Smith et al., 2020). Recent studies have also demonstrated the presence of mtDNA of the Japanese mitten crab (*Eriocheir japonica*) in European populations, including in the Netherlands, Belgium, and the United Kingdom,

indicating possible introgression between the two species (Hayer et al., 2019; Homberger et al., 2022; Ironside et al., 2025). Although the high prevalence of Japanese mtDNA could be attributed to random processes, it is more likely explained by selective advantages as a result of introgression (Ewers et al. in prep.). The specific fitness benefits (e.g., increased environmental tolerance, higher fecundity) associated with the Japanese mtDNA remain uncertain and warrant further research.

One potential pathway for new introductions of the Chinese mitten crab in Belgium is the commercial exploitation of the species in the Netherlands. Although European legislation generally prohibits the translocation and sale of live mitten crabs (European Commission, 2014), the Netherlands has been granted an exemption and considers commercial harvest and sale within its borders a form of population control (CLO, 2023). While most (online) Belgian and Dutch (shell)fish vendors and restaurants appear to comply with the European ban and do not offer live mitten crabs for sale in Belgium, it remains possible to purchase them through various formal and informal channels (Moerkerke, 2025). Dutch fishermen have reportedly sold live mitten crabs to vendors in Flanders, and interviews with vendors and fishermen often indicated a lack of awareness regarding the ecological risks posed by such practices, including the potential for further spread and genetic exchange among populations (Moerkerke, 2025). Although rare, the deliberate release of Chinese mitten crabs in Flemish waters appears to occur as well (Moerkerke, 2025), and poses an additional pathway. These findings highlight the need for greater outreach and education about the ecological consequences of invasive species, not only among the general public but also among stakeholders such as fishermen, vendors, and restaurant owners, even in regions where the species is already well established. Furthermore, active enforcement of existing regulations in Belgium appears to be still limited and could be improved.

#### **7.4.2 Ecosystem restoration**

Aquatic habitats have been extensively altered by humans through flow modification, chemical pollution, eutrophication, and the construction of artificial structures, often resulting in degraded ecological quality (Reid et al., 2019). In Flanders, for example, only one (0.5 %) out of 195 rivers currently meets the standards of the European Union's Water Framework Directive for 'good ecological and chemical status' (VMM, 2025). Non-native invasive species often show greater tolerance to such disturbed environments where native species tend to decline (i.e., 'disturbance hypothesis'), allowing them to establish (González-Ortegón and Moreno-Andrés, 2021; Jeschke and Heger, 2018). Furthermore, the presence of non-native invasive species in these habitats introduces additional disturbance and is believed to facilitate further

invasions (i.e., ‘invasion meltdown’) (Jeschke and Heger, 2018; Ricciardi, 2001). Artificial structures have also been identified as potential stepping stones that facilitate the spread of non-native invasive species across hard-substrate environments (Frehse et al., 2025). Collectively, these findings demonstrate that highly modified and degraded habitats open the door for the establishment and dominance of non-native species, at the cost of native biodiversity.

Therefore, restoring aquatic systems to good ecological quality could reduce the establishment risk, abundance, and impact of aquatic non-native invasive species such as the Chinese mitten crab, and should be a priority. Enhanced habitat heterogeneity and the presence of competitive or predatory native species have been shown to limit the abundance and distribution of non-native invasive species (Alexander et al., 2015; Astorg et al., 2021; DeRivera et al., 2005; Jensen et al., 2007; Kestrup and Ricciardi, 2009). Given the direct and indirect benefits on native biodiversity, ecosystem restoration should be integrated into invasive species management.

#### **7.4.3 Application of Chinese mitten crab traps**

The application of crab traps in Flanders shows promising results in limiting the upstream dispersal of Chinese mitten crabs and bringing down local densities. Particularly at the locations Grobbendonk and Merelbeke, large numbers of crabs have been captured, while bycatch has been minimal (Chapter 2; Schoelynck et al., 2021). During fish monitoring upstream of the crab trap in Kalken in 2020, significantly fewer crabs were caught compared to the year before (prior to trap installation) (Breine et al., 2021), which is a positive indication. Long-term monitoring of crab abundance and key ecosystem properties (e.g., macrophyte and macroinvertebrate diversity and abundance, water quality) across multiple sites is necessary to evaluate the effectiveness of the trap in reducing the upstream density and ecological impact of Chinese mitten crabs. Furthermore, several practical and scientific questions need to be resolved to allow the application of these traps on a wide scale.

Further research is required to determine under which environmental and hydrological conditions traps can be effectively installed, as well as to identify the factors that influence trapping efficiency, both positively and negatively. Additionally, it has not been determined if and how the trap can be modified to be applied in different site conditions, e.g., large tidal influence, wide rivers, and high riverbanks. While field and experimental observations demonstrate that many fish species can pass the trap without difficulty (H. Keirsebelik pers. obs., P. Van Loon and P. Boets pers. comm.),

further experimental research is needed to confirm that the trap does not pose a migration barrier for bottom-dwelling fish species or species with limited swimming ability.

The trap design with return tubes provides an innovative approach to safeguard upstream habitats from high densities while keeping maintenance costs low (i.e., no need to empty the trap or discard crabs) (D'hondt et al., 2023, 2021). However, the downstream consequences on crab density and ecological impact are still unknown and warrant further investigation (D'hondt et al., 2023).

Aside from crab traps, additional management measures can be used to limit nuisance. Low-cost movement barriers consisting of smooth plastic or metal surfaces installed along engineered or natural riverbanks can effectively prevent crabs from emerging from the water and dispersing over land at locations where they cause disturbance (D'hondt et al., 2021; Panning, 1938). Furthermore, barrier-guiding systems with pitfall traps can be used on land to intercept crabs (D'hondt et al., 2021).

Fyke nets, condo traps, and cages can be useful to monitor the presence of Chinese mitten crabs, as they are easy to use and applicable at most locations (Hewitt and McDonald, 2013; Keirsebelik et al., 2020; Rudnick et al., 2005b). Additionally, Chinese mitten crabs can also be caught through trawling, in estuaries, bays or marine environments (Rudnick et al., 2000). However, the catch efficiency of these methods for mitten crabs is variable, and the selectivity is relatively low (Keirsebelik et al., 2020; Rudnick et al., 2000). Therefore, they are not suitable for application on a wide scale to reduce the density of established Chinese mitten crab populations (Keirsebelik et al., 2020).

Finally, it remains important to continue exploring new and complementary approaches to management. For instance, an innovative method has been developed to produce sterile males of the non-native invasive American bullfrog (*Lithobates catesbeianus*) (Descamps, 2022), which could potentially be interesting for other invasive species as well. In addition, targeted removal of mature crabs at breeding sites, habitat modification (Lemmers et al., 2022), or biological control (Bojko, 2017) could be investigated. Public participation, for instance, engaging anglers and local communities in reporting or removing crabs, could further support these efforts (Hänfling et al., 2011).

#### 7.4.4 Post-capture handling and disposal

As more traps are deployed across the region and in neighbouring countries as part of the Interreg project CLANCY (<https://www.interregnorthsea.eu/clancy>), the number of crabs captured will increase accordingly. In Flanders, captured crabs are most commonly euthanized by freezing in air (ANB and INBO, 2017; P. Van Loon pers. comm.). Although freezing does not induce behavioural signs of distress, it takes a long time until death (Conte et al., 2021). While some studies suggest that certain species become insensible relatively quickly during freezing, this is not consistent across all crustaceans, and prolonged neural activity has been observed in some cases (Conte et al., 2021; Weineck et al., 2018). Currently, electrical stunning is considered the most humane method to minimize or eliminate suffering before death (Conte et al., 2021). However, its effectiveness in crabs is inconclusive, as neural activity may persist although individuals are immobilised (Weineck et al., 2018), and the procedure can lead to a high incidence of limb loss (Conte et al., 2021). Therefore, more research is needed to develop and standardize optimal animal welfare protocols for decapod crustaceans.

Finally, the question of what to do with the large biomass of trapped crabs remains open. While providing the dead crabs as feed for zoo animals has been a viable solution, the biomass that can be processed in this way is limited (D'hondt et al., 2021). Further research in alternative uses, such as processing crabs into fertilizer or other products, is essential to ensure that crab trapping can be viable on a wide scale and in the long term.

*We conclude that preventing new introductions and secondary spread of the Chinese mitten crab is crucial, both in regions that are suitable for establishment but where the species is not yet present, and in areas with established populations. Key pathways that should be monitored are ballast water, escapes from trade, and intentional introductions for cultivation. Ecosystem restoration toward a more natural state can help to minimise the establishment rate and impact on non-native invasive species like the Chinese mitten crab. The crab traps specifically designed to catch Chinese mitten crabs show great potential to reduce population density and impact in areas where the species is established, and could prevent upstream spread in other areas. However, further research is required to support its wide-scale application. Post-capture handling also requires further investigation, particularly regarding the humane killing methods and the sustainable use of the biomass.*





# 8

## Supplementary materials

## 8.1 Supplementary materials Chapter 2

### List S. 1 Data sources for distribution maps Chinese mitten crab in Belgium (1933 – 2025)

- Barbier Y, Coupremagne M, Branquart E, Vanderhoeven S (2025). DEMNA-DNE : Early warning system on Introduced Species in Wallonia. Version 1.903. Service Public de Wallonie – Département d’Etude du Milieu Naturel et Agricole (SPW – DEMNA). Occurrence dataset <https://doi.org/10.15468/dc2dyg> accessed via GBIF.org on 2025-07-07.
- Boets P, Brosens D, Lock K, Adriaens T, Aelterman B, Mertens J, Goethals P L (2021). Alien macroinvertebrates in Flanders, Belgium. Version 1.9. Ghent University. Occurrence dataset <https://doi.org/10.15468/xjtfoo> accessed via GBIF.org on 2025-07-07.
- Breine J, Brosens D, Desmet P (2025). VIS - Estuarine monitoring in Flanders, Belgium (post 2013). Version 1.16. Research Institute for Nature and Forest (INBO). Sampling event dataset <https://doi.org/10.15468/jhv16z> accessed via GBIF.org on 2025-07-07.
- Breine J, Verreycken H, De Boeck T, Brosens D, Desmet P (2025). VIS - Fishes in estuarine waters in Flanders, Belgium. Version 9.8. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/estwpt> accessed via GBIF.org on 2025-07-07.
- Buyse D (2022). Visdoorgangen - Fish passage places in Flanders, Belgium. Version 15.4. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/92ylpd> accessed via GBIF.org on 2025-07-07.
- Buyse D, Brosens D (2021). Fish damage at pump stations. Version 1.14. Research Institute for Nature and Forest (INBO). Sampling event dataset <https://doi.org/10.15468/hjh68q> accessed via GBIF.org on 2025-07-07.
- Coeck J, Pauwels I, Brosens D, Van Wichelen J, Van den Neucker T, Vandamme L (2025). VIS - Fish species introduction and recovery evaluation in streams and rivers in Flanders, Belgium. Version 1.8. Research Institute for Nature and Forest (INBO). Sampling event dataset <https://doi.org/10.15468/9u495r> accessed via GBIF.org on 2025-07-07.
- Devisscher S, Adriaens T, Brosens D, Desmet P (2023). Invasive species - Chinese mitten crab (*Eriocheir sinensis*) in Flanders, Belgium. Version 1.9. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/eakzzv> accessed via GBIF.org on 2025-07-07.
- Devisscher S, Adriaens T, Louette G, Brosens D, Desmet P (2025). Invasive species - American bullfrog (*Lithobates catesbeianus*) in Flanders, Belgium. Version 1.18. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/2hqqn> accessed via GBIF.org on 2025-07-07.
- Devisscher S, Brosens D, Brosens D (2025). Invasive species - American bullfrog (*Lithobates catesbeianus*) in Flanders, Belgium (post 2018). Version 1.94. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/daf62d> accessed via GBIF.org on 2025-07-07.
- iNaturalist contributors, iNaturalist (2025). iNaturalist Research-grade Observations. iNaturalist.org. Occurrence dataset <https://doi.org/10.15468/ab3s5x> accessed via GBIF.org

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Leloup E (1943). La propagation du crabe chinois en Belgique pendant les années 1939-1942. *Bull. du Musée R. d'Histoire Nat. Belgique* 19, 1–4.

Mahieu F, Boets P, Zoeter Vanpoucke M, Reyserhove L, Oldoni D (2024). Monitoring of fishes and crustaceans by Province East Flanders in Flanders, Belgium. Version 1.101. Province East Flanders. Sampling event dataset <https://doi.org/10.15468/ap9ejd> accessed via GBIF.org on 2025-07-07.

Mahieu F, Van Roeyen K, Oldoni D, Reyserhove L (2025). Monitoring of invasive alien species by the Province East Flanders, Belgium. Version 1.146. Province East Flanders. Occurrence dataset <https://doi.org/10.15468/29cggg> accessed via GBIF.org on 2025-07-07.

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Slootmaekers D, Gevens F, Reyserhove L, Oldoni D, Desmet P (2025). VMM - Rat control occurrences in Flanders, Belgium. Version 1.178. Flanders Environment Agency (VMM). Occurrence dataset <https://doi.org/10.15468/wqzva> accessed via GBIF.org on 2025-07-07.

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Van Thuyne G, Breine J, Brosens D (2025). VIS - Reference freshwater monitoring in Flanders, Belgium (post 2013). Version 1.14. Research Institute for Nature and Forest (INBO). Sampling event dataset <https://doi.org/10.15468/klly8u> accessed via GBIF.org on 2025-07-07.

Van Thuyne G, Breine J, Verreycken H, De Boeck T, Brosens D, Desmet P (2021). VIS - Fishes in inland waters in Flanders, Belgium. Version 9.7. Research Institute for Nature and Forest (INBO). Occurrence dataset <https://doi.org/10.15468/gzyxyd> accessed via GBIF.org on 2025-07-07.

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**Table S. 1** Results from the GLM with negative binomial distribution (log link) comparing the catch between months in Grobbendonk. Estimated marginal means (response scale) are shown for each month, along with the standard error (SE), 95% confidence interval (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey’s adjustment. Letter groupings reflect months that are not significantly different from each other.

Month	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Jan	97	52	34	275	a
Feb	1,505	570	716	3,163	cd
Mar	17,517	6,605	8,365	36,680	e
Apr	114,297	43,218	54,472	239,825	f
May	126,479	47,578	60,509	264,373	f
Jun	6,936	2,735	3,202	15,022	de
Jul	180	87	70	462	ab
Aug	214	127	67	685	abc
Sep	897	345	422	1,906	bc
Oct	497	191	234	1,053	abc
Nov	489	188	230	1,040	abc
Dec	428	179	189	972	abc

**Table S. 2** Results from the GLM with negative binomial distribution (log link) comparing the catch between months in Kalken. Estimated marginal means (response scale) are shown for each month, along with the standard error (SE), 95 % confidence interval (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey’s adjustment. Letter groupings reflect months that are not significantly different from each other.

Month	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Jan	90	21	58	141	a
Feb	162	26	118	222	ab
Mar	364	58	267	498	c
Apr	573	107	397	827	cd
May	406	75	282	583	c
Jun	404	109	237	685	bcd

Oct	1,150	312	676	1,958	d
Nov	827	222	489	1,401	cd
Dec	664	178	392	1,123	cd

**Table S. 3** Results from the GLM with negative binomial distribution (log link) comparing the catch between months in Lippenbroek. Estimated marginal means (response scale) are shown for each month, along with the standard error (SE), 95 % confidence interval (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey's adjustment. Letter groupings reflect months that are not significantly different from each other.

Month	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Jan	329	138	145	748	e
Feb	117	47	53	255	de
Mar	199	79	91	434	e
Apr	139	56	64	304	de
May	28	12	12	63	bcd
Jun	4	2	1	10	ab
Jul	4	2	2	12	ab
Aug	1	1	0	5	a
Sep	13	6	6	32	abc
Oct	10	5	4	25	ab
Nov	12	4	6	25	ab
Dec	71	24	38	136	cde

**Table S. 4** Results from the GLM with negative binomial distribution (log link) comparing the catch between months in Merelbeke. Estimated marginal means (response scale) are shown for each month, along with the standard error (SE), 95 % confidence interval (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey's adjustment. Letter groupings reflect months that are not significantly different from each other.

Month	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Jan	78	28	39	156	a
Feb	4,557	1,585	2,305	9,011	b
Mar	39,582	13,771	20,015	78,278	c
Apr	108,672	37,437	55,319	213,479	c
May	51,384	18,197	25,667	102,867	c
Jun	5,147	1,774	2,619	10,116	b
Jul	2,280	785	1,161	4,477	b

Aug	1,223	433	611	2,447	b
Sep	2,896	1,002	1,471	5,705	b
Oct	1,877	646	956	3,685	b
Nov	2,687	928	1,366	5,289	b
Dec	142	50	72	282	a

**Table S. 5** Results from the GLMM with a Gamma distribution (log link) comparing carapace width (mm) between locations and months. Estimated marginal means (response scale) are shown for each month per location, along with standard errors (SE), 95 % confidence intervals (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey’s adjustment. Letter groupings reflect months that are not significantly different from each other within each location.

Location	Month	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Grobbendonk	Jan	37.18	2.39	32.77	42.18	e
Grobbendonk	Feb	23.98	0.81	22.44	25.62	b
Grobbendonk	Mar	22.41	0.73	21.01	23.90	a
Grobbendonk	Apr	21.82	0.71	20.47	23.25	a
Grobbendonk	May	24.09	0.79	22.59	25.68	b
Grobbendonk	Jun	26.74	0.88	25.07	28.53	c
Grobbendonk	Jul	27.41	0.97	25.57	29.39	cd
Grobbendonk	Aug	34.17	2.51	29.58	39.46	de
Grobbendonk	Sep	45.80	1.55	42.87	48.94	f
Grobbendonk	Oct	51.69	1.75	48.37	55.24	h
Grobbendonk	Nov	53.10	1.76	49.75	56.67	h
Grobbendonk	Dec	48.98	1.66	45.83	52.34	g
Kalken	Jan	21.87	0.94	20.09	23.80	ab
Kalken	Feb	26.89	3.66	20.60	35.10	abcde
Kalken	Mar	21.26	0.77	19.81	22.83	a
Kalken	Apr	23.41	0.90	21.71	25.25	b
Kalken	May	26.99	1.03	25.04	29.10	c
Kalken	Jun	39.06	1.44	36.34	41.99	e
Kalken	Jul	NA	NA	NA	NA	
Kalken	Aug	41.11	1.74	37.83	44.68	e
Kalken	Sep	50.64	1.81	47.21	54.33	f
Kalken	Oct	55.26	2.44	50.69	60.24	f
Kalken	Nov	37.84	1.38	35.22	40.65	e
Kalken	Dec	29.80	1.07	27.77	31.98	d

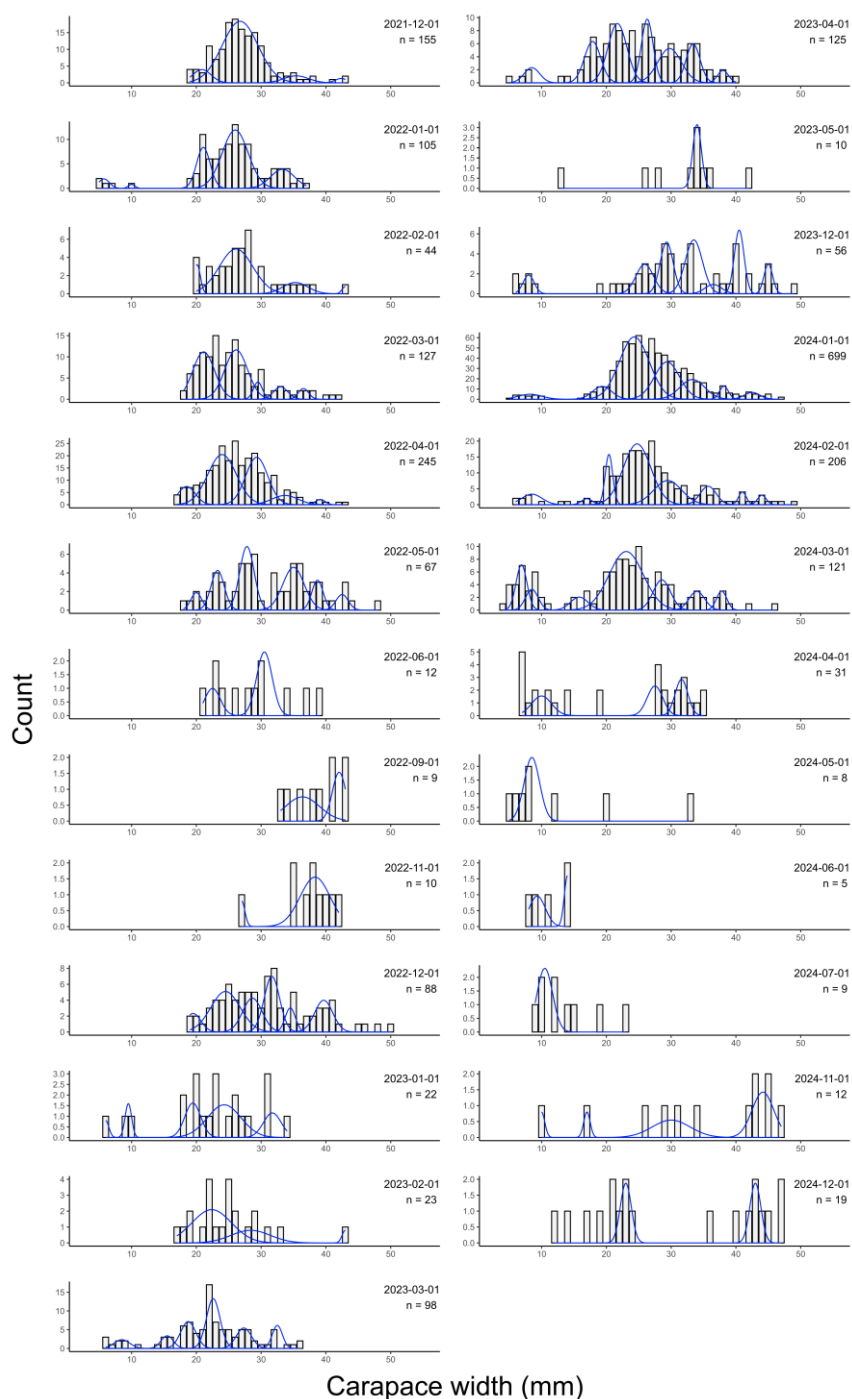
Lippenbroek	Jan	23.81	0.79	22.31	25.41	c
Lippenbroek	Feb	23.87	0.84	22.29	25.57	c
Lippenbroek	Mar	21.52	0.74	20.12	23.02	b
Lippenbroek	Apr	24.12	0.82	22.56	25.79	c
Lippenbroek	May	27.11	1.10	25.03	29.36	d
Lippenbroek	Jun	21.19	1.36	18.69	24.03	bc
Lippenbroek	Jul	15.37	1.17	13.24	17.85	a
Lippenbroek	Aug	31.21	4.24	23.91	40.74	bcdef
Lippenbroek	Sep	33.69	1.91	30.15	37.64	f
Lippenbroek	Oct	24.79	1.56	21.92	28.04	bcd
Lippenbroek	Nov	32.41	1.86	28.95	36.27	ef
Lippenbroek	Dec	28.04	0.97	26.20	30.00	de
Merelbeke	Jan	20.47	1.34	18.00	23.28	abcde
Merelbeke	Feb	19.19	0.67	17.91	20.55	ab
Merelbeke	Mar	18.86	0.63	17.68	20.13	a
Merelbeke	Apr	19.50	0.65	18.26	20.81	ab
Merelbeke	May	21.61	0.72	20.24	23.07	c
Merelbeke	Jun	24.50	0.83	22.92	26.19	ef
Merelbeke	Jul	22.79	0.81	21.26	24.43	cd
Merelbeke	Aug	26.58	0.98	24.72	28.57	g
Merelbeke	Sep	25.62	0.89	23.95	27.42	fg
Merelbeke	Oct	23.19	0.76	21.74	24.73	d
Merelbeke	Nov	22.80	0.75	21.37	24.33	d
Merelbeke	Dec	20.90	0.85	19.29	22.64	bc

**Table S. 6** Results from the GLMM with a Gamma distribution (log link) comparing carapace width (mm) between locations and months. Estimated marginal means (response scale) are shown for each location per month, along with standard errors (SE), 95 % confidence intervals (CI), and significance groupings ( $p < 0.05$ ) based on pairwise comparisons using Tukey’s adjustment. Letter groupings reflect locations that are not significantly different from each other within each month.

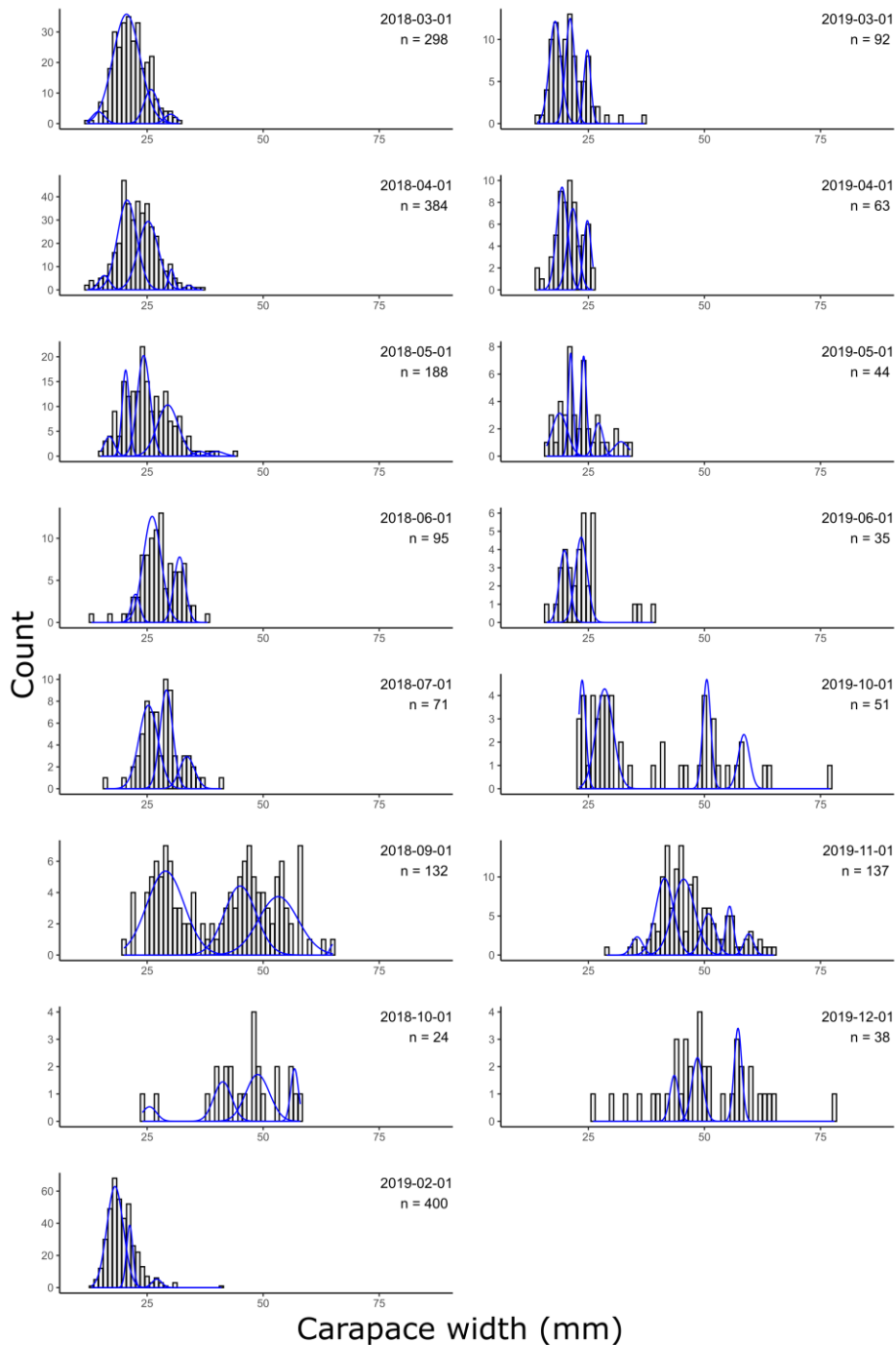
Month	Location	Estimated mean	SE	95% CI Lower	95% CI Upper	Group
Jan	Grobbendonk	37.18	2.39	32.77	42.18	c
Jan	Kalken	21.87	0.94	20.09	23.80	a
Jan	Lippenbroek	23.81	0.79	22.31	25.41	b
Jan	Merelbeke	20.47	1.34	18.00	23.28	a
Feb	Grobbendonk	23.98	0.81	22.44	25.62	b

Feb	Kalken	26.89	3.66	20.60	35.10	ab
Feb	Lippenbroek	23.87	0.84	22.29	25.57	b
Feb	Merelbeke	19.19	0.67	17.91	20.55	a
Mar	Grobbendonk	22.41	0.73	21.01	23.90	c
Mar	Kalken	21.26	0.77	19.81	22.83	b
Mar	Lippenbroek	21.52	0.74	20.12	23.02	b
Mar	Merelbeke	18.86	0.63	17.68	20.13	a
Apr	Grobbendonk	21.82	0.71	20.47	23.25	b
Apr	Kalken	23.41	0.90	21.71	25.25	c
Apr	Lippenbroek	24.12	0.82	22.56	25.79	c
Apr	Merelbeke	19.50	0.65	18.26	20.81	a
May	Grobbendonk	24.09	0.79	22.59	25.68	b
May	Kalken	26.99	1.03	25.04	29.10	c
May	Lippenbroek	27.11	1.10	25.03	29.36	c
May	Merelbeke	21.61	0.72	20.24	23.07	a
Jun	Grobbendonk	26.74	0.88	25.07	28.53	b
Jun	Kalken	39.06	1.44	36.34	41.99	c
Jun	Lippenbroek	21.19	1.36	18.69	24.03	a
Jun	Merelbeke	24.50	0.83	22.92	26.19	a
Jul	Grobbendonk	27.41	0.97	25.57	29.39	c
Jul	Kalken					
Jul	Lippenbroek	15.37	1.17	13.24	17.85	a
Jul	Merelbeke	22.79	0.81	21.26	24.43	b
Aug	Grobbendonk	34.17	2.51	29.58	39.46	b
Aug	Kalken	41.11	1.74	37.83	44.68	c
Aug	Lippenbroek	31.21	4.24	23.91	40.74	abc
Aug	Merelbeke	26.58	0.98	24.72	28.57	a
Sep	Grobbendonk	45.80	1.55	42.87	48.94	c
Sep	Kalken	50.64	1.81	47.21	54.33	d
Sep	Lippenbroek	33.69	1.91	30.15	37.64	b
Sep	Merelbeke	25.62	0.89	23.95	27.42	a
Oct	Grobbendonk	51.69	1.75	48.37	55.24	b
Oct	Kalken	55.26	2.44	50.69	60.24	b
Oct	Lippenbroek	24.79	1.56	21.92	28.04	a
Oct	Merelbeke	23.19	0.76	21.74	24.73	a
Nov	Grobbendonk	53.10	1.76	49.75	56.67	d
Nov	Kalken	37.84	1.38	35.22	40.65	c

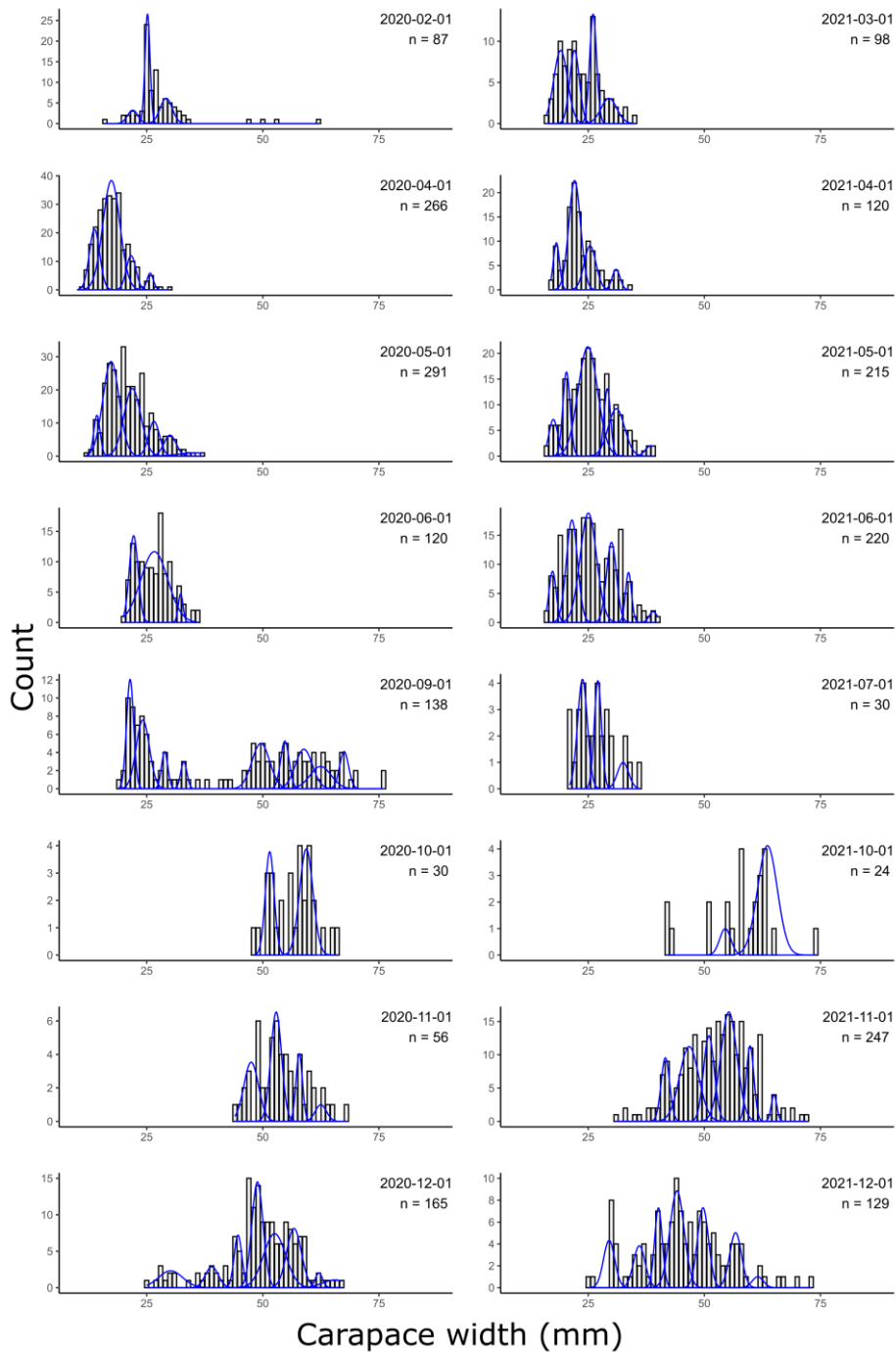
Nov	Lippenbroek	32.41	1.86	28.95	36.27	b
Nov	Merelbeke	22.80	0.75	21.37	24.33	a
Dec	Grobbendonk	48.98	1.66	45.83	52.34	d
Dec	Kalken	29.80	1.07	27.77	31.98	c
Dec	Lippenbroek	28.04	0.97	26.20	30.00	b
Dec	Merelbeke	20.90	0.85	19.29	22.64	a



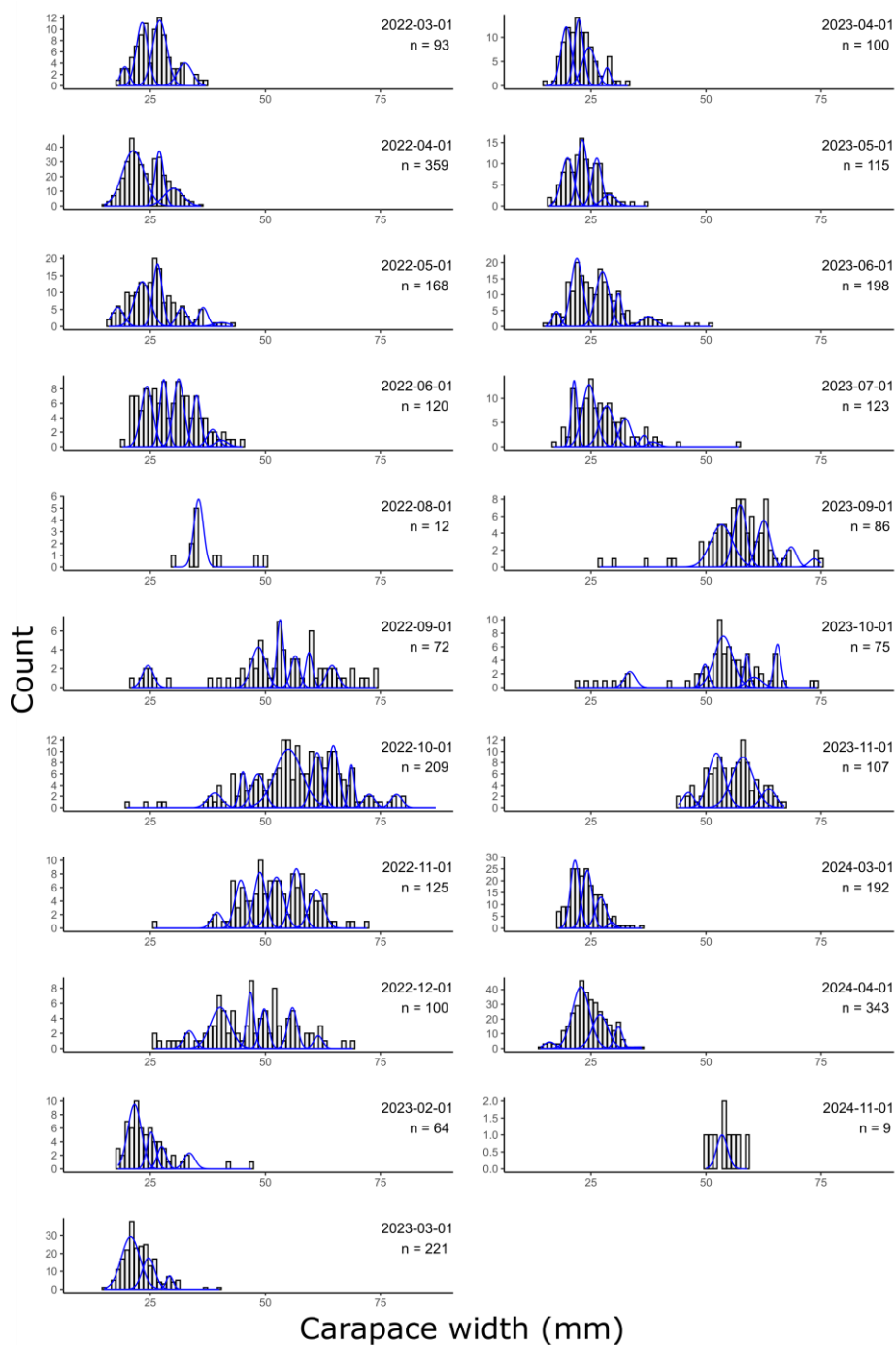
**Fig. S. 1** Monthly size-frequency distributions at the location Lippenbroek between November 2021 and December 2024, with indication of cohorts identified using Bhattacharya's method.



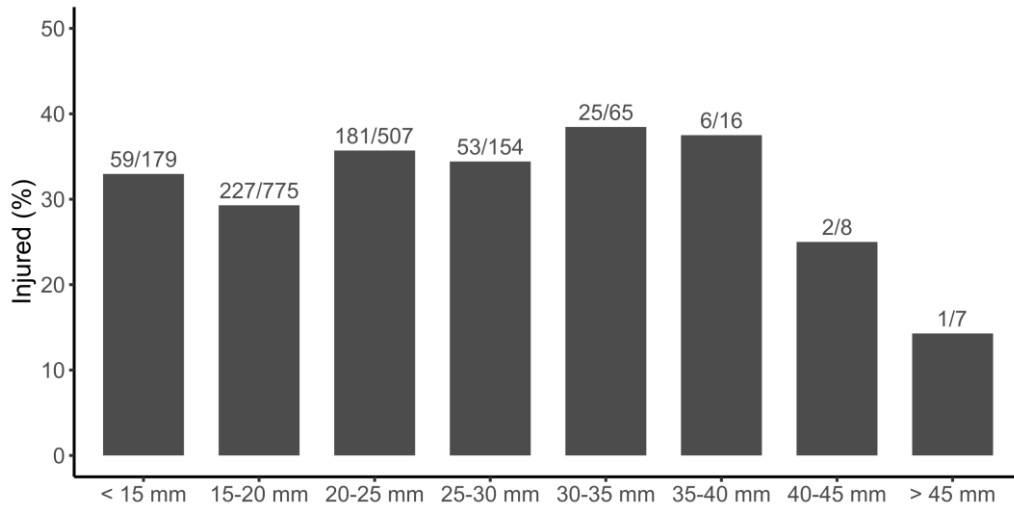
**Fig. S. 2** Monthly size-frequency distributions at the location Grobbendonk (part I) between March 2018 and December 2019, with indication of cohorts identified using Bhattacharya's method.



**Fig. S. 3** Monthly size-frequency distributions at the location Grobbendonk (part II) between February 2020 and December 2021, with indication of cohorts identified using Bhattacharya's method.



**Fig. S. 4** Monthly size-frequency distributions at the location Grobbendonk (part III) between March 2022 and November 2024, with indication of cohorts identified using Bhattacharya's method.



**Fig. S. 5** Percentage of injured crabs (i.e. individuals with missing appendages) per size class in 2023. Numbers above each bar represent the number of crabs with autotomy and the total number of crabs analysed.

## 8.2 Supplementary materials Chapter 3

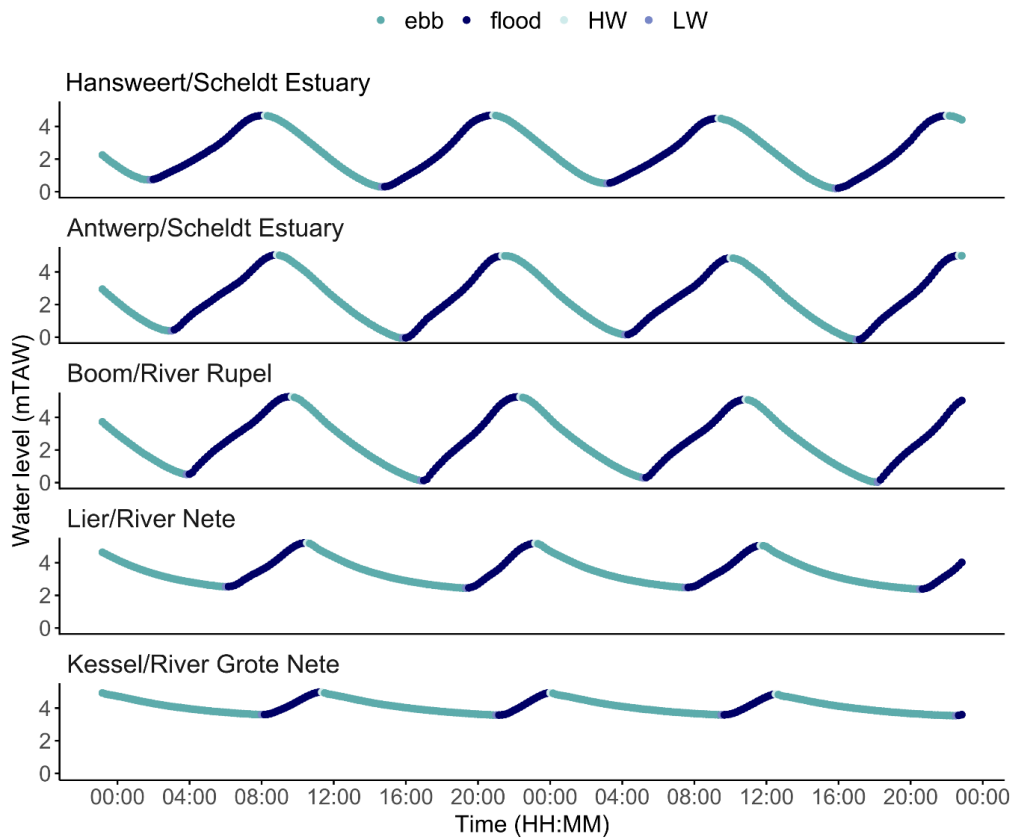
During preliminary tests, 18 adult Chinese mitten crabs (mean carapace width  $\pm$  SD:  $63.07 \pm 5.81$  mm, 4 female and 14 male) were tagged to test survival and tag retention. The dorsal carapace was dried and sanded superficially with a rotary tool. Next, a dummy tag (InnovaSea Systems Inc. V9-1x,  $9 \times 24$  mm, weight in air 3.6 g, weight in water 2.0 g) was attached according to three different methods (six crabs per method): (1) the tag was glued to the carapace with quick-setting cyanoacrylate (Pattex, super glue ultra gel) (**Fig. S. 6a**), (2) the method described in Section 3.2.3 using a combination of cyanoacrylate and Velcro but without tie wrap (**Fig. S. 6b**), and (3) the tag was glued with a trimethoxyvinylsilane based adhesive (Tec7) on the carapace and an extra layer of adhesive was put around the tag (**Fig. S. 6c**). Each tag was kept in place manually for 10 minutes. Every crab received a unique marking with nail polish, to make sure that the individual crabs could be recognised in case a tag would fall off. Crabs were placed individually in a bucket with a small layer of tap water for 30 minutes to allow the adhesives to cure. The whole tagging procedure including the drying time took about 1 h per crab.



**Fig. S. 6** The three different adhesion methods tested: (a) the first method using cyanoacrylate, (b) the second method using a combination of cyanoacrylate and Velcro and (c) the third method using trimethoxyvinylsilane.

After tagging, crabs were kept together in three tanks (circular, 2 m diameter). Three different set-ups were used to investigate the effect of salinity and hard structures on tag retention: (1) freshwater (tap water) and no structures, (2) freshwater, sand bottom and multiple hard structures, and (3) salt water (34 ‰), sand bottom and multiple hard structures. Every tank held two crabs per adhesion method. The tanks had a water height of 1.5 m and were aerated with an air stone. The hard structures included multiple large stones, wood and hard plastic, where crabs could crawl under. The salt in the third tank was added gradually over 14 days to allow the crabs to adjust to the salinity. All crabs were fed regularly with tree leaves. The experiment lasted for more than two months.

No crabs died during the experiment. Tag retention was 67 % for the first method, 83 % for method two and 0 % for method three. The second method gave the best results, with one tag lost in salt water after 69 days. The other tags were still firmly attached with the Velcro onto the carapace. Therefore it was decided to use this method in the field study. The method was slightly altered by adding a tie wrap to improve the attachment of the tag to the Velcro strip.



**Fig. S. 7** Snapshot (2021-01-08 until 2021-01-09, hour in UTC) of water level data of five different tidal measuring stations throughout the study area shows that the duration of the tidal phases becomes increasingly asymmetrical moving landward. HW indicates high water, LW indicates low water. Note that the tidal range reaches a maximum near Antwerp.

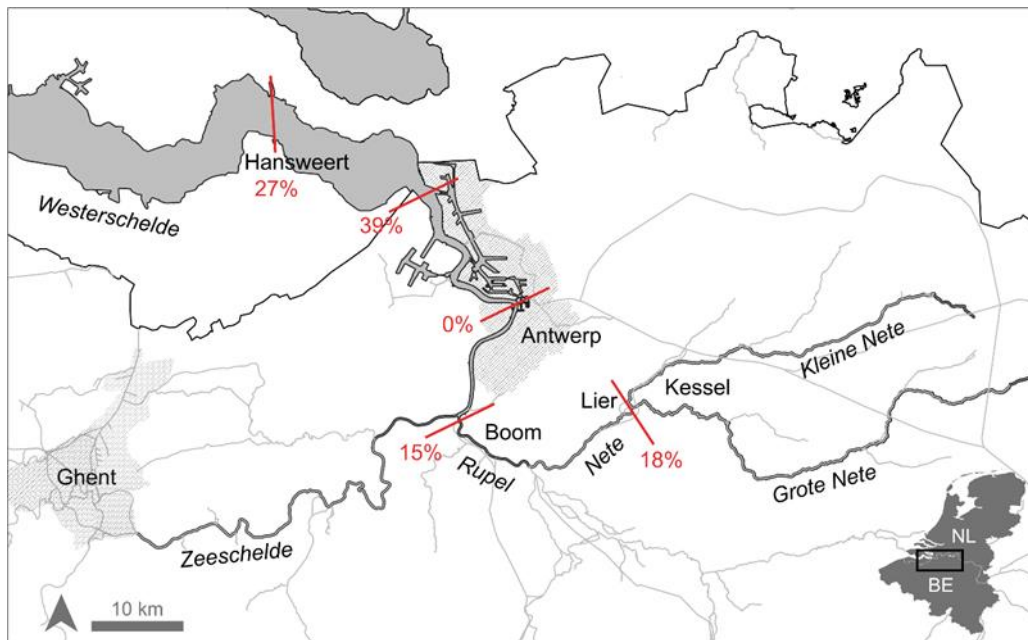
**Table S. 7** Overview of the tags, biometric measurements and release location of each Chinese mitten crab, with F = female, M = male, CW = carapace width and WW = wet weight.

ID	Tag			Crab			Release		
	Type	Sex	CW (mm)	WW (g)	Date	Location	River	Coordinates	
9830	ID-2LP6	F	64.40	117.87	2020-10-11	Grobbedonk	Kleine Nete	51°11'37.3" N 4°45'14.1" E	
9832	ID-2LP6	F	66.73	133.78	2020-10-11	Retie	Kleine Nete	51°14'38.3" N 5°03'07.3" E	
9836	ID-2LP6	F	64.18	119.46	2020-10-11	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9837	ID-2LP6	F	67.47	141.29	2020-10-11	Zammel	Grote Nete	51°05'28.1" N 4°56'37.9" E	
9840	ID-2LP6	F	63.65	110.06	2020-10-11	Lier	Nete	51°07'01.5" N 4°32'59.5" E	
9843	ID-2LP6	F	63.51	116.88	2020-10-11	Lier	Nete	51°07'53.4" N 4°34'52.2" E	
9845	ID-2LP6	F	65.08	121.62	2020-10-11	Lier	Nete	51°07'53.4" N 4°34'52.2" E	
9846	ID-2LP6	F	66.97	133.04	2020-10-11	Niel	Rupel	51°06'18.8" N 4°19'16.4" E	
9847	ID-2LP6	F	59.65	90.26	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9848	ID-2LP6	F	62.74	109.69	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9851	ID-2LP6	F	61.94	105.71	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9850	ID-2LP6	F	61.46	101.57	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9855	ID-2LP6	M	73.82	203.6	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9854	ID-2LP6	M	63.03	117.57	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9856	ID-2LP6	F	64.60	121.76	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9857	ID-2LP6	M	62.71	115.54	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9859	ID-2LP6	M	62.05	123.69	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	
9860	ID-2LP6	M	58.11	94.62	2021-10-27	Geel	Grote Nete	51°08'42.5" N 5°00'14.8" E	

3735	V9AP-2x-BLU-1	M	69.22	170.09	2021-11-09	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
3733	V9AP-2x-BLU-1	F	62.89	108.89	2021-11-09	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
3723	V9AP-2x-BLU-1	F	66.03	126.63	2021-11-20	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
3725	V9AP-2x-BLU-1	M	62.21	117.25	2021-11-20	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
3727	V9AP-2x-BLU-1	M	68.95	151.86	2021-11-20	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
3729	V9AP-2x-BLU-1	F	64.81	114.98	2021-11-20	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6925	ID-HP9	M	68.15	155.55	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6926	ID-HP9	F	67.67	134.44	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6927	ID-HP9	M	78.08	228.32	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6928	ID-HP9	M	70.22	171.88	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6929	ID-HP9	F	65.06	117.44	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6930	ID-HP9	M	69.62	149.92	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6931	ID-HP9	F	66.96	126.83	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6932	ID-HP9	F	65.34	118.02	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6933	ID-HP9	F	64.16	117.49	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E
6934	ID-HP9	M	65.84	128.75	2022-11-11	Geel	Grote Nete	51°08'17.4" N 4°59'46.5" E

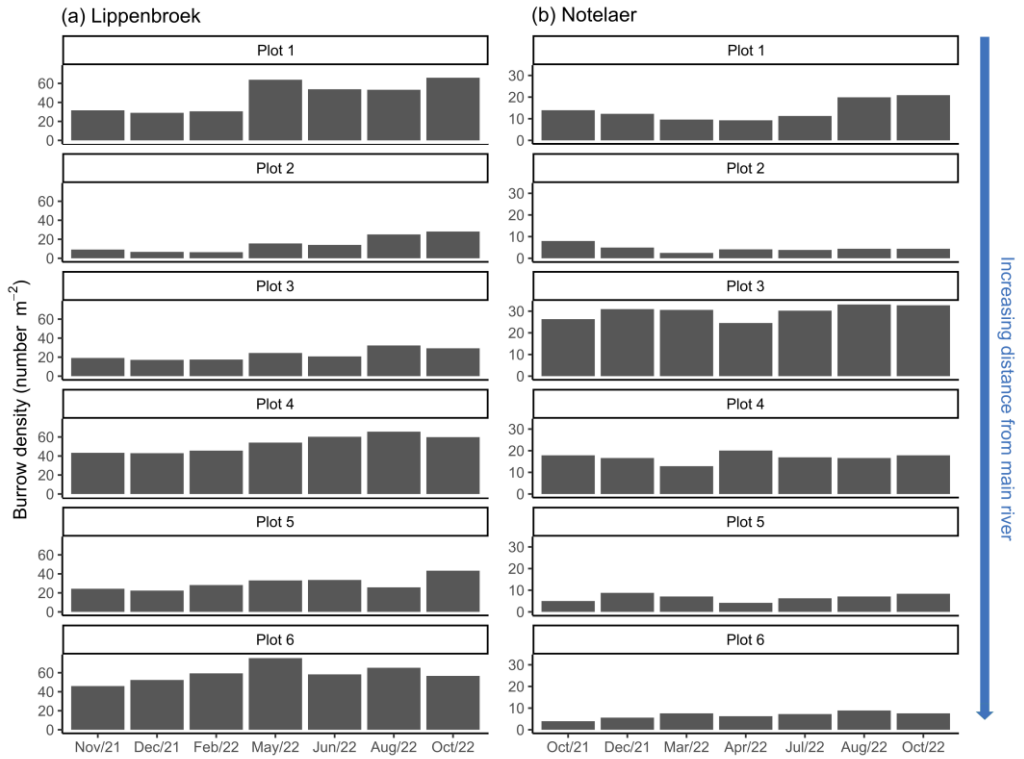
**Table S. 8** Overview of total distance, time and detections of each tagged Chinese mitten crab, with F = female and M = male, total time in days, and total distance in km.

Tag ID	Sex	First detection	Last detection	Total time	Total detections	Total distance
9830	F	2020-10-22	2021-03-26	155	482	101
9832	F	2020-10-22	2020-10-22	NA	0	NA
9836	F	2020-10-22	2021-03-28	157	1964	126
9837	F	2020-10-22	2021-08-19	301	362	117
9840	F	2020-10-22	2021-01-15	85	110	40
9843	F	2020-10-22	2020-12-13	52	269	19
9845	F	2020-10-22	2021-04-11	171	542	61
9846	F	2020-10-22	2020-12-07	46	32	19
9847	F	2021-10-27	2022-02-11	107	1006	86
9848	F	2021-10-27	2021-11-09	13	1166	42
9850	F	2021-10-27	2022-01-19	84	1445	55
9851	F	2021-10-27	2022-05-16	201	2958	100
9854	M	2021-10-27	2021-11-16	20	1443	42
9855	M	2021-10-27	2021-12-01	35	110	12
9856	F	2021-10-27	2021-10-29	2	16	1
9857	M	2021-10-27	2021-12-19	53	210	12
9859	M	2021-10-27	2022-01-10	75	1910	46
9860	M	2021-10-27	2022-01-17	82	1083	55
3733	F	2021-11-09	2022-04-06	148	7366	99
3735	M	2021-11-09	2021-12-17	38	1986	54
3723	F	2021-11-20	2022-03-18	118	5971	124
3725	M	2021-11-20	2022-02-09	81	3382	99
3727	M	2021-11-20	2022-04-05	136	2543	99
3729	F	2021-11-20	2022-02-02	74	3204	85
6925	M	2022-11-11	2023-02-04	85	6877	85
6926	F	2022-11-11	2022-11-22	11	443	35
6927	M	2022-11-11	2023-05-13	183	2002	126
6928	M	2022-11-11	2023-06-18	219	5574	124
6929	F	2022-11-11	2023-04-23	162	4960	124
6930	M	2022-11-11	2023-01-19	69	4042	85
6931	F	2022-11-11	2023-04-22	162	10285	125
6932	F	2022-11-11	2023-04-27	167	8093	85
6933	F	2022-11-11	2023-05-03	173	3537	124
6934	M	2022-11-11	2023-04-05	145	4734	99
<b>Average ± SD</b>				109 ± 69	2731 ± 2684	76 ± 40

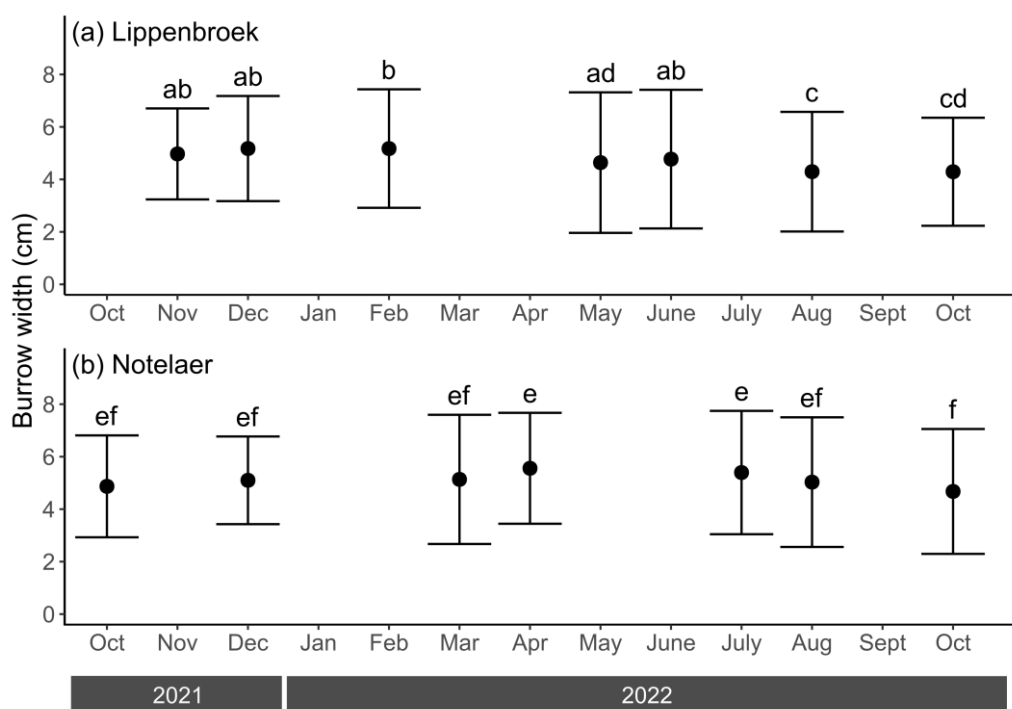


**Fig. S. 8** Map of the study area showing the migration endpoints of tagged Chinese mitten crabs (indicated in red). Of the tracked crabs, 18 % (n = 6) were last detected within the River Grote Nete, and 15 % (n = 5) within the Rivers Nete or Rupel. None (0 %) were lost between the confluence of the Rivers Scheldt and Rupel and the city of Antwerp. A total of 39 % (n = 13) were last detected near Antwerp, while 27 % (n = 9) were last recorded near Hansweert, both locations within the Scheldt Estuary.

### 8.3 Supplementary materials Chapter 5



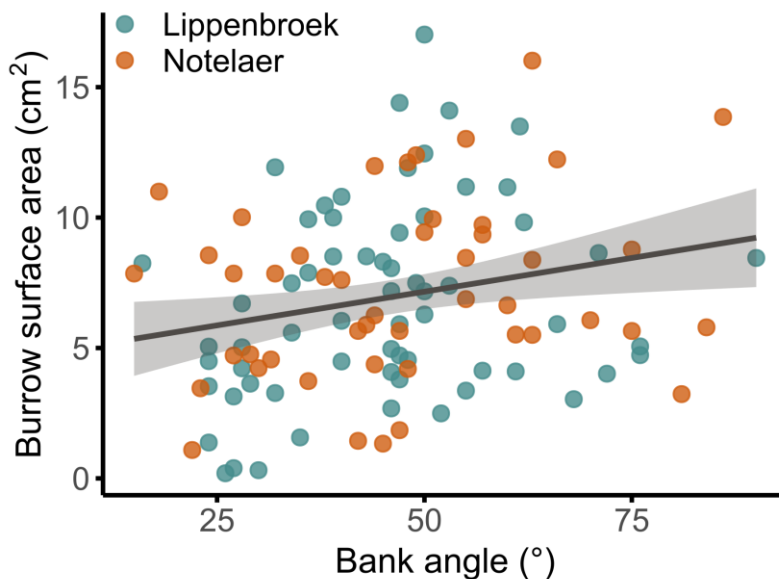
**Fig. S. 9** Monthly burrow density per study plot at the restored tidal area (a) Lippenbroek, and the natural tidal area (b) Notelaer.



**Fig. S. 10** Monthly mean burrow width (black circles  $\pm$  SD) at each location, based on six plots per time point. Letters above error bars indicate statistically significant ( $p < 0.05$ ) differences in mean burrow width between months within each location. Panel (a) shows the restored tidal area, Lippenbroek, and panel (b) shows the natural tidal area, Notelaer.

**Table S. 9** Average  $\pm$  standard deviation (SD) and range (in brackets) of environmental variables measured in September 2023 in the restored (Lippenbroek,  $n = 66$ ) and natural (Notelaer,  $n = 52$ ) tidal marshes.

	Lippenbroek	Notelaer
Bank angle ( $^{\circ}$ )	45 $\pm$ 15 (16 - 90)	45 $\pm$ 18 (12 - 86)
Creek width (m)	7.5 $\pm$ 1.5 (4.6 - 11.6)	4.1 $\pm$ 1.2 (2.1 - 7.2)
Distance from main river (m)	180 $\pm$ 108 (0 - 352)	65 $\pm$ 43 (0 - 144)
BDD ( $\text{g cm}^{-3}$ )	0.73 $\pm$ 0.20 (0.34 - 1.16)	0.60 $\pm$ 0.12 (0.34 - 0.93)
OM (%)	7 $\pm$ 4 (3 - 38)	9 $\pm$ 2 (5 - 14)
d50 ( $\mu\text{m}$ )	32 $\pm$ 13 (13 - 71)	25 $\pm$ 12 (10 - 53)
Clay (%)	4.1 $\pm$ 1.5 (1.7 - 6.7)	4.7 $\pm$ 1.4 (1.1 - 7.3)
Silt (%)	70.1 $\pm$ 9.7 (42.6 - 89.3)	72.8 $\pm$ 8.7 (54.7 - 87.0)
Sand (%)	25.8 $\pm$ 10.6 (4.8 - 55.7)	22.6 $\pm$ 9.5 (6.2 - 42.7)

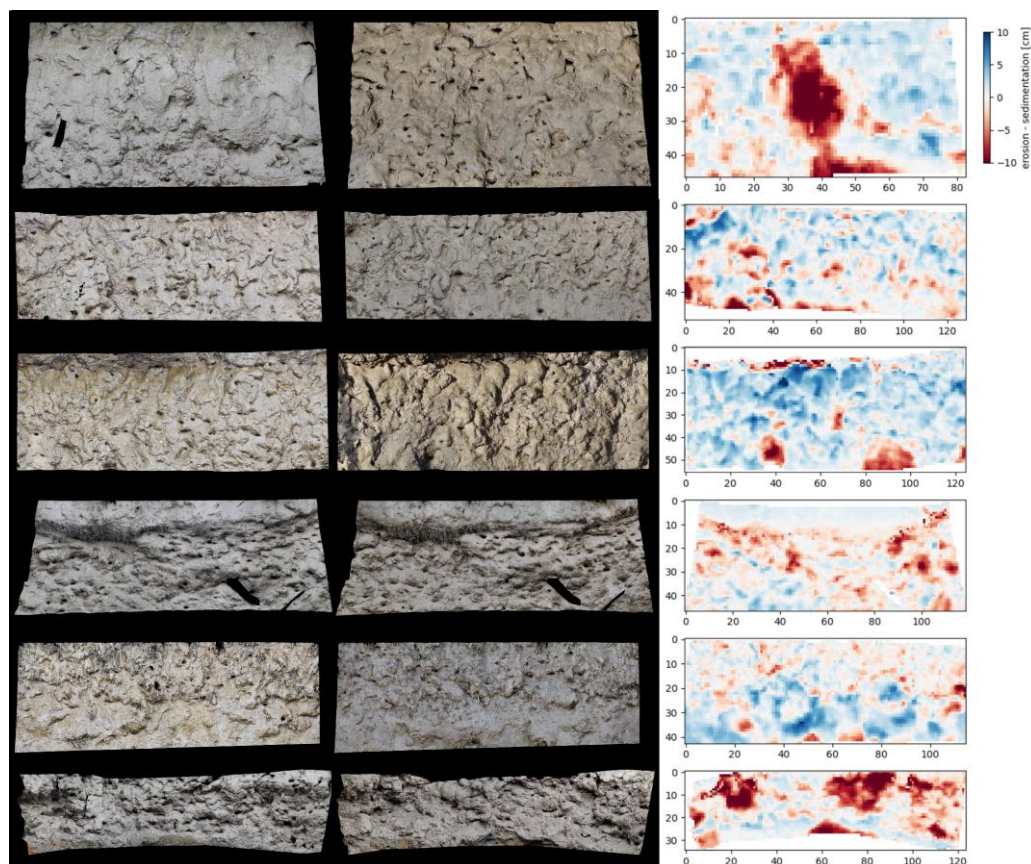


**Fig. S. 11** Relation between burrow opening surface area (cm<sup>2</sup>) and bank angle (°). The predicted mean and its 95 % confidence interval are shown as a line and ribbon in grey (two locations combined). The dots represent the actual values measured in the field. Blue indicates location Lippenbroek, a restored tidal marsh, and orange location Notelaer, a natural tidal marsh.

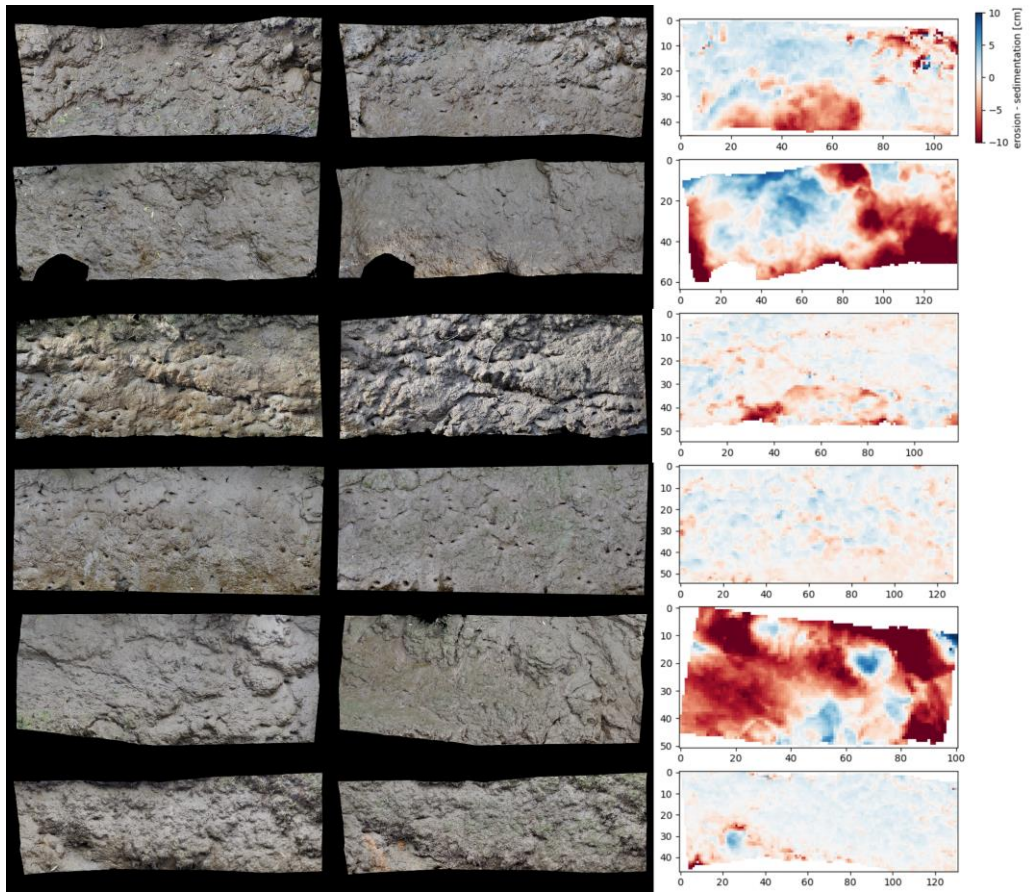
**Table S. 10** Average  $\pm$  standard deviation (SD) and range (in brackets) of environmental variables measured in November 2021 in the restored (Lippenbroek, n = 6) and natural (Notelaer, n = 6) tidal marshes.

	Lippenbroek	Notelaer
Bank angle (°)	54 $\pm$ 8 (44 - 64)	50 $\pm$ 7 (38 - 55)
Creek width (m)	7.5 $\pm$ 2.8 (2.7 - 10.8)	4.9 $\pm$ 0.7 (4.2 - 6.2)
Average creek depth (m)	1.3 $\pm$ 0.5 (0.8 - 2.0)	1.0 $\pm$ 0.2 (0.7 - 1.4)
Max creek depth (m)	1.8 $\pm$ 0.6 (1.1 - 2.7)	1.7 $\pm$ 0.3 (1.5 - 2.1)
Distance from main river (m)	248 $\pm$ 258 (29 - 622)	93 $\pm$ 45 (30 - 148)
BDD (g cm <sup>-3</sup> )	0.91 $\pm$ 0.18 (0.63 - 1.11)	0.85 $\pm$ 0.04 (0.82 - 0.92)
OM (%)	5.8 $\pm$ 2.2 (3.3 - 8.6)	6.7 $\pm$ 0.9 (5.8 - 8.0)
d50 ( $\mu$ m)	29 $\pm$ 11 (17 - 46)	28 $\pm$ 3 (24 - 32)
Clay (%)	4.7 $\pm$ 0.5 (4.0 - 5.3)	5.0 $\pm$ 0.2 (4.6 - 5.2)
Silt (%)	70.2 $\pm$ 10.8 (56.7 - 85.0)	67.4 $\pm$ 3.7 (61.5 - 70.6)
Sand (%)	25.1 $\pm$ 11.1 (10.3 - 39.3)	27.7 $\pm$ 3.6 (24.6 - 33.6)

Inundation frequency (middle of the plot, %)	$87.2 \pm 3.5$ (84.0 - 93.2)	$80.8 \pm 6.0$ (74.0 - 88.2)
Average inundation time per tidal cycle (middle of the plot, minutes)	$312 \pm 20$ (295 - 346)	$107 \pm 9$ (98 - 119)



**Fig. S. 12** Visualisation of the 3D point cloud models of the six study plots at Lippenbroek (plots 1 to 6, top to bottom). The left panels show the model from November 2021, and the middle panels from October 2022. The right panels visualise the height difference between the two models at a  $0.025 \times 0.025$  m resolution. Negative (red) values indicate erosion, while positive values (blue) indicate sedimentation.



**Fig. S. 13** Visualisation of the 3D point cloud models of the six study plots at Notelaer (plots 1 to 6, top to bottom). The left panels show the model from October 2021, and the middle panels from October 2022. The right panels visualise the height difference between the two models at a  $0.025 \times 0.025$  m resolution. Negative (red) values indicate erosion, while positive values (blue) indicate sedimentation.



# 9

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