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Reproduction in crabs:

strategies, invasiveness and environmental influences thereon

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This thesis was conducted under the auspices of the Research School for Socio-Economic and Natural Sciences of the Environment (SENSE).

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Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. dr. M.J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 16 April 2013
at 4 p.m. in the Aula.

Anneke Maria van den Brink
Reproduction in crabs: strategies, invasiveness and environmental influences thereon
164 pages

PhD thesis, Wageningen University, Wageningen. The Netherlands (2013)
With references, with summary in English and Dutch

ISBN: 978-94-6173-523-2

For my three true loves:
Jonathon, Sebastian and Mathias Hutchens

Preface

It has been many years since I first started studying crabs. Now that my current study draws to a close I can't help but think how those feisty little creatures have somehow become part of me, from the same silly jokes I hear a thousand times to the insessent urge to look under rocks on the shore to see what creatures I can find. From the beginning I found those little, sideways walking, weapon wielding crustaceans intriguing, particularly the big attitude they have despite their small size. More than once blood was drawn from my finger by a tightly clenched cheliped as my study subject made it clear that it would not go down without a fight, even against something many times bigger and stronger than itself, and sometimes it would win its freedom. I suppose you could say that they taught me that one should not give up the fight in the face of seemingly insurmountable odds.

Thus closes a chapter in my life. I look forward to new challenges and continuing research in the furture.

Anneke van den Brink

Cover art thanks to Mare and Nanne van den Heuvel

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Chapter 1:

General Introduction

BACKGROUND

Crab reproductive biology is a complicated and multi-faceted concept. Strategies, behaviour and physiology are inextricably interconnected and vary widely between species. The variation in strategies lies predominantly with the allocation of energy between growth and reproduction. Some species separate growth and reproductive phases, while others intersperse the phases throughout their life.

This thesis delves into the world of crab reproduction and investigates the various features of the reproductive biology of crabs with the aim of identifying the consequences of these variations to the individual crab and the population in which it exists. The two primary study species, *Halicarcinus cookii* and *Hemigrapsus takanoi* are similar in size and habitat preferences, but represent different reproductive strategies. These species are used as examples to broaden the current knowledge of influences on, and consequences of their reproductive strategies. Both physiological and external influences on reproduction, and their effects are considered along with population dynamics and ecology.

Growth and reproduction in crabs

Reproduction and growth are competing factors for energy, both of which are dependent on the moult cycle in crustaceans. The moult cycle in crustaceans is highly energy-demanding and correlated with the seasons. Although the relative importance of environmental changes may vary among different species and environments, breeding cycles are generally linked with seasonal changes in such a way that offspring are produced at a time most favourable to their survival (Raviv et al., 2008).

Moulting and reproduction are coordinated in a wide variety of ways in decapod crustaceans. The physiological nature of a species regulates the reproductive cycle, both by internal cues (e.g. moult cycle interval, integumental reproductive-related machinery, energy allocation, maternal care and embryonic development of offspring) and by external cues (e.g. habitat and season) (Raviv et al., 2008). McLay and Lopez Greco (2011) suggested that the reproductive strategy in crabs depends on three factors: whether the species shows indeterminate or determinate growth; hard or soft shell mating, and whether the seminal receptacle is dorsal or ventral.

Crabs with indeterminate growth continue to moult and grow throughout their lives. As an individual female grows, she can produce more eggs per brood and cross a size threshold to avoid some predators as well as increase competitiveness for food and space. However, with continual moulting the crabs are regularly vulnerable to predators and damage while soft shelled. Determinate growth occurs in species that experience a terminal, pubertal moult after which they are reproductively active but no longer grow. While these species avoid the regular vulnerable soft shelled stage as adults, they are limited in growth to the size at the terminal moult.

In some crab species, the female is only physically able to mate while soft shelled after mating. For internal fertilisation in crabs the male must physically insert his gonopods into the female's gonopore to deliver sperm. For crustaceans this means that the exoskeleton of the female must be flexible enough to receive the gonopod. In species with soft shell mating, mating occurs only while the females exoskeleton has not yet hardened directly after moulting. In species with hard shell mating, mating is possible while the female's integument is hard, this may be due to continuously open gonopores or temporary decalcification of an operculum covering the gonopore.

Seminal receptacles are structures within the female's body that collect and store sperm so that the female has a ready supply to fertilise her eggs. The unfertilised eggs travel through the oviduct into the seminal receptacle where they are fertilised and are then extruded through the vagina to the vaginal opening (through which sperm is delivered during mating) and into the external brood chamber. In species with dorsal seminal receptacles the oviduct attaches to the seminal receptacle at the opposite end to the vagina, thus the eggs are fertilised by the oldest stored sperm. In species with ventral seminal receptacles the oviduct connects with the seminal receptacle at the same end as the vagina so that the eggs are fertilised by the newest of the stored sperm. Some species have an intermediate type of seminal receptacles where the oviduct is attached near the middle of the seminal receptacle (McLay & López Greco, 2011).

The different reproductive strategies of crustaceans defined by the combination of growth strategy, hard or soft shell mating and ventral or dorsal seminal receptacles were divided into six groups by McLay and Lopez Greco (2011) (Table 1.1).

Table 1.1 Reproductive strategies of crustaceans (from McLay and Lopez Greco (2011))

Strategy	**Growth**	**Mating**	**Seminal receptacle**	**Example**
1	Determinate	Soft-shelled	Dorsal	Portunoids
2	Determinate	Hard-shelled	Ventral	Majoids
3	Indeterminate	Soft-shelled	Dorsal	Eriphioids
4	Indeterminate	Hard-shelled	Dorsal	Xanthoids
5	Indeterminate	Soft-shelled	Ventral	Cacroids
6	Indeterminate	Hard-shelled	Ventral	Grapsoid-Ocypodoids

Of the primary study species of this thesis, *Halicarcinus cookii* is an example of strategy 2. This is a relatively rare strategy and one not often studied, *H. cookii* is also endemic, with no record of it spreading beyond its native range, therefore in this thesis, the strategy of *H. cookii* is intensively investigated. *Hemigrapsus takanoi* is an example of strategy 6, a widespread strategy common in invasive crab species and one completely different to that of *H. cookii*

Sexual selection

Competition over mates takes many forms and has consequences that can be a driving force in sexual selection (Andersson & Iwasa, 1996). Sexual selection involves the selection for biological traits resulting from intra-specific competition for reproductive fitness (Andersson, 1994, Andersson & Iwasa, 1996). Each individual organism is expected to maximise its reproductive fitness; a measure of the reproductive success of an individual relative to that of its conspecifics (Andersson & Iwasa, 1996). Strong competition between individuals is therefore expected when a resource, such as mates, used by one individual is consequently less available to others (Andersson, 1994, Emlen & Oring, 1977).

In species with internal fertilisation, females and males differ in their strategies to maximise fitness, leading to a conflict of interests during interactions (Andersson, 1994, Andersson & Iwasa, 1996). According to

Bateman's theory, females gain fitness through investing a lot of resources (time and energy) into the survival of offspring through the production of few large, energy rich gametes (eggs) and development of zygotes through maternal brood care (Andersson, 1994, Andersson & Iwasa, 1996, Rolff, 2002). In contrast, males gain fitness by increasing mating rates, investing energy into many small, energy-poor gametes (sperm) with the sole purpose of transferring genetic material to as many eggs as possible (Andersson, 1994, Andersson & Iwasa, 1996, Rolff, 2002). Because of this, females and males differ in the resources limiting their reproductive success; females by energy resources for the production of eggs, and males by the number of eggs they can fertilise, which is essentially the number of females with whom they can mate (Andersson, 1994, Andersson & Iwasa, 1996). Traditionally, populations are considered egg-limited with no sperm-limitation. Therefore, competition over mates is seen primarily among males in a population as a result of their struggle to produce a maximum number of offspring with a limited number of mates, or in other words, to monopolise mates (Dunnington, 1999, Emlen & Oring, 1977). However, if sperm is in short supply then the primary aim of females first and foremost is to attract a male(s) who will provide sufficient sperm to fertilize all of her eggs (McLay & Lopez Greco, 2011). If there is sperm limitation in the form of insufficient males with whom to mate, the female can increase her reproductive fitness by mating with more than one male and storing sperm from each mating encounter so that she has a supply available when needed.

Several factors can influence the intensity of male-male competition for paternity of offspring in any population and the consequent mating strategies adopted by the individuals. By competing to monopolise the environmental resources that females require for reproduction, such as shelter and feeding sites, males can increase their own reproductive success. In this case, the spatial and temporal dispersion pattern of these key resources can influence the ability of males to monopolise them, and therefore resource distribution can influence the reproductive success of males and the intensity of competition among them (Emlen & Oring, 1977). This strategy of male territorial defence is seen in the intertidal fiddler crabs, *Uca* spp., which compete for burrows in which to mate and even fill in the burrows of other males {Zucker, 1977 #216}.

Population structure and dynamics can influence the level of competition in a population. Emlen and Oring (1977) emphasised the importance of the ratio of fertilisable females to sexually active males and termed this the operational sex ratio (OSR). The OSR indicates the degree to which an individual can monopolise mates in a population. When there is a skewed operational sex ratio, where receptive adults of one sex significantly outnumber those of the other, intra-sexual competition in the favoured sex will intensify as mates become limiting (Zimmer, 2001). Thus the greater the imbalance in the ratio, the stronger the competition for mates among members of the majority sex (Debuse et al., 1999, Emlen & Oring, 1977, Zimmer, 2001). Kvarnemo et al. (1995) showed that intrasexual competition was highest between male sand gobies, *Pomatoschistus minutus*, in a male biased OSR, and between females in a female biased OSR. This shows that competition for mates is a plastic behaviour and can be adjusted according to the OSR.

The number of fertilisable females in a population may be dependent on the biology of the species. For many species, particularly in the Brachyura, the synchrony of female receptivity to mating largely influences the number of fertilisable females available in the population at any time. If all females in a population are concurrently sexually receptive, such as in *Hemigrapsus sexdentatus* (Brockerhoff & McLay, 2005, McCurdy et al., 2000), breeding opportunities may be limited to only a short, synchronised period of time when the females are receptive, resulting in male-male competition for females being intense only during this time and very low at other times (Mathews, 2002). However, if female receptivity to mating is asynchronous, as seen in the snow crab, *Chionoecetes opilio* (Rondeau & Sainte-Marie, 2001), males are more able to monopolise mates, increasing the overall intensity of competition (Emlen & Oring, 1977).

Competition for paternity of offspring can continue after copulation in the form of sperm competition. Sperm competition occurs when a female can store sperm, mates with more than one male and when there is a delay between copulation and fertilisation (Danielsson, 1998, Parker, 1970, Parker, 1974). Andersson and Iwasa (1996) suggested that sperm competition decreased the realised reproductive rate of males compared to females, and may have been an indirect influence on the intensity of sexual selection. In species that do not store sperm, such as the spiny lobsters *Panulirus argus* and *Jasus edwardsii* (MacDiarmid & Butler, 1999), the female must mate and fertilise her eggs immediately or possibly lose the opportunity to fertilise her eggs. With this strategy the male can easily ensure his paternity of the female's next brood by guarding her until she lays her eggs. However, in species with polyandrous females, and where the female can store the sperm of several males for long periods of time, as is common in the Brachyura (Hartnoll, 1969), the male has no certainty of his contribution to egg fertilisation. In these species, such as the snow crab *C. opilio* (Diesel, 1991), males may attempt to prevent or reduce the amount of sperm competition through mate guarding to prevent rival males mating with a particular female (Diesel, 1991, Smith, 1984), or seal the sperm of earlier mates off with sperm plugs as seen in the ghost spider crab, *Inachus phalangium* (Diesel, 1989).

The behavioural mating strategies adopted by individuals in a population are intended to increase their individual reproductive fitness. For males this means to monopolise mates and compete for mates when they are limited (Emlen & Oring, 1977). For females, the ability to store sperm and mate with more than one male allows them sufficient sperm supply to fertilise their eggs, and for some, the ability to choose between potential mates allows them to use sperm from the highest quality males.

Temperature

For crabs, the speed of egg and larval development has been shown to increase with temperature (Dawirs, 1985, Wear, 1974). Optimal temperature is reached when development is fastest, but before a point where mortality becomes too high. Depending on other life history traits such as adult growth, gonad development and survivorship of larvae, if higher temperatures increase the speed of egg and larval development to a point where more offspring can be produced per female per season, then populations and distributional ranges may increase dramatically as a result of the increased fecundity and allow species to colonise new environments whose temperature was previously below the crab's temperature threshold. The temperature ranges experienced by *Halicarcinus cookii* and *Hemigrapsus takanoi* therefore have an impact on the population size, distribution and success of the species.

According to the predictions by the KNMI'06 Climate Scenario W North Sea temperatures could increase from about 1°C up to 6°C by 2100 as a result of climate change (KNMI, 2011). Climate change is a complicated and multi-faceted concept. Although we know that it is occurring, we cannot precisely understand or predict what effects it will have on the natural marine environment. Numerous factors can all be affected in different ways and their interactions can vary, resulting in a vast number of different scenarios. The concept of climate change in the marine environment includes factors such as temperature increase, sea level rise, acidification, eutrophication, rainfall, storm frequency and intensity and can result in changes in population dynamics of resident species. The primary cause of climate change is believed to be escalating levels of carbon dioxide resulting from combustion of fossil fuels. In this thesis only temperature rise as a result of climate change is considered because it is the most direct factor influencing reproductive rate in crabs.

The imminent temperature increases predicted by climate change scientists are likely to affect local environments and the species within them. Temperature influences the physiological processes of crustaceans, with increased temperatures leading to increased growth and reproductive rates (Leffler, 1972). If growth and/or reproduction of a species are currently limited by low temperatures, an increase in temperature may lead to population growth and spread in these species. Conversely, species whose survival, growth and reproduction are dependent on colder water temperatures may experience population declines due to a warmer and less suitable environment.

Non-indigenous species

While *Halicarcinus cookii* is an endemic species, *Hemigrapsus takanoi* has been introduced widely throughout the world. The globalisation of human activities has led to the transportation of species outside of their native range and into new habitats where they would not naturally occur (Troost, 2010). Some introduced non-indigenous species may establish successfully in the new habitat to which they are transported, and some of these may pose a risk to the native environment or communities through habitat modification, predation or competition.

The means by which the introductions of non-indigenous species artificially enter a new environment are diverse and variable. Most vectors of transporting non-indigenous species apply to crustaceans due to the various breadth of life styles, physiological repertoires, and reproductive strategies shown in the taxon. The two most common vectors of transporting decapods by anthropogenic means is global shipping and the movement of shellfish for aquaculture purposes (Carlton, 2011). Other anthropogenic influences can help the establishment of an introduced species in a new environment, such as the removal of physical barriers (with eg: canals) and the addition of infrastructure, which provides new habitats for the organisms to colonise (eg: harbour structures, dykes or bridges).

Vessels involved in global shipping easily become fouled with various organisms attached to their hulls. Without proper cleaning or prevention methods, these organisms can be transported along with the vessel to new locations where major vector for the transport of non-indigenous species. Where a vessel takes up ballast water, it can also collect planktonic organisms (eg: algae or larval forms) and transport them until the vessel releases its ballast water in a new environment. If larval forms can survive in the ballast water until they are released, they enter a new environment where they could settle and establish.

The movement of shellfish poses a risk of introducing non-indigenous species that might become invasive and could have a negative impact on the functioning of the ecosystem. Such organisms can be unintentionally transported as fouling on shellfish shells, within the water trapped inside and surrounding the shellfish, or in the tare collected with the shellfish. Shellfish transport is second only to shipping (ballast and hull fouling) as the most important vector responsible for the primary introductions of NIS in European waters (Minchin & Gollasch, 2002, Wolff, 2005) and probably worldwide.

Other vectors, which are less common, but can lead to major invasions include bait worms packed with seaweed, movement of floating structures (pontoons, sea buoys, dry docks, sea planes etc), movement of contaminated equipment (for: fishing, aquaculture, diving, water sports, research, tourism etc), habitat restoration (movement of plants and soils), floating plastic debris (fouling) and canals (active transport of water through a canal and passive transport on vessels) (Carlton, 2011).

Crabs are well known throughout the world as successful invaders. There are currently 93 described brachyuran families which include 6793 described species (Ng et al., 2008). At present, 73 of these crab species (in 26 families) have invaded non-native areas (Brockerhoff & McLay, 2011). The families with the largest number of non-indigenous representatives are Portunidae (15), Grapsidae (6), Pilumnidae (6) and Epialtidae (5). Of these, species that have become established in their new environments belong to the Portunidae (10), Grapsidae (5), Panopeidae (4), Varunidae (3), Pilumnidae (3), Epialtidae (3) and Leucosiidae (3) (Brockerhoff & McLay, 2011).

Non-indigenous crabs have been recorded in most of the common coastal marine habitats around the world. Generally, they are found in sandy, muddy or rocky habitats where they can easily find both food and refuge. Some crabs present a significant environmental and economic problem (in many parts of the world) as they establish high population densities and compete with local fauna for food and shelter. The brush-clawed shore crab, *Hemigrapsus takanoi* is native to the North-West Pacific but has spread to the North-East Atlantic and North Sea (Brockerhoff & McLay, 2011). This species is thought to compete for food and shelter with native shore crabs on the rocky shore habitats, especially where it occurs in high densities (Gollasch, 1999, Noël et al., 1997). *Hemigrapsus takanoi* has recently been introduced and become established in the coastal and delta waters of the Netherlands (Breton et al., 2002, Dauvin, 2009). It is thought to be a threat to newly settled shellfish such as mussel and oyster spat through predation and therefore can potentially impact local aquaculture activities (DeGraaf & Tyrrell, 2004, deRivera et al., 2007, Grosholz & Ruiz, 1995, Jensen et al., 2002, Troost, 2010, Wallentinus & Nyberg, 2007). The blue crab, *Callinectes sapidus* Rathbun is native to the North and South West Atlantic, but has spread to the North-East Atlantic, Baltic, Black Sea, Mediterranean, North Sea and Japan. It has been reported to cause some loss in aquaculture as it feeds on fish caught in traps along with clams, mussels and oysters and damages fishing nets (Brockerhoff & McLay, 2011). The European green crab, *Carcinus maenas* (Linnaeus) is native to the East Atlantic, North Sea and Baltic, but has spread to the North and South West Atlantic, South Africa and the North and South Pacific. It has been reported to compete for food and habitat and cause declines of other crab and bivalve species including those in the mussel farming industry (Brockerhoff & McLay, 2011).

PRIMARY STUDY SPECIES

The two species primarily investigated in this thesis are *Halicarcinus cookii* and *Hemigrapsus takanoi*. *Halicarcinus cookii* is a predominantly unstudied species of which little is known of its life history. *Hemigrapsus takanoi* was only recently described as a separate species (Asakura & Watanabe, 2005) and therefore has also not been heavily studied. Both of these species are relatively small crabs of similar size and also share similar habitat preferences (intertidal, often among shellfish and/or macroalgae). However, these species represent very different reproductive strategies, distribution patterns and status in the investigated locations (*H. cookii* is endemic in New Zealand, while *H. takanoi* is introduced into Europe). These two species therefore provide an interesting contrast in life history patterns and the information present broadens the currently limited knowledge of their biology.

While not a primary study species, *Carcinus maenas* is also investigated in this thesis, particularly in Chapter 7. *Carcinus maenas* is a well-studied species with much knowledge available in the literature. While a successful introduced species throughout the world, in the Netherlands (where *H. takanoi* was investigated), it is native and exists symbiotically with the introduced *H. takanoi*. The information available about *C. maenas* provides a good basis for comparison with the lesser investigated *H. takanoi*.

Halicarcinus cookii

Halicarcinus cookii Filhol (1885) of the family Hymenosomatidae is commonly known as a pill-box or false-spider crab (McLay, 1988; Melrose, 1975). The vast majority of hymenosomatids are found in the Indo-Pacific region, most of which inhabit Australia and New Zealand, but the family is also represented in China and Japan, Africa, and New Caledonia. One species, *H. planatus*, is found as far as the sub-Antarctic region of the west coast of South America (Melrose, 1975). Hymenosomatids are among the smallest of the Brachyura, ranging from a carapace width of 3-26 mm (Lucas, 1980). They are characterised by their short life spans, poorly calcified carapaces which are dorsally flattened and triangular or sub-circular in shape, often forming a horizontal rostrum, and their relatively short chelipeds that are not particularly mobile (Lucas & Hodgkin, 1970, Melrose, 1975) (Figure 1.1).

The genus *Halicarcinus* is found both in Australasia and Japan, but species tend to be endemic with 10 of the 12 species in New Zealand being restricted to the mainland shores (Melrose, 1975). *Halicarcinus innominatus* is unique in its trans-Tasman distribution as it has been found in Tasmania, although this is believed to be due to human interference (Melrose, 1975). New Zealand hymenosomatids are generally sub-littoral, although *H. cookii*, *H. varius*, *H. pubescens* and *H. innominatus* can inhabit shores as high as the lower mid-littoral zone depending on algal cover (Melrose, 1975).

Halicarcinus cookii is endemic to New Zealand and is ubiquitous along the east coast of the North and South Islands, as well as the Chatham Islands and Stewart Island (McLay, 1988, Melrose, 1975). The species lives in intertidal and sub-littoral shallow water among seaweeds on the rocky shore and its microhabitat includes dense algal fronds and holdfasts (McLay, 1988, Melrose, 1975). *H. cookii* is an opportunistic carnivore and scavenger, with a diet consisting of molluscs, polychaetes (*Perinereis*, *Lumbriconereis* and *Neanthys*) and especially amphipods (McLay, 1988). Melrose (1975) found that, when deprived of prey, the crabs would also eat algae or *Zostera*.

Halicarcinus cookii is typical of hymenosomatids in size. Prior to this study, males were reported to measure up to 13 mm in carapace width and females up to 8.5 mm in carapace width (McLay, 1988, Melrose, 1975). Males have larger chelae compared with females. McLay (1988) stated that the high proportion of ovigerous females found at any time indicates that *H. cookii* is capable of continuous breeding and goes through a terminal/pubertal moult, after which females mate in the hard-shell condition. In Chapter 4 of this study *H. cookii* is also shown to have ventral seminal receptacles (strategy 2 according to McLay & Lopez Greco 2011).

Fig 1.1 Male (left) and female (right) *Halicarcinus cookii* (actual carapace width: male – up to 13 mm, females – up to 8.5 mm).

Hemigrapsus takanoi

Hemigrapsus takanoi is a small crab of the family Varunidae and is commonly known as the Asian brush-clawed shore crab. The crab was previously identified as *H. penicillatus* until 2005 when it was identified as a separate species (Asakura & Watanabe, 2005). *Hemigrapsus takanoi* is native to north-west Pacific waters, particularly around Japan, Taiwan, Korea and China, and is commonly found under boulders in intertidal areas of estuaries, lagoons, boulder beaches, and wave-sheltered rocky shores (Asakura & Watanabe, 2005).

Species from the genus *Hemigrapsus* are successful invaders and have been introduced and become established in various different locations throughout the world (Dauvin et al., 2009, Doi et al., 2009, Epifanio et al., 1998, Jensen et al., 2002, McDermott, 1998). *Hemigrapsus takanoi* has become established in the coastal waters of Europe (Dauvin, 2010). *Hemigrapsus takanoi* was probably introduced into Europe via ballast water or hull fouling, and spread to the Netherlands with the transport of the Pacific Oyster *Crassostrea gigas* (Thunberg, 1793) from France (Carlton, 2011, Noël et al., 1997). The introduction of *H. takanoi* presented the ecosystem with a new competitor for the native crabs, particularly the European green crab *Carcinus maenas*, itself an exceptionally successful invader elsewhere (Cohen et al., 1995, Grosholz & Ruiz, 1995, Jensen et al., 2002, Thresher et al., 2003) and a predator of various commercially important species including mussels (Dauvin et al., 2009).

Hemigrapsus takanoi is small for the genus, generally measuring about 25 mm in carapace width and can occur in a variety of colours ranging from white and light brown patched to dark brownish-green and can have stripes or spots. The carapace is square-shaped with three spines close to the eyes. Males have much larger chelae compared with females and have a patch of setae on the hinge of their chelae (Figure 1.2).

Hemigrapsus takanoi represents reproductive strategy 6 (McLay & López Greco, 2011) as it has indeterminate growth, hard shell mating and ventral seminal receptacles. In general females can produce 2-3 broods per year, sometimes in the same intermoult, over the warmer months (Dauvin et al., 2009, McDermott, 1998).

Fig. 1.2 Male (left) and female (right) *Hemigrapsus takanoi* (actual carapace width: approximately 25 mm).

Carcinus maenas

The green crab, *Carcinus maenas*, is native to Europe and Northern Africa, but has invaded the Atlantic and Pacific coasts of North America, South Africa, Australia, South America, and Asia. These invasions are predominantly attributed to human vectors (Klassen & Locke, 2007).

Carcinus maenas crabs live up to 4-7 years and can reach a maximum size of 9-10 cm carapace width, much larger than both *Halicarcinus cookii* and *Hemigrapsus takanoi* (Klassen & Locke, 2007) (Figure 1.3). Males and females are similar in size and shape. Female *C. maenas* lay their eggs in spring and after an interval depending on water temperature (Wear, 1974) the larvae hatch into the water column where they develop through four zoeal and one megalopa stage before settling as a first instar juvenile crab (Dawirs, 1985). After about seven instars the juveniles mature at about 2-3 years old depending on location and temperature range (Berrill, 1982). The species inhabits coastal waters and intertidal environments, where juveniles can typically be found and adults also inhabit shallow subtidal waters up to 30 meters of depth. They occupy a variety of habitats from rocky shore, areas with boulders, macro-algae, mussel beds or oyster reefs to sand or mudflats (Amaral et al. 2009; Almeida et al. 2011). The crabs are generally found from the high tide level to 5-6 m deep and have a wide range of abiotic tolerances, being able to survive in temperatures from 0-35°C and reproduce in temperatures 18-26°C (Klassen & Locke, 2007).

Carcinus maenas represents reproductive strategy 1 (McLay & López Greco, 2011) with determinate growth (but several adult instars, see the paper for further explaination), soft shell mating and dorsal seminal receptacles.

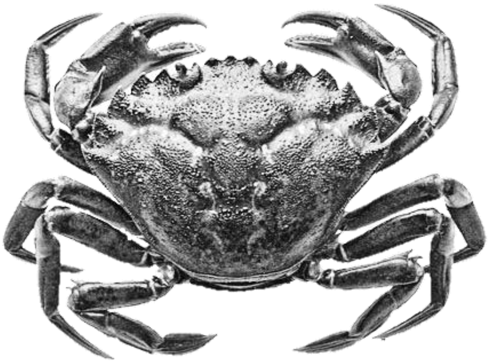

Fig. 1.3 Example of *Carcinus maenas* (actual carapace width: up to 10 cm) (photo from www.dfw.state.or.us).

THIS STUDY

This thesis is intended to address the one objective and two hypotheses outlined in Chapter 1:

1. To provide new information on the reproductive strategy, growth patterns, sexual selection, behaviour and population dynamics of the rarely studied crab species, *Halicarcinus cookii*.

2. H_0: Temperature has no effect on reproductive rates in crabs and global temperature rises will not influence reproductive rates to a point where population changes and invasive success of non-native species are affected.

3. H_0: The arrival, presence and effect of *Hemigrapsus takanoi* in the Dutch delta waters has had no effect on the native green crab *Carcinus maenas*.

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Chapter 2:

Relative growth and size at sexual maturity in *Halicarcinus cookii* (Brachyura: Hymenosomatidae): why are some crabs precocious moulters?

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Journal of the Marine Biological Association of the United Kingdom 2009, 89(4): 743–752.

ABSTRACT

The small intertidal New Zealand crab, *Halicarcinus cookii*, undergoes a terminal pubertal moult, but this moult takes place over a wide range of pre-moult sizes. Relative growth of the abdomen width in immature females is positively allometric, but negatively allometric in mature females. Male abdomen growth is negatively allometric. Growth of the cheliped propodus in males is positively allometric, but in females it is negatively allometric or isometric. Overlap in the size-range of mature and pre-pubertal immature female *H. cookii* is 72% and in other hymenosomatids it can be as high as 87%. This overlap is probably the result of crabs having a variable number of pre-pubertal instars, but seasonal change in water temperature, with crabs moulting to a smaller final size during colder months and to a larger size during the warmer months is also possible. The net reproductive rate (Ro) of early and delayed moulters is compared and for *H. cookii* Ro e / Ro d = 1.0 so the size overlap is stable.

INTRODUCTION

Relative growth of certain body dimensions can be used to distinguish between immature and mature crabs in a population. Growth is discontinuous and maturation can be easily broken down into stages, each of which can have distinct relative growth rates (Hartnoll, 1982). Structures that grow proportionately relative body size are isometric, while those that grow faster or slower, are positively or negatively allometric respectively. The most important change in life phase is when the crab becomes sexually active and is able to reproduce, this is known as the 'moult of puberty' (Hartnoll, 1969) or the 'critical moult' (Hartnoll, 1978). The pubertal moult is far more obvious in females than in males as it can involve allometric changes in the pleopods (including greater setation), sternum and most obviously, width of the abdomen (Hartnoll, 1969). Less obviously, males of many species experience an allometric increase in chelae size at the onset of reproductive maturity (Hartnoll, 1982). The larger abdomen of mature female crabs offer various degrees of protection to the embryos carried on the pleopods. In hymenosomatids and leucosiids we find the highest level of protection inside a brood chamber that fits closely against the sternum. Many other female crabs provide very little protection (see Thompson & McLay, 2005). Thus female sexual maturity is easily detected. Generally, male maturity is indicated by the size of the chelae: after the puberty moult, there is generally a very pronounced increase in relative size of the chelae (Hartnoll, 1982). Males often use chelae in territorial defence, combat and courtship, such as in Uca spp. (Hartnoll, 1969), and carrying or grasping the female during pre- or post-copulatory guarding, such as *Halicarcinus innominatus* (Dunnington, 1999). In hymenosomatids male chela size and female abdomen size are not always good guides to sexual maturity because pre-pubertal individuals can mate (Lucas, 1980). Such precocious maturation enables sperm transfer, but females must wait until after the pubertal moult to fertilize and lay eggs.

The only hymenosomatids that have been subjected to detailed morphological measurement are Amarinus laevis (Lucas & Hodgkin, 1970) and *Halicarcinus planatus* (Richer de Forges, 1977), *H. innominatus* (Dunnington, 1999), *H. varius* (Hosie, 2004) and *Rhynchoplax coralicola* (Gao et al., 1994).

The pattern of relative growth is affected by whether crabs have determinate or indeterminate growth. In the first hymenosomatid to be intensively studied, *Hymenosoma orbiculare* from South Africa, Broekhuysen (1955) showed that captive male and female crabs continued to moult after sexual maturity. Lucas (1980) also reported that *Elamenopsis lineata* from Australia and New Caledonia continued to moult after maturation, although no data were given. Although most hymenosomatids studied have determinate growth, these two species (and perhaps the genera they belong to) have indeterminate growth.

Most hymenosomatids probably have determinate growth so that after 8–12 moults crabs reach their adult size and cease moulting. This has been established for some species of *Halicarcinus* and *Rhynchoplax* (Lucas, 1980; Gao et al., 1994; Gao & Watanabe, 1998). For these species the dimensions of secondary sexual characters must be attained during a single moult to the adult instar, whereas *Hymenosoma* and *Elamenopsis* can continue to grow during adulthood. Unfortunately there have been no studies of relative growth on species in these two latter genera.

The aims of this study were to measure relative growth of secondary sexual characters in *Halicarcinus cookii*, a hymenosomatid crab endemic to New Zealand, and to measure the degree of size overlap between immature and mature crabs by investigating allometric changes during the terminal moult.

Furthermore, we compare maturity overlap with that found in other hymenosomatids and employ the concept of net reproductive rate (Ro) to test for the evolutionary stability of this overlap.

MATERIALS AND METHODS

Relative growth

Halicarcinus cookii were collected intertidally from Atia Point and First Bay, on the Kaikoura Peninsula (42°23'S 173°42'E), New Zealand, and in the laboratory measurements were made using MitutoyoTM Vernier callipers, accurate to 0.01 mm. Carapace width (CW) was used as the reference dimension and was measured across the widest part of the carapace (Figure 2.1A). Abdomen width was measured across the width of the 5th segment for both sexes (Figure 2.1B & C). Propodus height (PH) measured the maximum height of the largest cheliped (Figure 2.1D). Propodus length (PL) was measured across the longest point from the carpal–propodal joint to the tip of the fixed finger of the largest cheliped (Figure 2.1E). Analyses of relative growth were conducted using the power model equation:

$$Y = aX^{b},$$

where Y is the dimension of interest, X is the reference dimension (CW in all cases), a is the y-intercept and b is the relative growth rate (Hartnoll, 1978, 1982). The constants in this equation were estimated by linear regression using natural log (Ln) transformed data:

$$\text{Ln } Y = \text{Ln } a + b(\text{Ln } X)$$

Regression lines were compared using ANCOVA to identify the level and significance of allometric growth for each dimension. When $b = 1.0$ growth is isometric (both X and Y grow at the same relative rate), whereas when $b < 1.0$ growth is negatively allometric (Y grows less than X) or $b > 1.0$ (Y grows faster than X) and is positively allometric. Nineteen juvenile females were collected at different times during the summer months and followed through their pubertal moult. Measurements of the carapace width (CW) and abdomen width (AW) were taken before and after the pubertal moult to produce a percentage moult increment (increase in size from immaturity to maturity). The percentage moult. increment (PMI) was estimated using the formula:

$$\text{PMI} = (\text{post-moult } Y - \text{pre-moult } Y)/(\text{pre-moult } Y) \times 100$$

where Y is either CW or AW.

Effects of variable size at maturation

In hymenosomatids there is considerable overlap in size between mature and immature phases, which implies that crabs moult to maturity at different sizes. For *Halicarcinus cookii* we were only able to estimate this overlap for females. The percentage overlap in CW of mature and immature crabs can be estimated from:

$$\% \text{ overlap} = [(CWImax - CWImin) / (CWMmax - CWMmin)] \times 100,$$

and assuming the relationship between PMI and CW is linear:

$$CWImin = (CWMmin)/(1 + (PMI/100)),$$

where CWImax = maximum CW of immature females; CWImin = minimum CW of an immature female moulting to maturity; CWMmax = maximum CW of mature females; CWMmin = minimum CW of mature females; PMI =% moult increment of CW.

We also calculate the ratio CWMmax/CWMmin as an index of overlap. If the percentage increment of the pubertal moult is the same for all sizes, then as long as this is the final moult, the ratio will be an indicator of the size-range over which penultimate crabs moulted. It has the advantage of being easier to determine, but does not work if growth is indeterminate.

The effect of variation in the CW at which maturation occurs on lifetime larval production was investigated using the concept of net reproductive rate (Ro). The net reproductive rate is given by

$$Ro = \Sigma lxmx,$$

where lx is the proportion surviving to age x and mx is brood size at age x. When Ro = 1.0 the group exactly replaces itself. So if we assume that precocious moulters and delayed moulters have the same net reproductive rate, then $R_{o\,e} = R_{o\,d}$

$$\Sigma l_e m_e = \Sigma l_d m_d \text{ and}$$
$$1{:}0 = \Sigma\, l_e m_e / \Sigma\, l_d m_d$$

If me and md are fixed such that $m_e < m_d$ then we can estimate how many broods the delayed moulters have to produce to equal the sum of the broods produced by the early moulters, i.e. $R_{o\,e} = R_{o\,d}$. For the purposes of this estimate we assume that survivorship between broods, l_x, is constant (=0.5) and identical for precocious and delayed moulters. The maximum number of broods produced was set at 6 because at 20–30 days per brood cycle (depending upon temperature) 120–180 days would exceed the adult life span. The values of m_x were obtained using an equation giving the relationship between brood size and CW (see Van den Brink & McLay, 2009).

In order for there to be a stable size-range of maturation to sexual maturity the long term average of the ratio $R_{o\,e} / R_{o\,d} = 1.0$, otherwise all crabs would moult at the smallest or at the largest size. Therefore we tested whether the ratio was significantly different from 1.0 for *H. cookii*.

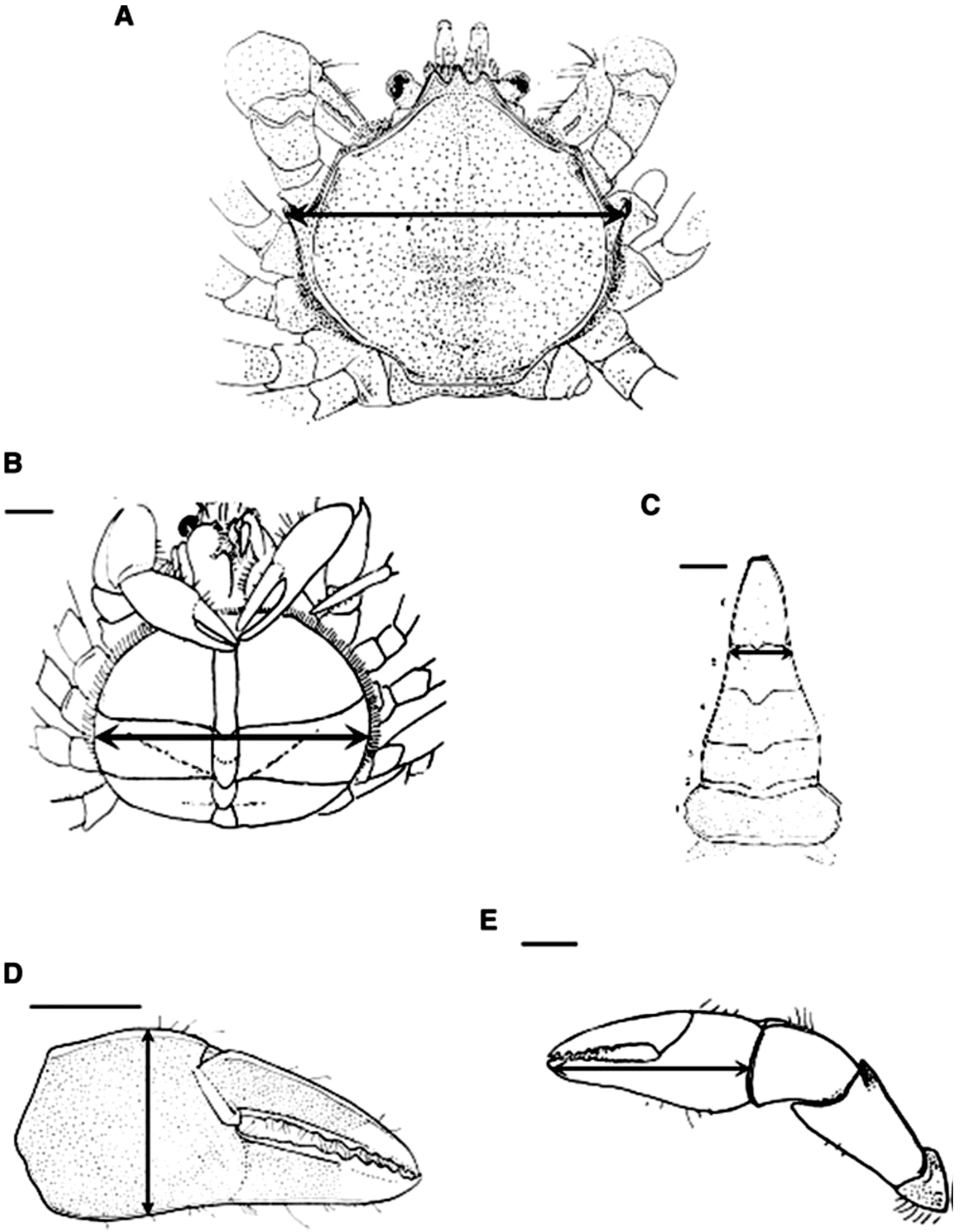

Fig. 2.1. Dimensions measured to investigate relative growth in *Halicarcinus cookii*. (A) Carapace width (dorsal view); (B) abdomen width of female; (C) abdomen width of male; (D) propodus height; and (E) propodus length. Scales represent 1 mm (from Melrose, 1975).

RESULTS

Brachyurans normally have two-phase postlarval development with the phases separated by a pubertal moult when relative growth of secondary sexual characters can change (Hartnoll, 1978). In *Halicarcinus cookii* the two phases are evident in changes to the abdomen width of females, but growth of male abdomen and chelipeds is continuous so that only one phase is evident. Since there was no obvious discontinuity in growth rates in males, which would suggest a single large change in increment at puberty, all measurements for males were pooled into a single group for all analyses.

Abdomen width

Female *H. cookii* showed a significant change in allometric abdomen growth from positive (b = 1.48) prior to the pubertal moult to negative (b = 0.85) when adult (Table 2.1; Figure 2.2). As the maturity moult is also a terminal moult, there is no opportunity for allometric growth in the final instar. However, mature females show an allometry of size, equivalent to allometric growth and can be termed ‘apparent growth’, as a result of females undergoing the pubertal moult over a range of sizes (Hartnoll, 1982). This ‘apparent growth’ is achieved in a single instar whereas relative growth of immature crabs is the result of real growth. Males showed negative allometric abdomen growth (b = 0.80) significantly different from 1.0 for both immature and mature crabs, without discontinuity. Males always had smaller abdomens than even immature females (Figure 2.2). Regression slopes of allometric abdomen growth are significantly different from 1.0 in all groups (Table 2.1). Relative growth rates of AW were also significantly different between all groups (df = 1, 182 (males); 58 (immature females); 203 (mature females), P < 0.001 in all cases) (Table 2.2). Increase in AW is limited by how wide the sternum is because the abdomen is coapted (Guinot & Bouchard, 1998, p. 614) to fit tightly against the sternum to form the brood chamber.

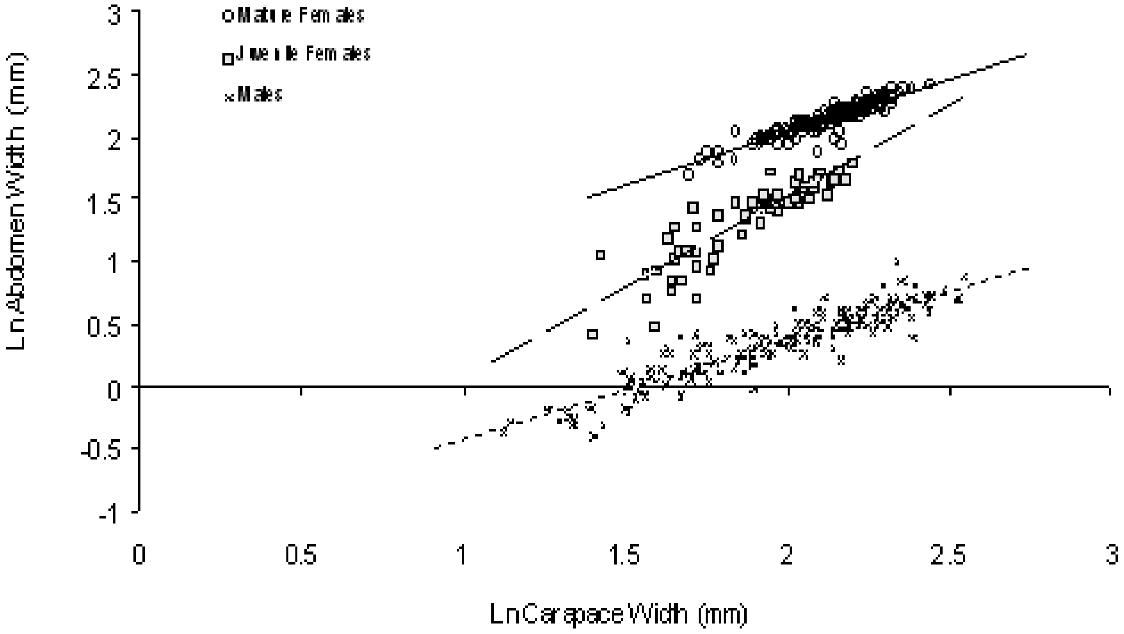

Fig. 2.2. Relative growth rate of the abdomen width to carapace width in *Halicarcinus cookii*. Ln abdomen width plotted against Ln carapace width, N=184 males, 205 mature females and 62 immature females. Regression equations and R^2 values are given in Table 2.1.

Propodus size

Both immature and mature females showed negative allometric growth in propodus length (b = 0.67 and 0.84 respectively) while males showed a positive allometric growth rate (b = 1.21) (Table 2.1). There appears to be little difference in propodus length between male and female mature groups (Figure 2.3), however, propodus length was still significantly different between the three groups, being longest in males and shortest in immature females (df = 1, 182 (males); 58 (immature females); 203 (mature females), $P < 0.001$ in all cases) (Table 2.2).

Males showed significantly positive allometric growth in propodus height (b = 1.45) while in females, growth in propodus height changed from negatively allometric in immature females (b = 0.89) to isometric in mature females (1.07) (Figure 2.4; Table 2.1). Relative growth in propodus height was significantly different between all groups (df = 1, 182 (males); 58 (immature females); 203 (mature females), $P < 0.001$ in all cases) (Table 2.2).

There was no discontinuity in the relationship between propodus height and carapace width in males (Figure 2.4), as is common in brachyurans, so there was no indication of the size when males mature. An arbitrary criterion was therefore established to separate mature from immature males for convenience. Male dissections suggest that males in a size range of 6–8 mm CW begin to produce. This provides an estimate of CWMmin but we cannot measure CWImax for males. For calculation of sex ratios, male H. *cookii* were regarded as immature with a CW > 7 mm and mature with CW > 7 mm.

Table 2.1. Equations for linear regression of relative growth of abdomen width (AW), proprodus length (PL) and propodus height (PH) for *Halicarcinus cookii*. A, y-intercept; b, relative growth rate; R^2, determination coefficient; N, sample size, F, F value; S, significant ($P < 0.05$) ("–" and "+" indicate negative and positive allometry respectively); and NS, not significant ($P > 0.05$).

Dimension	**Regression Equation LnY = (b)Ln X + Ln a**	**R^2**	**n**	**F-value**	**Significance of Allometry**
Abdomen Width					
Females	In AW = (0.8495)In CW + 0.3468	0.84	205	36.28	S -
Immature females	In AW = (1.4755)In CW - 1.4364	0.79	62	59.49	S +
Males	In AW = (0.7993)In CW - 1.2268	0.84	184	550.6	S -
Chela					
Propodus Length					
Females	In PL = (0.8483)In CW - 0.2979	0.64	205	199.9	S -
Immature females	In PL = (0.6784)In CW + 0.0290	0.66	62	150.6	S -
Males	In PL = (1.2113)In CW - 0.8326	0.94	184	516.81	S +
Propodus Height					
Females	In PH = (1.0747)In CW - 1.8421	0.66	205	12.1	NS
Immature females	In PH = (0.892)In CW - 1.4186	0.67	62	87.06	S -
Males	In PH = (1.4518)In CW - 2.1763	0.93	184	1414.7	S +

Table 2.2. Analysis of covariance of allometric growth rates of abdomen width, propodus length and propodus height of both male and female *Halicarcinus cookii*, F, F statistic; df, degrees of freedom; *P*, level of significance.

Dimension	**F-value**	**df**	**p-value**
Abdomen Width	111.61	2, 447	$p < 0.001$
Propodus Length	320.473	2, 447	$p < 0.001$
Propodus Height	1929.335	2, 447	$p < 0.001$

Table 2.3. Percentage overlap in size at which hymenosomatid crabs undergo their pubertal moult in relation to the adult size-range, i.e. pre-moult size range/post-moult size-range and the ratio of maximum mature carapace with (CWM_{max}) to minimum mature carapace (CWM_{min}) width.

Species	**Males**		**Females**		**Source**
	Size Overlap (%)	**CWMmax /CWMmin**	**Size Overlap (%)**	**CWMmax /CWMmin**	
Amarinus laevis	79.6	1.8	61.1	1.42	Lucas & Hodgkin, 1970
Hymenosoma orbiculare	??	??	44.4	1.8	Broekhuysen, 1955
Rhynchoplax coralicola	80	1.48	48	1.6	Gao etal, 1994
Halicarcinus planatus	49.8	1.85	87.3	1.74	Richer de Forges, 1977
Halicarcinus innominatus	64.3	3	78.1	2.27	Dunnington, 1999
Halicarcinus varius	68.6	2.5	59.3	2.17	Hosie, 2004
Halicarcinus cookii	??	??	72.5	2.11	Present study
Means	68.5	2.13	64.4	1.87	

By monitoring 19 females in their penultimate instar through their maturity moult, it was possible to produce the percentage moult increments (PMI) for the carapace width and abdomen width. The PMI for carapace width ranged from 5.8%–41.29% with a mean of 17.96% (±2.55). This suggests that on average females increased close to 20% in overall size over the pubertal moult. In contrast, the PMI for abdomen width ranged from 46.115–146.58% with a mean of 96.13% (±6.02). Therefore, while increasing in overall size by approximately 20%, females increased their abdomen width by close to 100%. The pubertal moult marks the transition to sexual maturity when the female produces her first clutch of eggs that will be stored in the abdominal brood chamber, the size of which is determined by the moult increment (coaptation of sternum and abdomen; see Guinot & Bouchard, 1998).

A plot of measurements of pre- and post-pubertal moult females with the regression lines from the allometric growth study (see Figure 2.2) shows females monitored through their pubertal moult in the laboratory and regression lines derived from females found in the field (Figure 2.5) are close. The slope of these lines is 1.4755 for immature crabs (positively allometric) but only 0.85 for mature females (negatively allometric). This difference is explained by the negative relationship between the moult increments and pre-moult CW with larger crabs tending to have smaller increments (see Figure 2.6).

Size at the pubertal moult

There was a substantial overlap in CWofmature and immature females. Using abdomen width, immature females ranged in CW from 4.05–9.01 mm while mature females ranged from 5.44–11.51 mm. The pre-moult CW of the smallest mature female (5.44 mm) must be (5.44/1.18) = 4.61 mm, rather than the 5.51 mm CW of the smallest penultimate female found in the field. This calculation provides a range for the penultimate instar pre-moult CW of 4.61–9.01 mm. For females the overlap between mature and immature phases is:

Overlap = [(9:01 - 4:61)/(11:51 – 5:44)] × 100 = 72:5%:

How many times would the smallest crab that made the pubertal moult have to moult in order to reach the maximum immature size (i.e. grow from 4.61 to 9.01 mm)? The mean moult increment of the 19 captive pubertal females was 18%, so beginning with CW 4.61 and growing at 18% per moult we could have up to 4 additional prepbertal instars of CW 5.44 mm, 6.42 mm, 7.57 mm and 8.94 mm. The relationship between brood size and CW is Ln m = 1.17 + 0.9408 Ln CW (R^2 = 0.69) (see Van den Brink & McLay, 2009). For the smallest mature female *H. cookii* me = 316 eggs/brood so that over 6 brood cycles, with only half of the females surviving to produce the next brood, an early moulter would produce 622 larvae. But if females delayed the pubertal moult, and reproduced at the largest CW, so that md = 2818 eggs/brood, and commenced their first brood in the fourth brood cycle (half-way) they could produce approximately the same number of larvae (617), even though their number had been reduced to only 12.5% (0.5 × 0.5 × 0.5). Given these assumptions the ratio $R_{o\ e}$/ $R_{o\ d}$ = 622/617 =1.01 is not significantly different from 1.0.

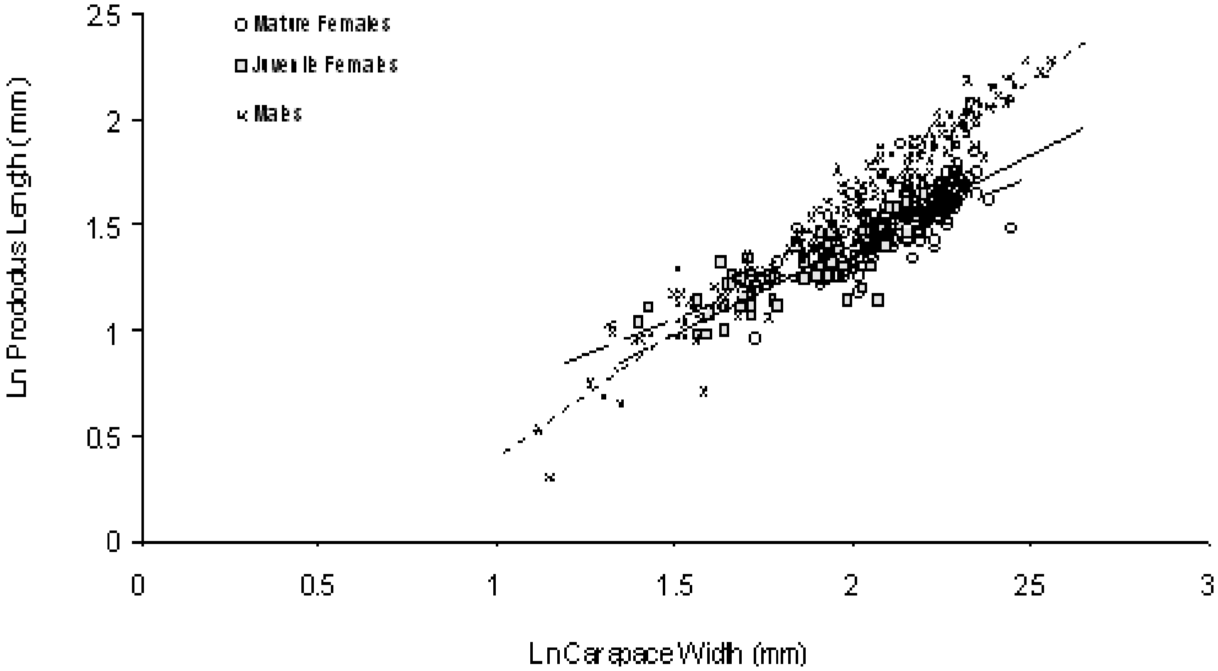

Fig. 2.3. Relative growth rate of propodus length to carapace width in *Halicarcinus cookii*. Ln propodus length plotted against Ln carapace width, N = 184 males, 205 mature females and 62 immature females. Regression equations and R^2 values are given in Table 2.1.

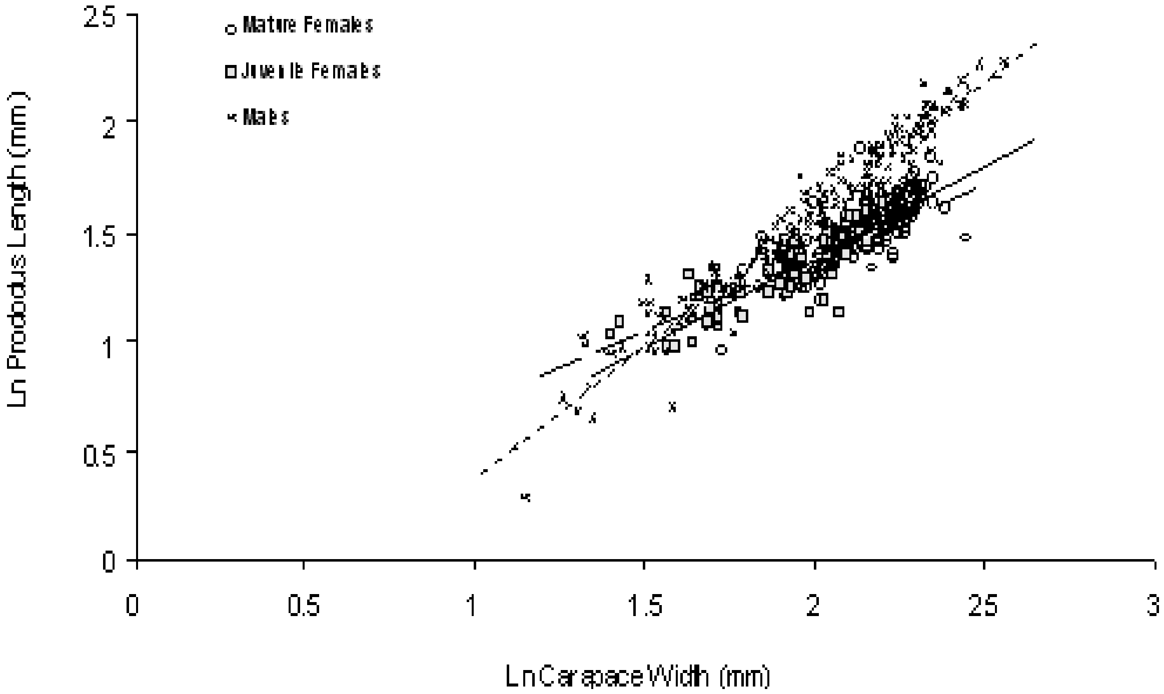

Fig. 2.4. Relative growth rate of propodus height to carapace width in *Halicarcinus cookii*. Ln propodus width plotted against Ln carapace width, N = 184 males, 205 mature females and 62 immature females. R^2 values and regression equations are given in Table 2.1.

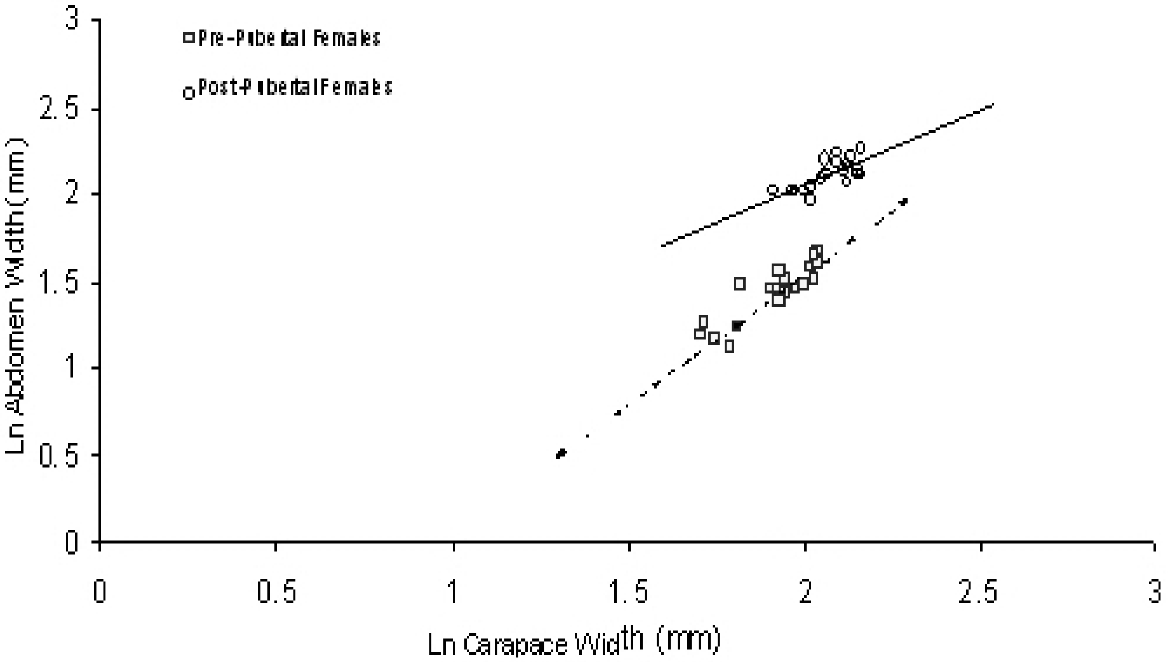

Fig. 2.5. Comparisons of the Ln carapace width to Ln abdomen width of *Halicarcinus cookii* females before and after their pubertal moult. N = 19. Regression lines are taken from allometric growth rates (Figure 2.2). Regression equations and R^2 values are given in Table 2.1.

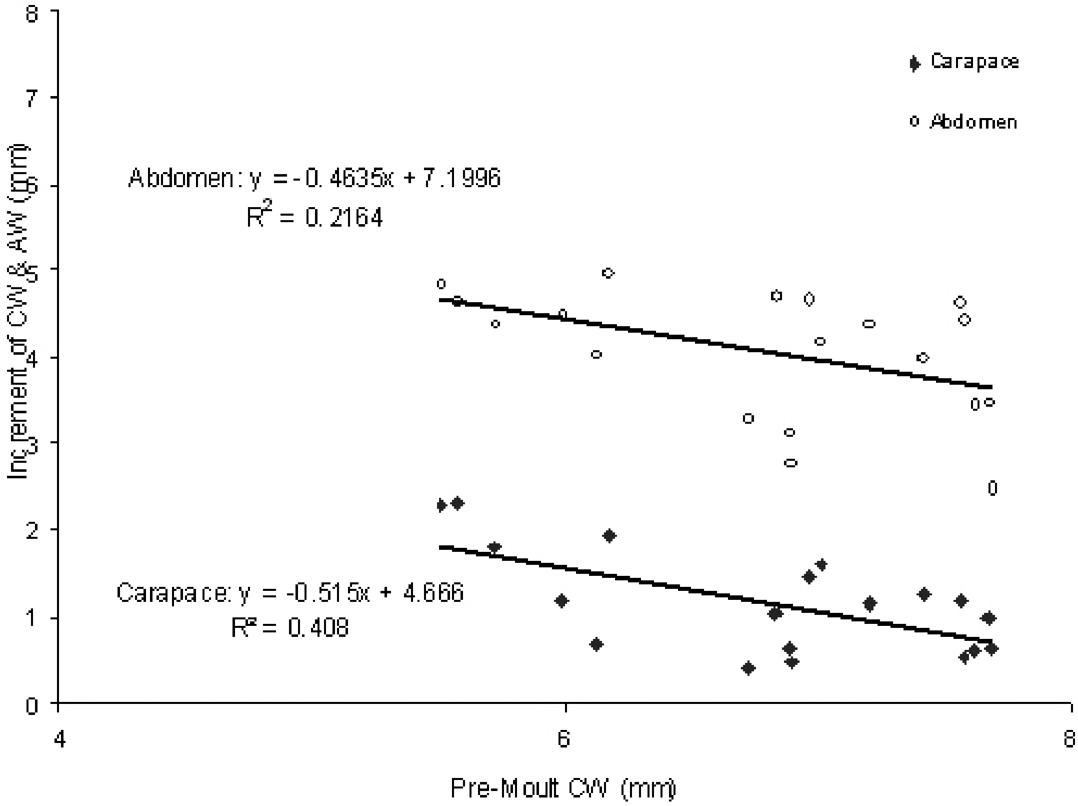

Fig. 2.6. Growth in abdomen width (AW) and carapace width (CW) in relation to pre-moult carapace width at the pubertal moult in *Halicarcinus cookii*. Equations of regression lines fitted to data (N = 19) and R^2 shown above (see Figure 2.5 for original data).

DISCUSSION

Relative growth

Secondary sexual characteristics are the result of allometric growth and in most crabs are indicators of ability to produce gametes. For females this usually involves the abdomen and for males chelae size relative to carapace width (Watson, 1970; Comeau & Conan, 1986, 1992; Sainte-Marie & Hazel, 1992; Stevens et al., 1993; Claxton et al., 1994). For species with indeterminate growth, separate phases of relative growth are the result of a sequence of several moults, but in crabs such as hymenosomatids and majids, which have determinate growth, the apparent 'growth' of mature animals is the result of a single pubertal moult that occurs over a range of pre-moult sizes.

The relative growth of abdomen width in *Halicarcinus cookii* males was negatively allometric. The male abdomen changes little throughout development, remaining triangular and covering only the gonopods and a small part of the sternum. In contrast, two distinct phases of abdomen growth were seen in females. Immature females showed positive allometry in abdomen width, while after the pubertal moult abdomen width became negatively allometric and the abdomen changed in shape. In female *H. cookii*, the abdomen changes from being flat and only partially covering the sternum, to convex and completely covering the sternum, creating a brood chamber in which the embryos will be incubated. Thus the size of the brood chamber is set by the sternal area because the abdomen is coapted (Guinot & Bouchard, 1998, p. 614) to fit tightly against the sternum. Immature and mature females are easily recognized in the field. Similar patterns of abdominal growth are seen in other hymenosomatids such as *Rhynchoplax coralicola* (Gao et al., 1994), *Amarinus laevis* (Lucas & Hodgkin, 1970a), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004).

For chelipeds, *H. cookii* males showed strong positive allometry of both propodus length and height throughout development. This pattern is typical of brachyurans (Hartnoll, 1978, 1982, 1985) and was reported for the hymenosomatids *Rhynchoplax coralicola* (Gao et al., 1994), *Amarinus laevis* (Lucas & Hodgkin, 1970), *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). Female *H. cookii* showed negative or isometric growth in their chelipeds. Overall male chelae size increases at a much higher rate than in females, resulting in males having obviously larger chelipeds than females. In male *H. cookii*, there was no distinguishable difference in chelae size relative to carapace width that would indicate more than one growth phase. Male *H. cookii* use their chelipeds to guard females which they mate with, so for them size does matter .

Chelae morphology can also be used as an indication of male maturity in some brachyurans. Changes in the size, shape, setation and dentition of the chelae over the maturity moult have been used to distinguish between immature and mature males of the Majidae (Hartnoll, 1965; Comeau & Conan, 1992; Claxton et al., 1994). Over the maturity moult, males of the hymenosomatid *A. laevis* develop pulvini (prominent sacs) between the fingers of the chelae (Lucas & Hodgkin, 1970) and similar sacs are seen in *Hemigrapsus sexdentatus* (McLay, 1988). Males of *Elamena* spp., *Cyclohombronia depressum*, *Halicarcinus innominatus* and *H. varius* develop an extra tooth on the dactyl of the cheliped that becomes more pronounced and is an indication of maturity (Melrose, 1975; Lucas, 1980). Male H. *cookii*, however, lack this tooth and show no distinct change in morphology of the cheliped fingers throughout development (Melrose, 1975).

Secondary sexual characters normally indicate ability to mate, however Lucas (1980) reported that in the hymenosomatids *Amarinus lacustris* and *A. paralacustris*, males classified by chelae size as immature, were capable of ‘copulating’. Also in *Chionoecetes opilio*, morphologically immature males have been reported to produce sperm (Comeau & Conan, 1992), but were unable to successfully restrain and therefore mate with, multiparous females in the wild (Stevens et al., 1993; Claxton et al., 1994, respectively). However, immature males of *C. bairdi* can restrain soft, primiparous females long enough to copulate (Paul & Paul, 1992). If cheliped size is not an adaptation primarily for restraining females, competition between males is a likely causal factor for the development of such weapons for purposes of fending off rival males (Andersson, 1994). In *H. cookii*, chelipeds are not used in courting displays, but they are used in agonistic encounters between males. Males with larger chelipeds have an advantage over those with smaller chelipeds, providing one selective force favouring large chelipeds in males. With their precocious mating habits hymenosomatids, are an interesting case where lack of fully developed secondary sexual characters does not necessarily mean that the crabs cannot mate. Female *H. cookii*, in their penultimate instar, are attractive to males and can copulate so that both sexes are precocious maters.

In most hymenosomatids, the pubertal moult is also the terminal moult, after which growth no longer occurs. This cessation in growth restricts the individual from shedding damaged or parasitized integument, and replacing lost limbs (Lucas, 1980). A terminal, pubertal moult for both males and females is thought to occur in *Amarinus* and *Halicarcinus* species (Lucas, 1980). Such a terminal moult was obvious in female *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). Similarly, a terminal, pubertal moult was obvious in female H. *cookii*, but there was little evidence suggesting a terminal moult in males. Only one phase of cheliped growth is evident, which could be interpreted as indicating that males do not have determinate growth. However, no captive large male *H. cookii* were observed to moult and no limb buds were apparent in crabs that had lost pereopods. If male *H. cookii* did not experience a terminal moult, they would be expected to grow much larger than females. As this was not observed, a terminal moult in males can probably be assumed to occur.

Mature and immature size overlap in hymenosomatids

For such a wide size-range over which maturation occurs there must be advantages to moulting at a small size as well as advantages to moulting at a large size. Perhaps moulting small means earlier maturation and more chances to reproduce, but moulting large means that females have a larger brood size and males get more copulations. However, the longer that maturation is delayed the fewer crabs that survive, but for both growth strategies to coexist $R_{o\,e}/R_{o\,d} = 1.0$.

Studies of seven hymenosomatids are available in which size overlap between mature and immature crabs can be assessed from relative growth data (Table 2.3). *Amarinus laevis*, *Hymenosoma orbiculare*, *Halicarcinus planatus* and *H. innominatus* are much larger than the others, growing to around þ20 mm CW, while *H. cookii* and *H. varius* grow to about 14 mm CW, but *Rhynchoplax coralicola* is very small only growing to around 4 mm CW. Typically hymenosomatid males grow larger than females: for the species listed in Table 2.3, males are on average 20% larger than females. For hymenosomatids with indirect development (the majority) there are normally 3 zoeal instars, the last of which moults to the first crab stage as there is no megalopa. The number of crab instars is variable in hymenosomatids: *A. lacustris* has 8, *A. paralacustris* can have between 10 to 12 or 13 instars (Lucas, 1980), and for *H. planatus* the pubertal moult is the 11th instar (Richer de Forges, 1977). The number of instars is evidently not fixed.

The overlap between immature and mature animals of the same CW is mostly more than 50% (Table 2.3), greater in males of *A. laevis, R. coralicola* and *H. varius* than in females, but in *H. innominatus* and *H. planatus* the reverse seems to be true. For all species the mean overlap for males is 68.5% and for females 64.4%. A similar pattern is revealed using the ratio CWMmax/CWMmin although the difference between males and females is accentuated (2.13 versus 1.87). Whatever the CW at which *H. cookii* makes its terminal moult, the longer it delays the smaller will be the moult increment, because of the negative relationship between MI and CW (see Figure 2.6). The other consequence for delaying the moult is that there are fewer survivors. In the case of *H. cookii* the largest mature crabs would have the same net reproductive rate (R_o) as the smallest mature crabs if they produced at least 3 broods, which is not unreasonable. With $R_{o\,e}/ R_{o\,d} = 1.0$ for *H. cookii* females the conditions for coexistence of both growth strategies seem to have been met. If this were not true then one would expect one extreme or the other to dominate. One would expect the same to hold true for males.

It seems unlikely that the large size-range over which the pubertal moult occurs could be solely due to the cumulative effects of variation in growth rate within a fixed number of instars. An alternative explanation is that individuals vary in the number of instars they take to reach sexual maturity (see discussion by Hartnoll, 1978, p. 291; Schejter & Spivak, 2005). Therefore, it is reasonable to hypothesize that *H. cookii* has a flexible moulting pattern and a variable number of moults before reaching maturity. If a 4.6 mm CW crab (the smallest size at which females undertake their pubertal moult) chose to continue moulting, increasing at 18% per moult we could have up to 4 additional pre-pubertal moults to reach the CW of the largest immature crab, 9.0 mm (instar sizes would be 5.44 mm, 6.42 mm, 7.57 mm and 8.94 mm). This spans the size-range where mature and immature crabs overlap. The answer to the question 'Why are some crabs precocious (or delayed) moulters?' can be answered for females by saying that they both have roughly the same net reproductive rate. The flexible moulting strategy may allow the crabs to take advantage of seasonal changes. This hypothesis could be tested by recording the number of moults by captive crabs, under natural conditions, during warm and cold months.

A complicating factor in the coexistence of multiple growth strategies is the occurrence of precocious mating. Mating can occur prior to the pubertal moult and females usually lay their first clutch of eggs within a couple days of moulting, indicating that their ovaries were well developed during the last immature instar. In some species precocious mating has been demonstrated for both males and females. Male *Amarinus lacustris* and *A. paralacustris* can impregnate females as early as 3 moults before the pubertal moult (Lucas, 1980). While the reproductive output of females can be easily estimated by the number of eggs produced, it is more difficult to estimate the number of fertilizations achieved by males. Females can only lay eggs after the pubertal moult, when they have a brood chamber, but males can begin spreading their 'seed' beforehand and so their reproductive output is not so closely tied to the pubertal moult. Sperm competition resulting from females storing sperm from multiple partners, adds to the problem of estimating the number of fertilizations achieved by a male. Male mate guarding behaviour, that affects sperm precedence in *H. cookii* and therefore fertilizations, is dealt with by Van den Brink & McLay (in preparation).

A feature of growth in hymenosomatids is that most species have determinate growth, but a few species have indeterminate growth. *Hymenosoma orbiculare* (Broekhuysen, 1955) and *Elamenopsis lineata* (Lucas, 1980) continue to grow after reaching maturity. In Hymenosoma moult increments decrease and inter-moult durations increase with crab size, slowing the rate of growth. Multiparous females delay laying their eggs until about 2 months after moulting–mating indicating that their ovaries were not mature at the time of mating. The significance of this observation is that mate attraction by females must be the result of a moult-linked signal rather than an ovarian maturation signal. Although there is a delay in fertilization of about 2 months, *H. orbiculare* has retained the ancestral link between moulting and mating (see Thompson & McLay, 2005), whereas the majority of hymenosomatids have a terminal moult and both sexes are hard-shelled when they mate. In these species mate attraction must be the result of an ovarian signal. This difference in mate attraction was an important step in the evolution of hymenosomatid mating strategies. Given that moulting þ mating (indeterminate growth) is the plesiomorphic state, the presence of species with no link between moulting and mating (determinate growth) suggests that determinate growth may well have evolved independently in the hymenosomatids, perhaps as part of the strategy of small body size and a frenetic pace of reproduction. Apart from hymenosomatids, determinate growth is common amongst the spider crabs (Majidae) (Hartnoll, 1978; Schejter & Spivak, 2005).

The overlap in size between immature and mature crabs should beminimal in species that have a fixed number of immature instars and indeterminate growth, perhaps as much as the percentage moult increment for the pubertal moult (15–25%), whereas in species with determinate growth and a variable number of instars, the overlap should be much greater. In *Hymenosoma orbiculare*, the only indeterminate growth species studied, the overlap is 44%, which is the smallest of all the species, but the ratio

CWMmax/CWMmin is close to the mean for all species (1.87) (see Table 2.3). Both of these measures of overlap are greater than might be expected, so that it is possible that this species also has a variable growth strategy. Overlap in CW between immature and mature instars may well be an ancestral feature of hymenosomatids, perhaps shared with majids (a symplesiomorphy).

An alternative explanation of the large size-range over which *H. cookii* moult to maturity is that the timing of the pubertal moult may be affected by season/temperature. During winter when growth is slow, crabs could moult to maturity when they are small, but in the warmer months they could grow larger and make the pubertal moult at a larger size. If this were true then one would expect to find seasonal changes in occurrence of the largest size-classes. The population of *H. cookii* at Kaikoura is unimodal dominated by crabs of CW 8–10 mm and with no evidence of cohorts that reach a larger size during the summer. The mean size throughout the year for both sexes ranges between 7 and 9.5 mm CW with a tendency to be lower during the colder months. For *Halicarcinus planatus*, from Kerguelen where temperatures are very low year round (mean monthly temperature range 1.78–7.38C), there is no evidence of seasonal occurrence of larger crabs (Richer de Forges, 1977). However, in *Rhynchoplax coralicola* from Japan, which is a very small hymenosomatid (maximum CW 4.0 mm), there are clear seasonal changes in population structure with larger crabs becoming progressively more common during the summer (Gao et al., 1994). So seasonal changes might explain the moulting pattern and the 80% size overlap for males and 48% overlap for females in this species.

ACKNOWLEDGEMENTS

Thanks to Jack van Berkel at the Edward Percival Field Station for all his help, and Kimberley Seaward for keeping me sane. We would also like to thank two anonymous referees whose suggestions greatly improved this chapter.

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Chapter 3:

Competing for last place: Mating behaviour in a pill-box crab, *Halicarcinus cookii* (Brachyura: Hymenosomatidae)

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Zoologischer Anzeiger 2010, 249: 21–32

ABSTRACT

The mating strategy of *Halicarcinus cookii* was investigated to ascertain how males maximised their fitness through mate choice. An intertidal population at Kaikoura, New Zealand, was dominated by mature crabs of both sexes in summer and by immature crabs in the colder months. More than 95% of mature females were ovigerous with early stage and late stage broods found in almost every month, indicating that egg production and larval release is continuous. The operational sex ratio was less than 1 male/female in summer, but often more than 1.0 in the colder months. The gonosomatic index increased along with brood development so that as soon as zoeae were released, the next clutch of eggs was ready to be fertilised. Males searched for receptive females and began pre-copulatory mate guarding without any courtship display. They mated preferentially with late stage or non-ovigerous females: copulation duration was longest for stage 5 females as was post-copulatory guarding (mean 18.3 h). Late stage females were up to 14% of the female population. Mate attraction seems to be the result of an ovarian signal rather than from the developing brood. Manipulation of the sex ratio had effects upon copulation duration and post-copulatory guarding: presence of a rival male increased duration of guarding. Females showed precocious mating in the penultimate instar and were able to lay fertilised eggs after their pubertal moult in the absence of males. *Halicarcinus cookii* females have many mates, but males attempt to ensure paternity by preferentially pursuing mature females close to egg laying and by guarding these females after copulation. These behaviours are all elements of a competitive strategy to ensure that a male loses (not wins) the race to copulate because females have a ventral seminal receptacle, giving sperm precedence to the last male to mate. Male mating behaviour is a consequence and evolutionary response to female morphology.

INTRODUCTION

Brachyuran reproductive strategies should maximise individual reproductive fitness (Diesel, 1991). For males the aim is to fertilise as many eggs as possible and for females it is to maximise the supply and genetic diversity of the sperm. Sperm storage in brachyurans allows females to accumulate sperm and to produce multiple broods without re-mating (Diesel, 1991). Where sperm competition occurs males would be expected to employ mate guarding behaviour to minimise the risk of other males mating with the female.

For comparative purposes, we consider that the important reproductive characteristics of brachyurans are (1) whether growth is determinate or indeterminate; (2) whether mating is linked to moulting; and (3) the structure of the seminal receptacle. Of lesser importance is the allocation of energy to reproduction vs growth whose consequences are reflected in brood size and survival. In the Hymenosomatidae growth is determinate and mating is not linked to moulting (except in *Hymenosoma orbiculare*) (Lucas, 1980) so that after the pubertal moult females have no way of emptying the contents of their sperm storage organs by moulting. Of equal importance is the morphology of the seminal receptacle, in particular where is the oviduct connected. In *Halicarcinus cookii* the pubertal moult is terminal and, in the absence of sperm mixing, the last male to mate should fertilise the majority of the eggs in each clutch because the oviduct enters the receptacle in a ventral position (Van den Brink and McLay, 2009). However, mating with multiple males, and the absence of moulting, means that females accumulate sperm over time and males can never be entirely sure of paternity. The male mating strategy, to maximise the probability of fertilisation, must circumvent female morphology. These features of the seminal receptacle structure, the growth format and the absence of a link between moulting and mating (except for primiparous females in some species) are all part of the majoid mating strategy (see McLay and Lopez Greco, in prep.). We restrict our discussion and comparisons to species sharing these characters.

Species of the family Hymenosomatidae are commonly known as pill-box or false-spider crabs (Lucas, 1980; Melrose, 1975). The vast majority are found in the Indo-Pacific region, most of which inhabit Australia and New Zealand, but the family is also represented in China, Japan, Southeast Asia, Africa, and New Caledonia (Ng and Chuang, 1996). They are characterized by their small size, poorly calcified carapaces, which are dorsally flattened and triangular or sub-circular in shape, and short life-spans. *Halicarcinus cookii* is endemic to New Zealand and is ubiquitous along the east coast of the North and South Islands, as well as the Chatham Islands and Stewart Island. The species lives in intertidal and sub-littoral shallow water among seaweeds on the rocky shore and its microhabitat includes dense algal fronds and holdfasts (McLay, 1988; Melrose, 1975). *Halicarcinus cookii* is an opportunistic carnivore and scavenger, with a diet consisting of molluscs, polychaetes (Perinereis, Lumbriconereis and Neanthys) and especially amphipods (McLay, 1988). *H. cookii* is typical of hymenosomatids in size: males grow to a maximum CW of 14.0 mm and females a CW of 11.5 mm.

The aims of this study were to investigate the population dynamics of *H. cookii*, the relationship between ovarian and brood development, the mating behaviour, what makes females attractive to males and whether the operational sex ratio (mature males per mature female) influences mating behaviour or not.

MATERIALS AND METHODS

Population dynamics

Monthly samples of the population from two sites provided data for the calculation of sex ratios for 15 months from October 2004 until December 2005. Surveys occurred over two consecutive days each month and involved a 90 min haphazard search at Atia Point and First Bay, Kaikoura Peninsula (42°125'S, 173°142'E), New Zealand. Searches were focused on the mid to low shore in stony and sandy areas with abundant brown seaweeds (e.g. *Hormosira banksii* and *Cystophora* spp.). All *H. cookii* individuals found were collected and brought to the laboratory. In the laboratory carapace width (CW) was measured (as defined in McLay and Van den Brink, 2009, Fig. 3.1) and crabs were sexed and the stages of development of the brood carried by ovigerous females was recorded as stage 0–5. Stage 0 females are non-ovigerous; stage 1 eggs have bright orange yolk with little or no embryo cleavage; stage 2 eggs have orange yolk and obvious signs of cleavage; stage 3 eggs have orange yolk, chromatophores and the embryo makes up about 50% of the volume; stage 4 eggs have chromatophores, the embryo with eyespots and constituting 75% of the volume; and stage 5 eggs have less than 10% yolk, prominent eyespots, zoea fully developed and ready to hatch.

In both males and females the moult to maturity occurs over a range of sizes and probably instars (see McLay and Van den Brink, 2009). Operational sex ratios were determined as the ratio of mature males and mature females. Maturity in females was easily determined by the shape and larger size of the abdomen. Unfortunately male maturity was more difficult to determine: log-transformed cheliped data show positive allometry for both propodus length ($b=1.21$) and height ($b=1.45$) for all sizes, relative growth being continuous and without inflections or breaks in elevation. Examination of testes and vas deferens of 30 males, 4.4–11.4 mm CW, showed sperm to be present in males as small as 6.3 mm, but no sperm were found in some males up to 7.02 mm. Given that the smallest male observed to mate was 6.4 mm, we chose to regard 7 mm CW as being the minimum size of male maturity.

Gonosomatic index

Halicarcinus cookii female gonads were removed under a dissection microscope, placed on a glass slide, where excess water was blotted with a paper towel, and weighed (GW). The gonosomatic index (GSI) was calculated as the ratio between wet gonad weight (GW) and wet body weight (BW) as a percentage. The mean GSI was calculated for each brood stage ($n=13$, 19, 25, 16, 18 and 19 for stages 0–5, respectively) and results were compared using a one-way ANOVA. Variances for each brood stage were tested for homo- geneity using Cochran's test. Egg size was measured with an eyepiece micrometer (accurate to 0.03 mm) for 20 eggs at each stage of development. Assuming circularity, volume was estimated by $V=4/3\ (\pi r^3)$. For GSI and egg size females were sampled throughout the year although there was a summer bias because more suitable females were available. There was little seasonal variation in either variable

Mate guarding

Females were separated into the five brood stages. For each trial, a female carrying a brood of known stage and a male (of similar size but often larger because of size dimorphism) were selected and placed together in a 2 L container half-filled with fresh seawater. Each pair was monitored for copulation using a Panasonic™ WV-BP312 infrared video camera and a Panasonic™ AG-1070 time-lapse video recorder. A copulation was identified when the male had turned himself onto his dorsal side and positioned his sternum inside the brood chamber of the female whom he clasped from underneath with his legs. If copulation did occur, these were compared using one-way ANOVA and a χ^2 test for goodness of fit. The presence and duration of guarding behaviour was observed and recorded. Mate guarding was recorded when the male caged the female, with his sternum on the female's carapace, or simply when the male's legs or chelipeds remained in contact with the female.

The pair was monitored until the male lost physical contact with the female and therefore all mating behaviour between the pair was considered to have ended. Twenty trials for each brood stage (5) plus juveniles (CW 5–6 mm) and mature females without eggs were conducted producing a total of 140 trials (7 × 20). New crabs were used for each successive trial to maintain a degree of independence in the results. The frequencies and durations of mate guarding for each brood stage

Female choice

A small male ~8 mm CW and a large male >12 mm CW were tethered to opposite corners of a 2 L container filled with seawater. The female was placed in the middle inside a small transparent container with holes punctured in the sides so that the female experienced the same water as the males. After 10 min acclimatisation the container was removed allowing the female to freely roam the container (20 trials using new crabs each time). The size of the first male able to clutch the female was recorded.

Operational sex ratio

To investigate the effects of biased operational sex ratios (OSR) on mating behaviour we conducted experiments in which there was 1 male: 2 females, 1 male: 1 female and 2 males: 1 female, where all the females were carrying stage 5 eggs. Where 2 males or 2 females were present they were of matched CW. Timing began when one male clutched the female and ended when he lost physical contact with her. Observations of male–male interactions were also recorded. The duration of copulation and mate guarding in the presence of a second male was then compared to that of the single male trials.

Mate attraction

Females carrying stage 5 broods were subjected to the videoed guarding trial (see Mate Guarding, above), and then the embryos were removed with care to avoid any damage to the pleopods. The trial was then conducted again with a different male, and the same female without embryos.

To determine whether or not a male could distinguish between a female with developed ovaries and one with undeveloped ovaries, two females, one with a stage 5 brood, and one with a stage 1 brood were placed in a container, after the brood had been removed ($n=20$). A different male was introduced into the container and his choice of which female to mate with was recorded. The broods of stages 4 and 5 females were carefully removed and the females presented to males individually and observed using the time lapse video technique. The duration of copulation and mate guarding were recorded and compared between brood stages.

RESULTS

Population dynamics

During the summer months, the *Halicarcinus cookii* population was dominated by mature individuals in both sexes (Fig. 3.1). Mature males were most frequent in June and August 2005 (31.8% and 33.3%, respectively), and the least in January 2005 (14.4%). Immature males were most frequent in October 2004 (27%) and through the winter months of 2005 (22.7% in June through to 24.7% in October). Mature females were most frequent in spring and summer, reaching a peak of 77.5% of the population in January 2005 and were least frequent in the winter months at a low of 22.7% in June 2005. Immature females showed the opposite trend to mature females, being most frequent in May 2005 (41.4%) and least in January.

Mature females are broken down according to whether they were ovigerous or not and if so following their stage of brood development (Fig. 3.2). Of the mature females found, a mean of 96.4% (±1.07%) were ovigerous ranging from 85.9% in December 2005 to 100% in October 2004, March–July 2005 and September 2005. Stage 1 broods were recorded every month showing that egg production is continuous while stage 5 broods were found in every month, except June 2005, indicating that larvae are released year round. Therefore we can expect recruitment to be continuous. Overall most ovigerous females were carrying stage 1 broods (44.4±3.17%), followed by stage 2 broods (16.4±2.13%), stage 5 broods (14.1±1.33%), stage 3 broods (11.6±2.04%), and those carrying stage 4 broods were the least common (9.8±1.38%). Assuming the sampling was unbiased, these frequencies are an estimate of the relative duration of each stage. The longest is stage 1 and the shortest is stage 4.

The operational sex ratio (OSR, mature males per mature female) was similar to the population sex ratio (see Figure 3.3) and differed significantly from 1:1 in December 2004–April 2005 ($p<0.001$ in January– March 2005 and $p<0.05$ in December 2004 and April 2005) and November and December 2005 ($p<0.001$ in both cases). Generally the OSR was less than 1.0 during the warmer months and greater than or near 1.0 during the colder months (Figure 3.3). Stage 5 broods, the most desirable to males (see below), constitute up to 20% of the female population, but is outnumbered by males so that the OSR for these females ranges from 1.4 to 6.8 males per female.

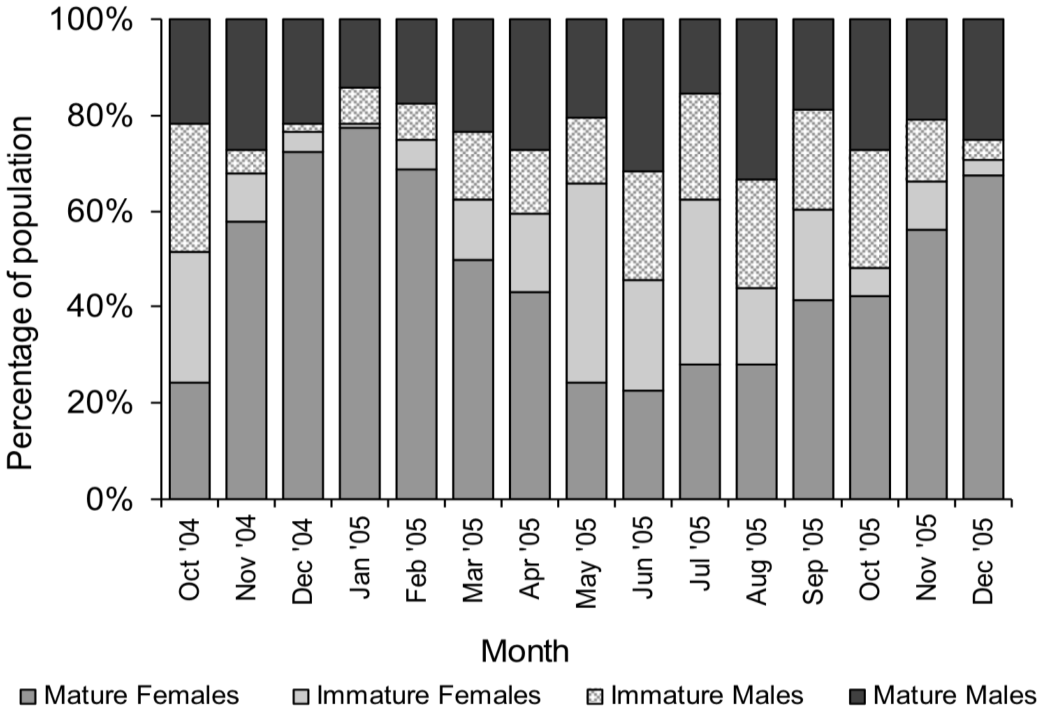

Fig. 3.1. Percentage of the population of *Halicarcinus cookii* sampled from October 2004 to December 2005 grouped according to reproductive status and sex (mature/immature males/females). Sample sizes are shown above the bar for each month.

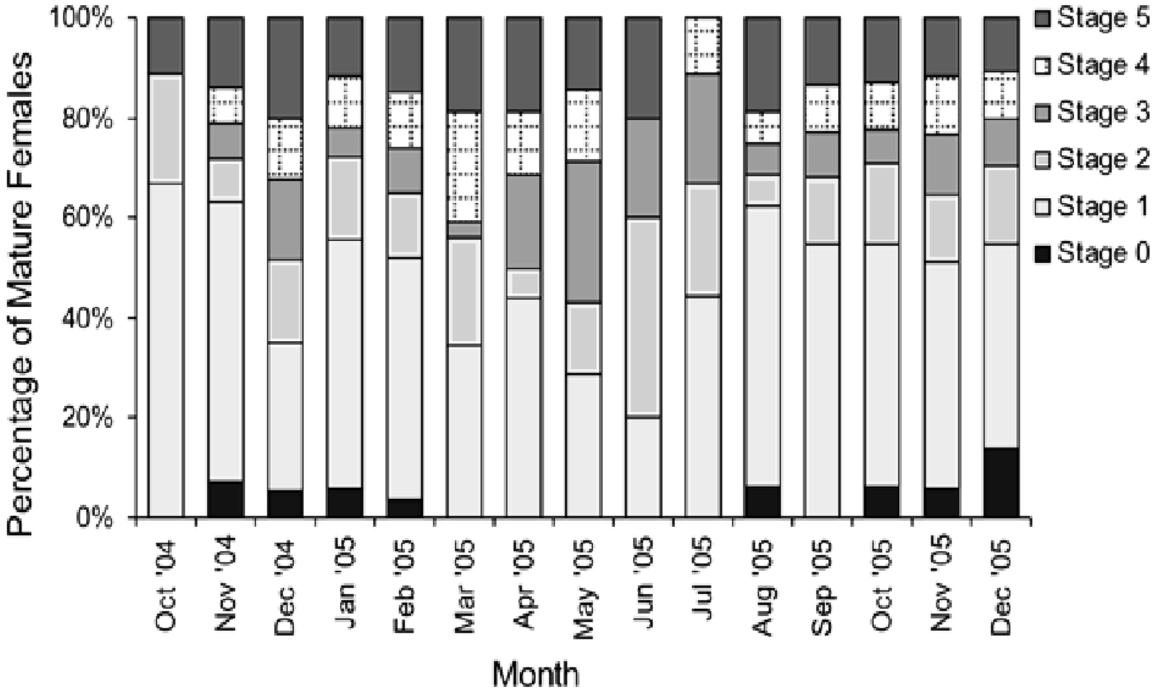

Fig. 3.2. Composition of the mature female population of *Halicarcinus cookii* sampled from October 2004 to December 2005 according to brood stage (0–5). Note: Stage 0 females are not ovigerous. Sample sizes are shown above the bar for each month.

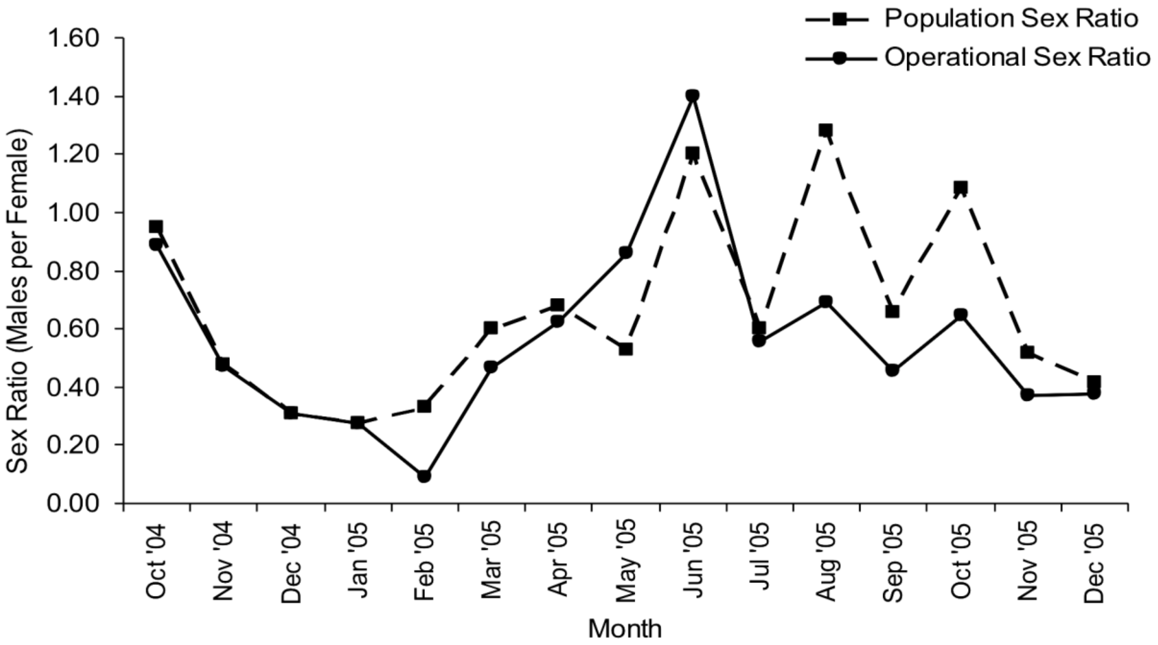

Fig. 3.3. Comparison of population sex ratio (dashed line) and operational sex ratio (solid line) of *Halicarcinus cookii* each month from October 2004 to December 2005. Ratios are presented as males per female.

Ovarian and brood development

During incubation mean egg diameter increased from stage 1 (0.363 mm) to stage 5 (0.452 mm). Mean volumes were: stage 1: 0.025 mL (mm^3), stage 2: 0.032 mL, stage 3: 0.039 mL, stage 4: 0.044 mL and stage 5: 0.049 mL, a significant increase in volume of 92% ($F_{4, 95}$=144.64, p<0.001).

A difference in ovary mass between females carrying different brood stages was easily observed through the semi-transparent exoskeleton: ovaries of females carrying early stage eggs were lighter green in colour and were much smaller than those of females carrying later stage eggs. Gonosomatic index increased with brood development: stage 1 females had the lowest mean GSI (2.22±0.26) and stage 5 females had the highest mean GSI (10.19±0.68) (Figure 3.4). There was a significant difference in GSI between females carrying broods at different stages (F5, 122=60.08, p<0.001). Female gonads therefore develop concurrently with broods and are at their maximum weight when the broods are closest to hatching. As soon as the zoeas hatch the next clutch of eggs is ready to be fertilised and laid.

Mating behaviour

Mating behaviour in *H. cookii* was observed to be relatively uniform, varying only slightly within an identifiable set of behaviours (Figure 3.5). No courtship displays were observed. Males searched for females rather than vice versa and made the decision of whether the female was worth pursuing or not early in the encounter. Females with stages 1 and 2 eggs were rejected almost always at the outset. There were two courses of action observed by the male after he showed interest in a female. A male might be attracted to a female almost immediately (a few seconds), advancing toward her and clutching on to her with quick, jerky pereopod movements as soon as physical contact was made. This occurred primarily when females had advanced stage eggs (stage 5). Alternatively, a male could have one or two legs in contact with the female for some time (often a few minutes) before deciding that the female was worthy of interest and then clutch on to her body, legs or chelipeds. In the absence of rival males, guarding could be intermittent with the male guarding for a few minutes, departing for a few minutes and then returning to guard again. Such male indecisiveness was seen when females had earlier stage eggs (stage 4).

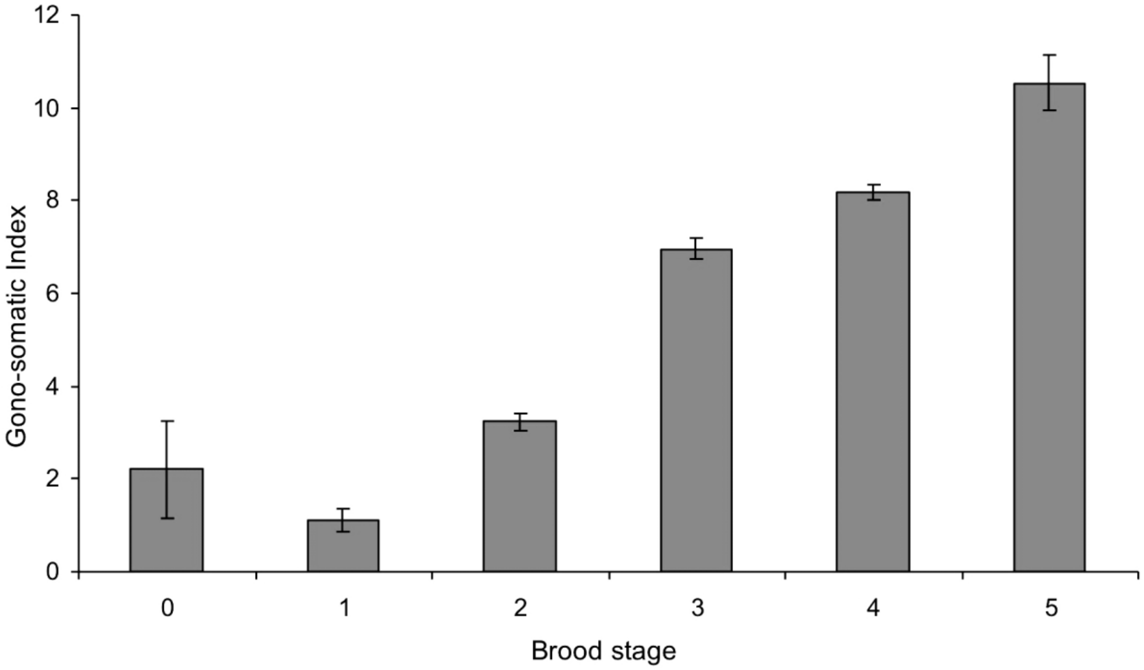

Fig. 3.4. Mean gonosomatic index (>1 S.E.) of female *Halicarcinus cookii* according to brood stage (0-5). Sample sizes are shown above each bar.

Pre-copulatory guarding involved the male on top of the female's carapace with his legs wrapped around the female's body and sometimes one or both of the male's chelae clutching her legs or chelipeds. The male usually faced the same direction as the submissive female, and would remain in the grasping position regardless of whether the female moved around the container or not. We found little evidence to support the idea of female choice. Sometimes the female became more sexually aggressive than the male by being more cooperative while at the same stage some females appeared to resist the advances of a male by avoiding physical contact or moving around and struggling when grasped by the male, sometimes even using their chelipeds in defence. In these cases the male would either give up on his advances or, if he could overpower her, grasp the female and mate with her regardless. A total of 8 cases (out of 20) of female resistance were observed, but in 4 of these the male was still able to mate with the female. Frequency of resistance did not seem to be related to female brood stage or size and there was some resistance at all stages, but the sample size was too small (only 8 of 140 cases) to draw a definite conclusion. Faced with the choice of a male larger than themselves and a male smaller than themselves, there was no significant difference in the female choice of mate size (Cochran's $Q=2$, $df=1$, $p<0.05$, $n=15$).

Copulation involved the male crawling beneath the female, turning onto the dorsal side of his carapace so that the pair was sternum to sternum, and inserting his abdomen between the female's sternum and abdomen, inside the brood chamber (Fig. 3.6A). The gonopods were inserted into the female gonopores without delay and presumably sperm transfer began immediately. Males mated most frequently with females carrying broods close to hatching (stages 4 and 5) followed by stage 3 females and those carrying no brood (stage 0) (Fig. 3.7). Time spent in copula was least with stage 0 females and greatest with stage 5 females and the mean copulation time was 37.5 min (Fig. 3.8). However, an ANOVA indicated that brood stage had no significant effect on the duration of copulation ($F5, 36=1.43$, $p<0.05$).

Mating was terminated by the male dismounting the female whereupon he would begin post-copulatory mate guarding (Fig. 3.5) by standing over the female and 'caging' her with his legs which could be followed by a period of pereopod contact (Fig. 3.6B). The occurrence of post-copulatory mate guarding and length of time this position was held depended on the stage of development of the brood that the female carried. Guarding was observed in the laboratory with females at all brood stages, but was most common with females carrying late stage broods (Fig. 3.9). Mate guarding is strongly dependent on female brood stage ($\chi2=34.847$, $df=6$, $p<0.001$). In the field at least 8 instances of post- copulatory guarding were observed in the field, all of which involved females carrying stage 5 broods. Duration of mate guarding was much longer for stage 5 females (18.3 h) than for stage 4 females (3.3 h) or earlier (Fig. 3.9). An ANOVA revealed a strongly significant difference in mate guarding durations according to brood stage ($p<0.001$). Mate guarding duration of stage 5 females was significantly different from all other stages (pO0.001 in all cases) while all other stages were not different ($p<0.05$ in all cases, Tukey's HSD test). Termination of post-copulatory mate guarding ended the liaison. The male would then depart and show no further interest in the female.

Juvenile females can mate in their penultimate instar: females collected in the field and isolated during their penultimate instar produced a clutch of eggs within 3 days of moulting: of 18 such females 8 produced fertilised broods. Eggs laid by the other 10 females were presumably unfertilised, as they were lost within 4 days. Thus precocious mating seems to be reasonably common in *H. cookii*.

Effects of manipulating the operational sex ratio (OSR)

Antagonistic behaviour was often observed when two males were in the presence of a desirable female. The males faced each other standing on the dactyli of their walking legs, lifting the front of the carapace upwards, spreading their chelipeds and occasionally clutching their opponent's pereopods (Figure 3.6C). Eventually one male would retreat or both appeared to lose interest without inflicting any damage.

When one male was in the presence of two stage 5 females, the male would generally mate with the first female he came across. Sometimes the male appeared to be undecided about which female to mate with and would attempt to clutch on to both of them, after which one would eventually be released and copulation with the other would begin. Copulation with both females occurred in about 40% of the trials, but more often the male would lose interest in both females after mating/guarding one, or clutch on to the second female but not copulate, sometimes alternating between the females. Occasionally one of the two females' broods would hatch, and she would lay a new brood, after which she was no longer attractive to the male. Males seem to be capable of only one copulation per 24 h.

Males copulated for a mean of only 6.5 min in the presence of two females while copulation lasted 33 and 31 min in the 1:1 and 2:1 situations, respectively (Figure 3.10). There was a significant difference in copulation duration between the three sex ratios ($F_{2, 30}=23.60$, $p<0.001$).

Post-copulatory guarding duration increased as the male to female ratio increased (Figure 3.10). With one male and two females present guarding lasted an average of 2.5 h, while in the 1:1 situation guarding lasted an average of 20 h and when 2 males were present guarding lasted an average of 29.6 h. There was a significant difference in guarding durations when all three sex ratio situations were considered ($F_{2,30}=30.8$, $p< 0.001$). A Tukey's HSD test for all three situations showed that the two female situation was significantly different from the other two situations ($p<0.001$), but the 1:1 and 2:1 situations were not significantly different ($p< 0.05$).

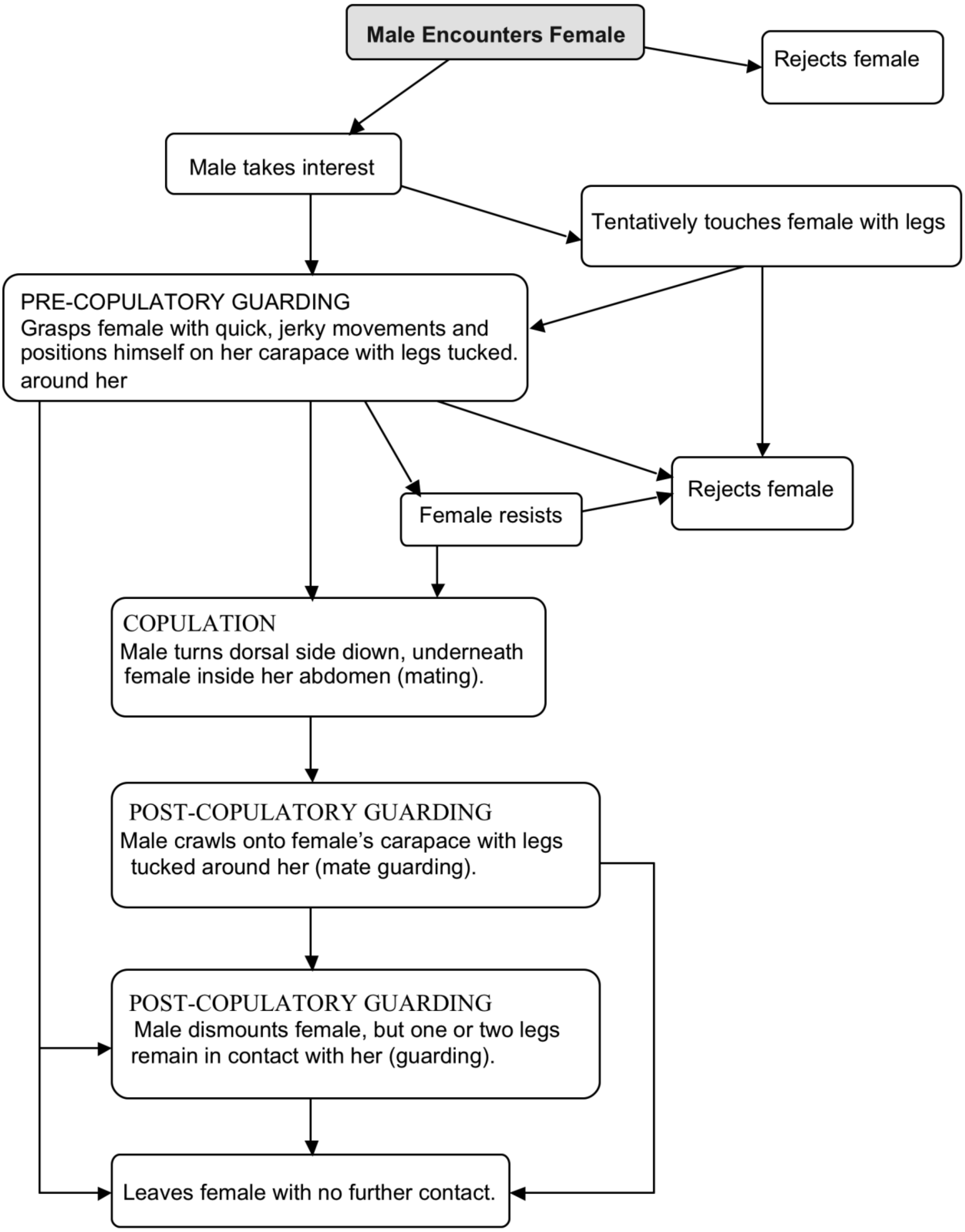

Fig. 3.5. Flow chart showing the behavioural sequence involved in the mating behaviour of *Halicarcinus cookii* as observed in the laboratory.

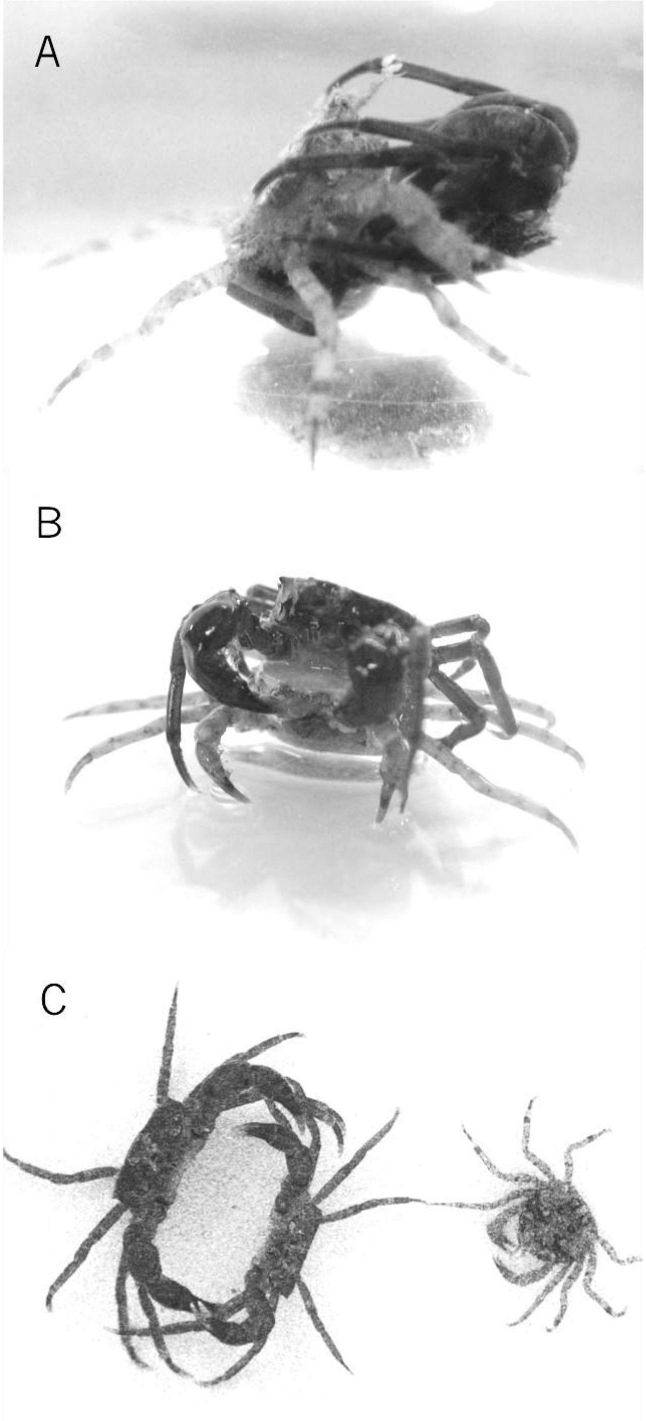

Fig. 3.6. (A) Side view of *Halicarcinus cookii* male and female in copulatory position with the male (right) turned onto the dorsal side of this carapace, grasping the female (left) with his first two pairs of walking legs (Chelipeds are folded and held close to his ventral side) and his abdomen inside the open female abdomen; (B) *Halicarcinus cookii* in typical post-copulatory guarding position with the male on the dorsal side of the female, caging and grasping her with his legs; and (C) antagonistic behaviour between two male *Halicarcinus cookii* in the presence of a desirable female (right). Males stand on dactyl tips with body almost vertical and engage each other's spread chelipeds.

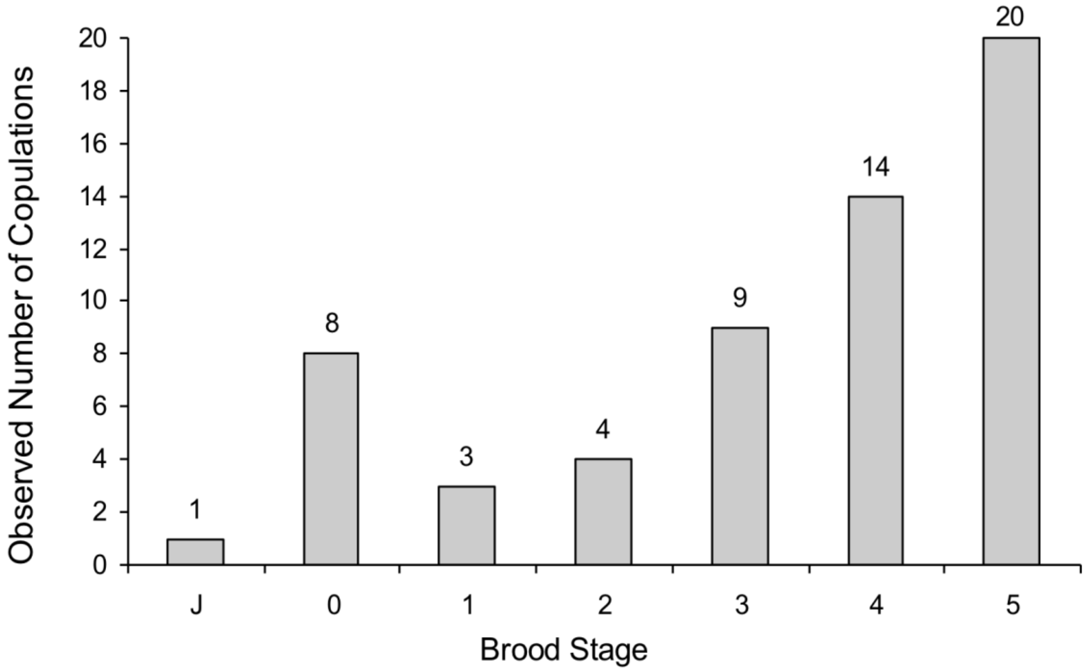

Fig. 3.7. Observed frequencies of *Halicarcinus cookii* encounters resulting in copulation between males and females carrying different brood stages (0-5) and juvenile females (J). Data labels indicate the number of observed cases. A total of 59 copulations were observed out of 140 trials (i.e. 20 trials per treatment).

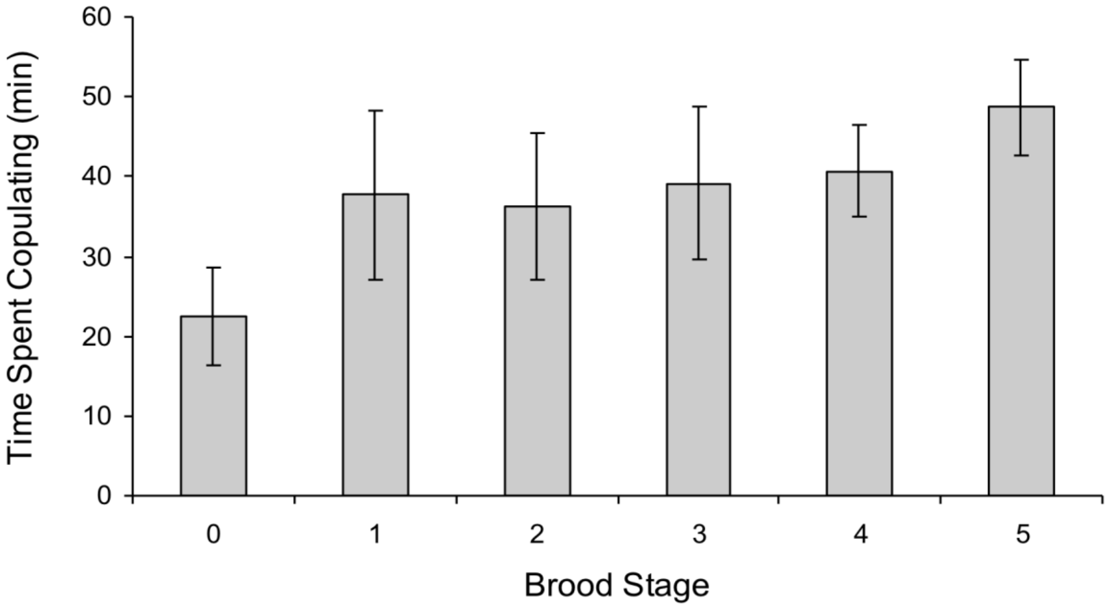

Fig. 3.8. Mean duration (min) of copulation (>1 S.E.) by males with *Halicarcinus cookii* females carrying different brood stages (0-5). Number of replicates = 20 for each brood stage.

Mate attraction

Male attraction to mature females could be based on chemical signals produced by the female that advertise the stage of her ovary development or on a chemical signal produced by the brooded eggs that advertise their stage of development. Both of these hypotheses were tested by comparing mate choice and guarding duration of females with and without broods.

Males preferentially chose to mate with females carrying late stage broods over early stage broods (see Figure 3.9) even after the embryos were removed (Cochran Q=7.12, df=1, $p<0.05$). Comparisons of mate choice (copulation) were made between females at stages 4 and 5 before and after their broods were removed. A factorial ANOVA showed that males copulated with females regardless of whether or not they carried embryos, and there was no significant difference in the occurrence of copulation depending on whether the female carried a brood or not or between the two brood stages ($F_{1,39}=3.35$, $p=0.075$ (brood stage); 1.92, $p< 0.05$ (brood presence) and 2.27, $p<0.05$ (brood stage × brood presence). A factorial ANOVA showed that the duration of copulation was marginally not significantly different between females carrying a brood and those in which it had been removed, but there was a significant difference between brood stages $F_{1,39}=19.01$, $p< 0.001$ (brood stage); 0.34, $p< 0.05$ (brood presence) and 0.6, $p< 0.05$ (brood stage × brood presence).

Males generally guarded females of both brood stages regardless of whether the females have had the brood removed or not. A factorial ANOVA showed no significant difference in the occurrence of mate guarding between females with and without a brood ($F_{1,32}=0.62$ (brood stage); 0.03 (brood presence) and 0.03 (brood stage × brood presence), $p< 0.05$ in all cases).

For mate guarding duration variances could not be homogenised so data were logged to produce the closest to homogeneous variances possible and the threshold of significance increased to $p< 0.01$. A factorial ANOVA showed a significant difference in mate guarding duration according to brood stage, but no significant difference according to egg presence ($F_{1,39}=9.92$, $p=0.003$ (brood stage), 6.9, $p< 0.01$ (brood presence) and 6.86, $p<0.01$ (brood stage × brood presence)). However, Tukey's HSD test showed that there was a significant difference in guarding time between stage 5 females with broods, and each of the other three conditions ($p< 0.01$ in all cases). A one-way ANOVA was then conducted to compare stage 5 females with and without broods and showed a significant difference in guarding duration ($F_{1,19}=18.81$, $p< 0.001$). Apart from male guarding of stage 5 females with broods, none of the measures provide any strong evidence of a brood signal and suggest an ovarian signal is the basis of mate attraction

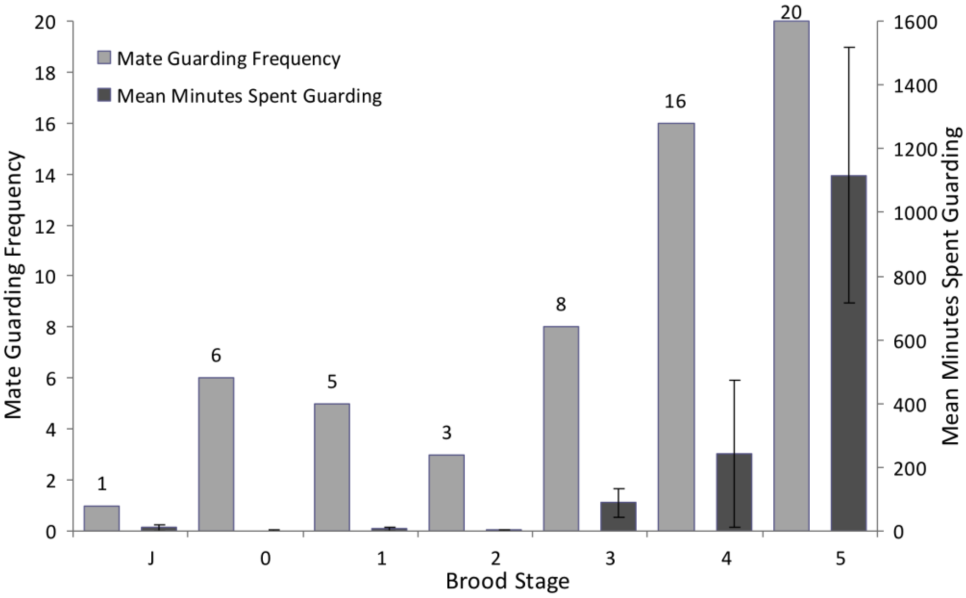

Fig. 3.9. Frequency and duration (min) of post-copulatory mate guarding (±1 SE) of *Halicarcinus cookii* females carrying different brood stages (J = juvenile, 0 = without eggs, 1–5 early through late development stages) out of 20 replicates for each brood stage. Data labels indicate actual number of females guarded out of 20.

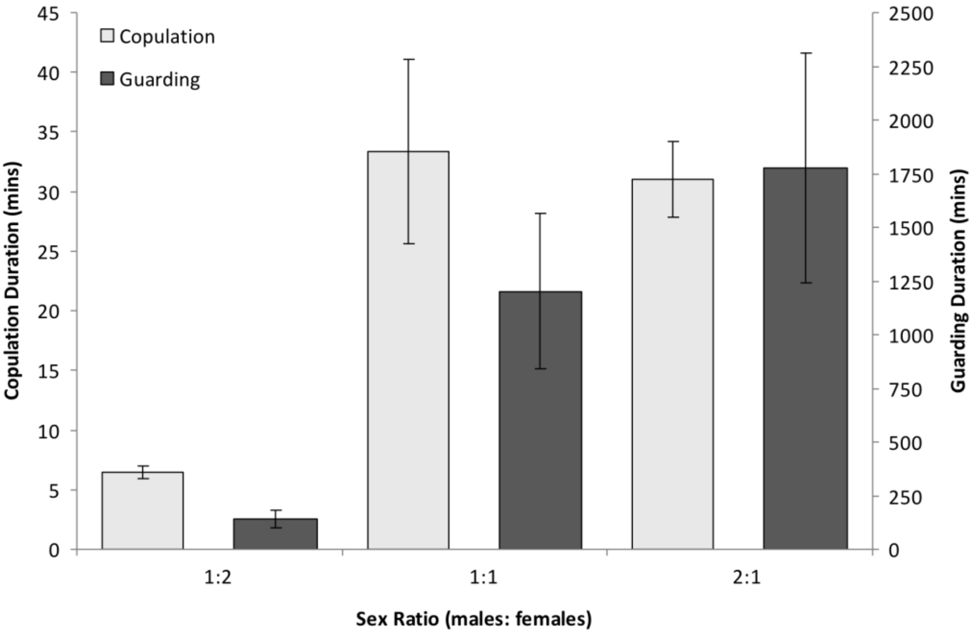

Fig. 3.10. Comparison of mean copulation and mate guarding durations (min) (±1 SE) between different sex ratio situations of *Halicarcinus cookii*. Females had stage 5 broods. Ratios are male per female. Number of replicates=20 for each sex ratio.

DISCUSSION

With ventral type seminal receptacles and absence of sperm mixing resulting in last male sperm precedence in *Halicarcinus cookii*, males would mate most efficiently (i.e. use their sperm and time) if they can ensure that they are the last to copulate with the female (Van den Brink and McLay, 2009). Males must then be able to discriminate between females who are likely to re-mate before laying a new brood and those with whom they are likely to be the last to mate. Females carrying stage 5 eggs constituted around 14% of the population of mature females. Since ovary development coincides with brood development, a female carrying a well-developed brood, which is close to hatching, is also closer to laying a new brood than a female carrying a less developed brood. Therefore, males are most likely to maximise paternity of a brood if they copulate with a female about to spawn thereby displacing any sperm that may be already in the seminal receptacle. *Halicarcinus cookii* males not only mate preferentially with stage 5 females, they also take longer over copulation. This may indicate that more sperm was transferred to a stage 5 female than to females at an earlier stage. If sperm is not transferred throughout coupling then part of this time may also be considered to be close contact mate guarding. Consequently, females carrying broods about to hatch (stage 5) are the most efficient and therefore attractive females for males to mate with because the last male's sperm has a smaller chance of being displaced away from the site of fertilisation. Furthermore, guarding a female after copulation can be an effective strategy for a male to protect his reproductive investment (Smith, 1984; Diesel, 1991). Post-copulatory mate guarding was longest for stage 5 *H. cookii* females with males spending 18.3 h on average compared to only 3.3 h for stage 4 females. Male guarding prevents females copulating with other males, always a danger because mature females remain able to mate throughout their lives, unlike varunid crabs, for example, where there is only a narrow window of opportunity (see Brockerhoff and McLay, 2005). However, guarding is costly in terms of lost feeding time and, primarily, loss of future mating opportunities. Therefore, it is most efficient to preferentially guard females requiring the least time investment.

The other way by which males could maximise their fertilisation would be for them to select larger females because they will produce more eggs to be fertilised (Hines, 1982). For *H. cookii* the size of females is limited by the range over which the terminal moult occurs (Van den Brink and McLay, 2009). The size range of mature females is CW=5.4–11.5 mm and the range of fecundity is 320–1780 eggs per female (Van den Brink and McLay, 2009). But is it worthwhile searching for a large female if a small female with late stage eggs is available? Even given a five-fold difference in fecundity, a bird in the hand may be worth two (or even more) in the bush! Males do not waste sperm and time on females who are likely to re-mate before a new brood is fertilised.

Brachyurans similar to *H. cookii* in having determinate growth and ventral seminal receptacles are *Chionoecetes opilio* (hard and soft shell mating), *Maja squinado* (only hard-shell mating), and *Inachus phalangium* (hard-shell mating). In all of these species, females accumulate sperm from many males because they do not moult so we might expect them to share some features of their mating strategy. *Chionoecetes opilio* and *M. squinado* are both much larger (~150–170 mm CL) and longer lived than *I. phalangium* and live over a wider depth range (Fonseca et al., 2008; Sainte-Marie et al., 2008). *C. opilio* undergo seasonal breeding migrations from deep to shallow water where the pubertal moult and mating occurs (Ennis et al., 1990). Aggregation or podding behaviour by *C. opilio* and *M. squinado* females results in large clumps of crabs with males lurking nearby (Sampedro and Gonzalez-Gurriaran, 2004). Female *C. opilio* can undergo their moult to maturity at any one of four different (VIII–XI) instars (Orensanz et al., 2007).

Males are attracted to females having their pubertal moult (primiparous) and to females that are releasing their larvae (Sainte- Marie et al., 2000). For a large part of the year, *C. opilio* males are segregated from females in deeper water.

Inachus phalangium is a similar size (~20 mm CL) to *H. cookii* and also lives in shallow sub-tidal waters where it lives in association with the anemone *Anemonia sulcata*, gaining protection from predators. Life expectancy is around 12–17 months (shorter for males than for females). Males show some remarkable breeding adaptations to the spatial arrangement of sedentary females and their unsynchronised breeding cycles: a male patrols an area containing up to 8 females and visits each one just before they release their larvae. A female may mate and be guarded by up to 4 males that can be displaced by a larger male so that the largest one copulates and guards her until spawning. In the seminal receptacle rival sperm is sealed off by seminal plasma layering. Once mature, females breed almost continuously and produce around 6 clutches before they die in the following year (Diesel, 1986, 1990, 1991). To explain this we have to assume that males have both a spatial and temporal memory that informs them of what is happening. Males will turn up to an anemone at the right time even though the female has died! The evolution of these remarkable reproductive traits seems to have been facilitated by the link to site-attached anemones and sedentary females.

As far as we know *H. cookii* does not have nearly such a sophisticated breeding system as *I. phalangium*. They are not closely associated with anemones, which are abundant on the shore at Kaikoura, and neither sex show any evidence of site attachment, so that we can only assume that males search randomly for females carrying late stage eggs. We could not find any evidence of sperm layering in the seminal receptacles. However, using the sterile male technique we have demonstrated that the last male to mate sires the large majority of a brood (Van den Brink and McLay, 2009). Sperm of males which mated earlier is only used if females do not mate again during incubation. Like *I. phalangium, H. cookii* females have a similar level of fecundity and breed almost continuously, except when the reproductive cycle slows in winter. Unlike *Chionoecetes* and *Maja*, *H. cookii* does not show any evidence of depth migration and does not indulge in podding behaviour. However, it is similar to *C. opilio* in being able to moult to maturity over a range of sizes (Van den Brink and McLay, 2009). Males of *H. cookii* and *Chionoecetes* spp. are all attracted to females carrying advanced stage eggs or releasing larvae and so have the same strategy of being the last male to mate.

Donaldson and Adams (1989) provide a detailed description of the mating behaviour of the tanner crab, *Chionoecetes bairdi*, with which we can compare *H. cookii*. The behaviour shown by *C. bairdi* is much more elaborate because males mate with primiparous (first time breeders) and multiparous (second and more) females. Males provide moulting assistance to primiparous females. Apart from the moulting preamble both species show the same sequence of male-initiated mate guarding, sternum to sternum copulation followed by further male guarding. Copulation with multiparous *C. bairdi* females lasted longer (mean 24.5 min) than with primiparous females (13.7 min). Copulation duration in *H. cookii* was somewhat longer, lasting 37.5 min on average, but tending to increase from 20 to 50 min with early to advanced broods. In *C. bairdi* post-copulatory guarding usually continued until the female laid her eggs. In *H. cookii* the average duration of guarding was 18.3 h and sometimes guarding was cut short by the eggs hatching and a new brood being laid, but in other cases the male departed 1 or 2 days prior to release of larvae, thereby risking his reproductive investment. The maximum length of post-copulatory guarding was 34.5 h. Duration of post-copulatory guarding may be related to sperm recharge time (see below).

In hymenosomatid crabs the link between mating and moulting may be variable. It is clear that at least in the genus *Halicarcinus* mating is between hard shelled males and females, but in *Amarinus* spp. and *H. orbiculare* the females may be soft-shelled (Lucas, 1980; Broekhuysen, 1955). In *Amarinus* the pubertal moult is terminal so it may be that they have a similar mating pattern to *C. opilio* (i.e. hard and soft shell mating), but in *H. orbiculare* moulting apparently continues after puberty (Broekhuysen, 1955). This is very likely the ancestral condition for the family.

Hymenosomatid crabs require us to revise our concept of brachyuran sexual maturity. *Halicarcinus cookii* females can mate before their pubertal moult and Lucas (1980) showed that Amarinus spp. females also show precocious mating and males are able to fertilise females as early as 3 instars before their pubertal moult. Prepubertal male H. planatus are also capable of mating (Richer de Forges, 1977). As a result, females can lay and fertilise their eggs without delay immediately after their pubertal moult. Eggs cannot be laid any earlier because prepubertal females do not have the abdominal brood chamber in which to attach and incubate them. Precocious mating and continuous reproduction may be a consequence of small body size.

The mating behaviour of *H. cookii* changes according to the OSR. A couple alone copulated on average for 33 min, but if another female was present it lasted only 6.5 min, while mate guarding was also reduced from 20 h to only 2.5 h. Sometimes the male attempted to control the behaviour of both females. If additional mating opportunities are available the male attempts to take advantage of them, but this may be limited by sperm supply and length of the recharge period. Given the small body size this may be an important limiting factor. When a rival male was present copulation duration was not significantly different (31 vs. 33 min), but mean duration of guarding increased from 20 to 29.6 h. In *C. opilio* dominant males inversely scale guard time and sperm allocation to the number of mating opportunities (Rondeau and Sainte-Marie, 2001). The presence of other males elevates the risk of overt sperm competition, but there is nothing that males can do about covert sperm competition caused by the accumulation of sperm of other males in the seminal receptacle. The mating behaviour of *H. cookii* is typical of the Hymenosomatidae (see Lucas, 1980; Dunnington, 1999; Hosie, 2004). After a terminal moult they are able to mate continuously in the hard-shell condition. Paternal success is maximised in *H. cookii* by a preference for copulating with, and guarding females closest to spawning, thereby reducing the risk of wasting sperm or losing future mating opportunities. Males will spend a token amount of time mating with and guarding females carrying early stage eggs, but clearly the male strategy is to move on in search of more profitable mating opportunities. Perhaps such mating pays off occasionally, if the female does not encounter another male, but the investment of both sperm and time into early stage females is risky. Guarding duration was significantly shorter in a female biased sex ratio, suggesting that male reproductive behaviour in *H. cookii* is flexible enough to change as the costs of guarding fluctuate in the population.

ACKNOWLEDGEMENTS

Kimberley Seaward provided valuable assistance and kept me (in)sane. Jack van Berkel, Edward Percival Field Station, provided moral support, copious advice, and constant back-chat. Thanks also to the many other people who gave vital help and support during the development of this part of the project.

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Chapter 4:

Use of the sterile male technique to investigate sperm competition, storage, and use in a pill-box crab *Halicarcinus cookii* (Brachyura: Hymenosomatidae)

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Journal of Crustacean Biology 2009, 29(1): 62–69

ABSTRACT

Halicarcinus cookii is a small intertidal New Zealand crab that has high levels of ovigerous females in all months. Continuous breeding requires continuous mating. Mating is not linked to moulting because the pubertal moult is terminal. Seminal receptacle fullness is modelled in terms of the difference between the rates of copulation and rates of brood production. It is estimated that females can fill their seminal receptacles with sperm after 3-4 copulations (depending on male size) and that approximately 15% of an ejaculate is used to fertilise each brood. Given the fact that females have multiple partners we asked the question: is it worthwhile for males to spend any time mate guarding? Using sterile males we showed that the majority of each brood is sired by the last male to mate. In females prevented from mating again, more of each successive brood is likely to be sired by earlier males who had mated with the female. This suggests that over periods exceeding one brood cycle (i.e., months) sperm slowly becomes mixed. Therefore, it is worthwhile for male *H. cookii* to invest time in guarding female partners.

INTRODUCTION

All brachyurans can store sperm for relatively long periods of time (often over successive moults, breeding cycles, or even years) so that the sperm from one male is likely to encounter viable sperm from previous mates inside the females seminal receptacle resulting in potentially high levels of sperm competition (Diesel, 1991). In crabs, sperm competition normally takes the form of sperm displacement from the site of fertilisation (Rorandelli et al., 2008). Brachyuran seminal receptacles can be divided into two groups according to their position relative to the oviduct. The proximity of the oviduct to the vagina influence which sperm is used to fertilize the eggs. Species with dorsal-type seminal receptacles, such as the portunids *Carcinus maenas* (Linnaeus, 1758) and *Callinectes sapidus* Rathbun, 1896 (Diesel, 1990, 1991), tend to use the oldest sperm (sperm from the first male to mate) first, whereas species with ventral-type seminal receptacles, such as the spider crabs *Chionoecetes opilio* (Fabricius, 1788) (Beninger et al., 1988), *Inachus phalangium* (Fabricius, 1775) (Diesel, 1989), and *Cancer magister* (Dana, 1852) (Jensen et al., 1996) as well as species in the Calappidae, Geryonidae, Leucosiidae and Corystidae (Diesel, 1991), use the sperm closest to the entrance (sperm from the last male to mate) to fertilize the eggs first (Diesel, 1991). Sperm storage, viability and competition have been investigated in various species including *Metopograpsus messor* (Forska˚ l, 1775) (Anilkumar et al., 1999), *C. opilio* (Beninger et al., 1988), *Menippe mercenaria* (Say, 1818) (Cheung, 1968) and *Callinectes sapidus* (Jivoff, 2003). All of these are relatively large, long lived crabs, which show seasonal breeding and may well survive to breed in more than one breeding season (year).

The importance of sperm competition depends upon what measures males take to control female mating and sperm storage. Copulatory mate guarding by males can restrict access to females while inside the seminal receptacles males can use seminal fluid to seal off rival sperm or sperm plugs to block the gonopore entrance (Diesel, 1990). All of these tactics are aimed at minimizing sperm mixing that could dilute a male's sperm. Sperm layering occurs in *Inachus phalangium* (Diesel, 1991) and *Chionoecetes opilio* (Sainte-Marie and Sainte-Marie, 1999) preventing sperm mixing. The sterile male technique, in which irradiated males are mated to females and then the number of infertile eggs counted, has been used by Koga et al. (1993) to investigate paternity in the ocypodid, *Scopimera globosa* (De Haan, 1835). The same technique was earlier used by Snedden (1990) to study sperm competition in *Orconectes rusticus* (Girard, 1852). In the present study we used sterilised male *Halicarcinus cookii* Filhol, 1885 (Hymenosomatidae) in an attempt to measure sperm mixing and paternity.

Hymenosomatids are a family of around 100 species of small, somewhat delicate crabs, from the Indo-West Pacific (Melrose, 1975; Lucas, 1980; Chuang and Ng, 1994; Ng and Chuang, 1996). They have small broods and are unusual in lacking a megalopa stage in their life cycle. Both males and females may reach sexual maturity and be capable of mating before the pubertal moult. They are able to store the spherical to ovoid sperm in the seminal receptacles, which are enlarged regions of the genital duct between the vagina and the oviduct, and fertilise many broods from a single copulation (Lucas, 1980), suggesting that there is a potential for male-male competition (including sperm competition) which may influence male behaviour and lead to greater dimorphism of secondary sexual characters. Hymenosomatid females have some remarkable adaptations to protect the small number of embryos that they carry: the abdomen totally encloses the pleonal sternal (brood) cavity and branchiosternal canals connect the brood cavity with the branchial chambers so that the embryos are irrigated by water from the branchial flow, similar to the brood protection provided by leucosiid females.

In contrast to the commonly studied larger crabs, species of *Halicarcinus* White, 1846 at Kaikoura, New Zealand rarely grow more than 15 mm CW and constitute populations in which there is a high level of turnover of short-lived individuals. Populations of *H. cookii*, *H. varius* (Dana, 1851) (Hosie, 2004) and *H. innominatus* Richardson, 1849 (Dunnington, 1999) at Kaikoura have breeding and recruitment throughout the year, although the highest levels of ovigerous females occurs in summer (December-February), but cohorts are not evident. Further evidence of the continuity of breeding is the fact that ovigerous females carry early and late stage broods year-round so that there is no brood synchrony in the population. Therefore the pace of reproduction in these hymenosomatids is frenetic compared to larger crabs. Casual observations suggest that in *H. innominatus* the seminal receptacles can make up more than 25% of female body weight (Rudi Diesel, personal communication).

Halicarcinus cookii is a small (max CW 13 mm) shallow water crab endemic to New Zealand. Both males and females have determinate growth with the pubertal moult being terminal. The terminal/pubertal moult is advantageous in that mature individuals are able to produce offspring continuously, but the penalty is that the number of offspring produced per brood is limited, due to small female body size, and remains roughly the same throughout her lifetime. Mating is not linked to moulting. There is little evidence of a discrete mating season and high proportions of ovigerous females produce successive broods throughout the year. Thus continuous mating, in the hard-shelled condition, is necessary to maintain the sperm supply necessary to fertilise the eggs. We used the following model of sperm dynamics in the female seminal receptacles:

$$\mathrm{SF} = (\mathrm{EJ} \times \mathrm{NC}) - (\mathrm{NB} \times \mathrm{SP})$$

Where SF = seminal receptacle fullness expressed as a percentage of maximum capacity, EJ = ejaculate volume, NC = number of copulations, NB = number of broods and SP = volume of sperm used to fertilise each brood. We attempt to explain seasonal variation in SF using this model.

The questions that we attempt to answer are: How much sperm mixing occurs? Is it better to be the first or the last male to mate with the female? In a population where females are ovigerous almost all the time and mating is continuous, is it worthwhile for males to invest any time at all in mate guarding?

MATERIALS AND METHODS

Sperm Supply

Seasonal variation in mating frequency was estimated by measuring the fullness of the seminal receptacles monthly from October 2004 (Spring) to December 2005 (Summer) and ejaculate size. All female crabs found during 90 min intertidal searches on two consecutive days at Atia Point and First Bay, Kaikoura, New Zealand were collected. Fullness of both seminal receptacles was estimated visually using a binocular microscope to look through the transparent sternum when the brood chamber was exposed by holding open the abdomen. Females were recorded as 0, 10, 25, 50, 75 or 100% full.

To investigate the number of times a female must mate to fill the seminal receptacles, 20 females were selected and their carapace measured. A digital photograph of the seminal receptacles was initially taken through a dissection microscope before the female was placed with a male of known carapace width in a 2 L container of seawater and monitored for mating.

After successfully mating, a second digital photograph of the seminal receptacles was taken and so on for subsequent matings. Photographs were analysed and compared using "ImageJ 1.33u". Size scales were set as evenly as possible for all photographs of a single female to ensure accurate comparison between photographs. Each individual seminal receptacles was outlined using a freehand selection option and the area was calculated. The area was outlined and measured at least ten times to allow for variance in freehand drawing. The mean area was added to that of the opposite seminal receptacles to produce an estimate of the area encompassed by the seminal receptacles. The percentage increase in area over successive photographs was produced using the formula:

$$\% \text{ change} = (A/B - 1) \times 100$$

where B is the estimate of the area of the seminal receptacles before copulation and A is the estimate of the area of the seminal receptacles after copulation.

Sperm Use

Twenty females mostly with seminal receptacles estimated to be 100% full, were selected and kept for 24 h with a selection of males (n ; 10) to provide opportunity to fill their seminal receptacles. The females were then isolated from males and kept in a separate tank for the remainder of the experiment. Females were tagged with commercial bee tags attached with super glue to their carapace. This allowed individual crabs to be monitored through each brood cycle. Every 3 or 4 days the brood stage of each crab was recorded. Eggs were not removed and counted in an attempt to avoid the extremely high mortality, potentially due to the stress of egg removal, experienced by Hosie (2004). Nevertheless there was some mortality, but there were still at least 14 crabs remaining. Prior to the experiment, a digital photograph of the seminal receptacles of each individual female was taken through a dissection microscope. After each female had laid a new brood another digital photograph of the seminal receptacles was taken to compare with the photographs taken during previous broods. This allowed a direct photographic comparison of sperm use and decrease in seminal receptacle area. A control experiment was also conducted for comparison, using the methods described above, but with a male constantly present in the holding tanks.

Sperm Storage

In theory, the sterile male method should be able to measure sperm mixing by measuring the proportion of the brood that does not develop. This is a surrogate for sperm mixing so that in the case of total mixing, the expected proportion of eggs fertilised is the proportion of the seminal receptacle contents provided by each male: for random mixing of the sperm of two males the expected value is 50%. However there are some limitations to this method because, as shown by the present study, eggs fertilized by the sterile male are easily lost from the brood chamber. Most of the ones left are going to be those fertilized by the other males because they stick to the pleopods. So it can be difficult to estimate what the total number of eggs was in the first place so that the proportion fertilized by the sterile male can be estimated.

To investigate sperm mixing and sperm priority in the seminal receptacles, 12 male crabs of various sizes were selected for sterilization by irradiation. Each male was labelled with a commercial bee tag attached with super glue.

These males were sterilized by exposure to gamma radiation, using 6 MV (mega volts) photons from a Varian 2100C/D linear accelerator (LINAC). The amount of MU (monitor units) required to deliver a dose of 40 Gy (gray, i.e., joules/kg) to the whole volume of water was calculated to be1960 MU delivered from each beam using measured dose data based on the set-up conditions: A 25 3 25 cm field was used which was large enough to completely cover the whole container of crabs, a total depth of 8 cm of water was used, and this was centered at the isocentre. The LINAC was calibrated to deliver 1cGy per MU (centi-gray/monitor unit) to the dmax (the point that would receive the maximum dose from a beam) 100 cm from the source of the radiation conditions (isocentre) at a depth of 1.5 cm in water for 6 MV photons.

Irradiated crabs were transported back to the laboratory where they were allowed to mate with females. As crab sperm are immotile, spermatozoa from irradiated males were assumed to have equal opportunity with those from healthy males to meet up with the eggs, thus producing an infertile brood. Thirty bee tag labelled non-ovigerous females with little or no sperm stored in their seminal receptacles were selected. The females were then separated into three groups of differing mating sequences: Group A: Females only mated with an irradiated male (I-I) (control), B: Females mated with an irradiated male followed by a healthy male (I-N), and C: Females mated with a healthy male followed by an irradiated male (N-I). If there is no sperm mixing then Group B females should produce a normal brood of healthy eggs while Group C females should not produce any healthy eggs at all.

Females were then monitored until they produced their next brood of eggs. Soon after a brood was laid, it was carefully removed from the pleopods, counted and the proportion of unfertilised eggs recorded. During the dissections of females described above, the number of unfertilized eggs was recorded for 10 females to control for females who have only mated with healthy males (N-N). However, due to the high level of mortality caused by either stress or fatal injury during egg removal, this approach was abandoned for a simple observation of broods produced.

The eggs were initially allowed to begin developing before examination. Unfortunately, unfertilised eggs were lost from the pleopods within the first few days after being laid. However, there was a difference in colour between fertilised and unfertilised eggs. Unfertilised eggs were a much paler and duller orange compared to healthy, fertilised eggs. This was then used to distinguish between eggs fertilised by irradiated and healthy sperm. Any mixing of sperm would be indicated by only the number of eggs remaining and developing in a single brood.

RESULTS

Filling of the seminal receptacles

Halicarcinus cookii have ventral-type seminal receptacles (Figure 4.1) with the oviduct entering close to the vagina. As new sperm is added older sperm gets displaced dorsally further away from the oviduct. The seminal receptacles increased from 0% to 100% after only three or four copulations (Figure 4.2) depending on male size. Figure 4.3 shows that the smallest male (CW = 8.4 mm) increased fullness by around 10% per copulation compared to the largest male tested (CW = 10.4 mm) which increased fullness by around 75%. An average sized male (CW = 9.5 mm, SE = 0.18) could increase fullness by approximately 40%. For the 20 females tested, there was an average of 37.7% (SE = 6.42, n = 9) increase in seminal receptacle surface area after each copulation. Percentage increase of seminal receptacle surface area ranged from 9.3% (as the female's third copulation with a male with carapace width of 8.4 mm), to 73.44% (as the female's second copulation with a male of carapace width of 10.2 mm).

Sperm Use

Although crabs in the sperm use experiment were only checked every three or four days and eggs were not removed, there was high mortality of females. This was most likely due to the stress caused by the experiment, or could be simply due to the fact that the experiment was conducted at the end of the summer season (February) and the females were potentially facing the natural end of their life span. Out of the original 20 females, only three survived to produce a third brood. Nevertheless, photographs of seminal receptacles were still analysed, including those of females only producing a second brood. Most females had an initial seminal receptacle fullness around 100%. There was a mean of -22.7% (SE = 2.09, n = 14) change in visible seminal receptacle surface area after the production of a new brood, ranging from -11.3% to -41.0%.

Females in the control treatment had a negligible mean change in seminal receptacle surface area of 7.51% (SE = 5.87, n = 14). However, 50% of these females increased in seminal receptacle surface area over successive broods with a mean increase of 24.32%. The remaining 50% decreased in seminal receptacle surface area by a mean of -9.31%. There was a significant difference in percent change in seminal receptacle surface area between the control and the experiment (F1, 26 = 23.5, P , 0.001).

After combining the results of the sperm supply and sperm use experiment, it was possible to make a first approximation estimate of the proportion of a single ejaculate that females use to fertilise a single brood. As egg numbers were not recorded in an attempt to avoid high mortality, brood size could not be used as a factor influencing the proportion of sperm used in fertilization. Nevertheless, there was a difference of 15% between percentage increase and percentage decrease (37.7-22.7%) of mean visible seminal receptacle surface area, which suggests that females use approximately 15% (SE = 3.15) of the ejaculate of one male to fertilise a single brood. Therefore, if a female has a seminal receptacle fullness visual estimate of 100%, she could theoretically fertilse at least 6 broods before the sperm supply may become exhausted. Given an average brood cycle length of 20-30 days, this would mean that the female would take 120-180 days after the pubertal moult to completely exhaust her stored sperm. Females would appear to have more than adequate insurance against the chance that they would not encounter another mate.

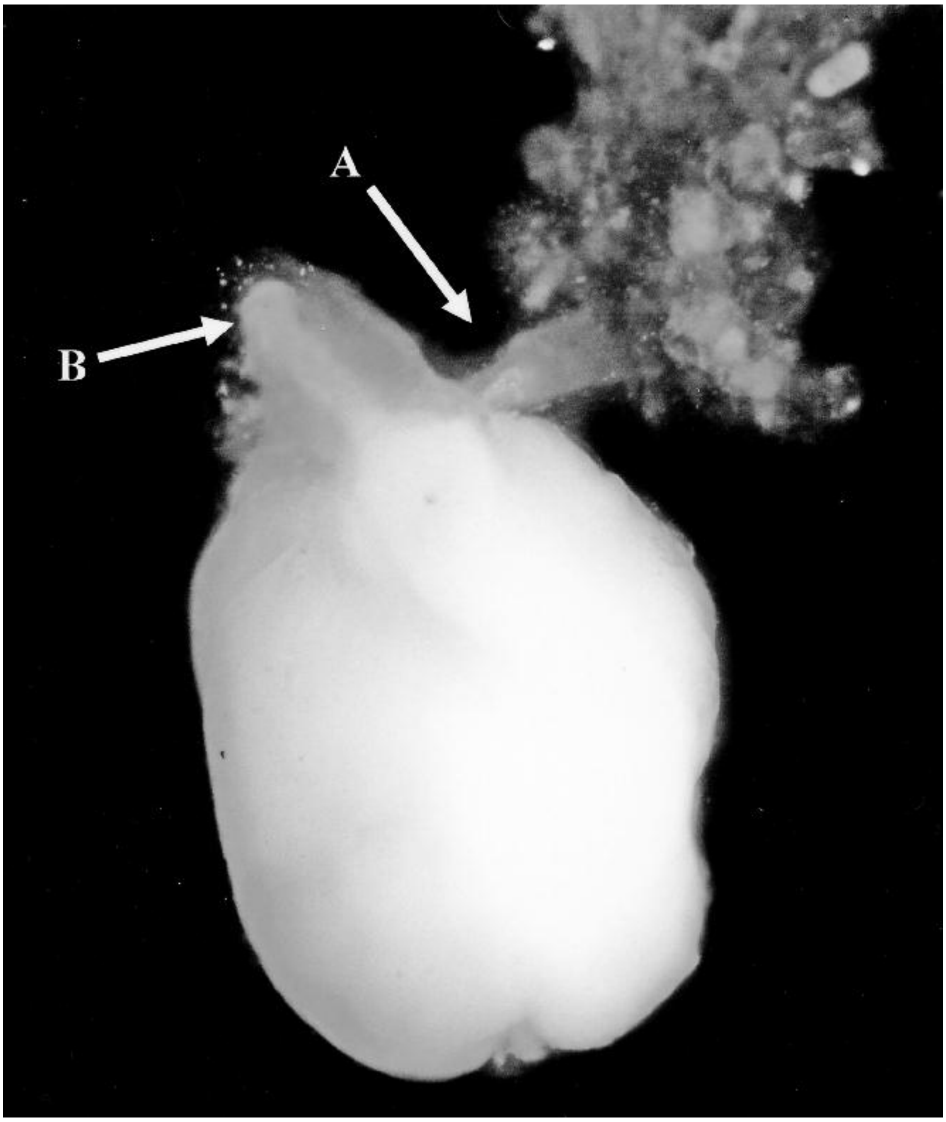

Fig. 4.1. A single seminal receptacle dissected from a female *H. cookii*. Note the oviduct, (A), through which eggs pass into the seminal receptacle and are laid out of the vaginal opening at (B).

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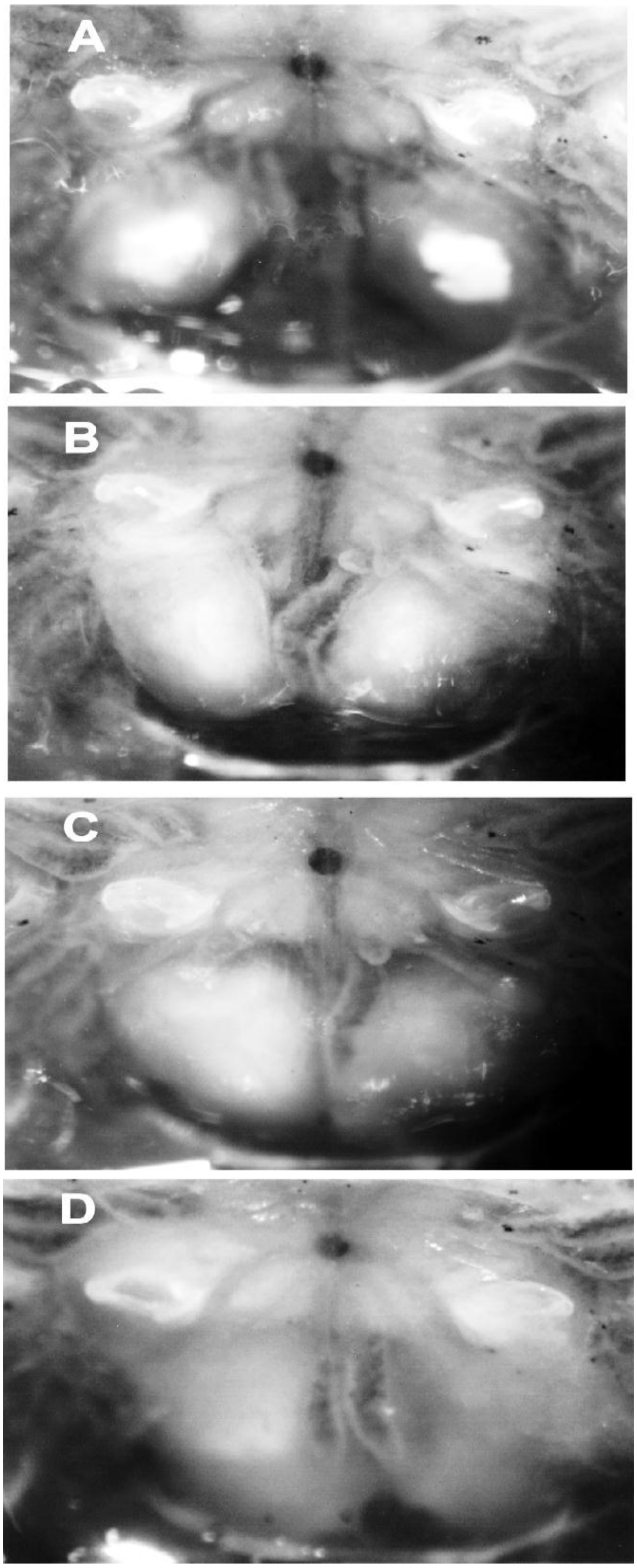
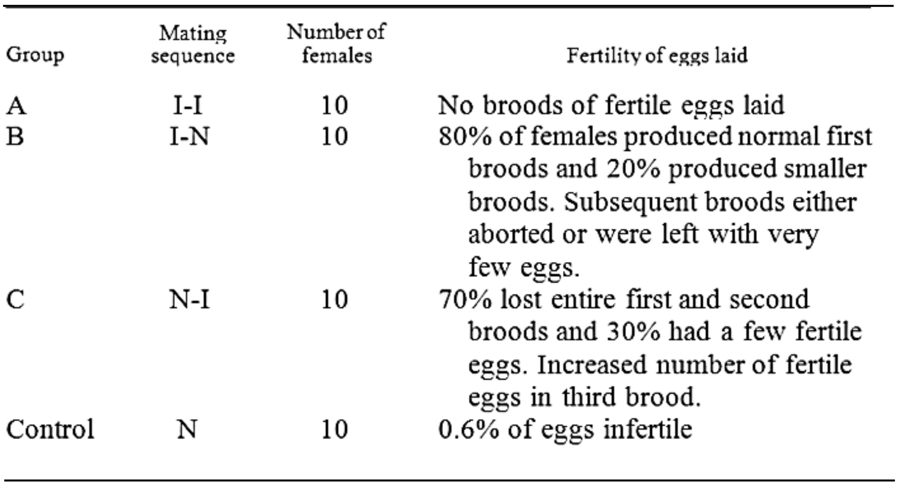


Fig. 4.2. Digital photographs of the seminal receptacles of a single *H. cookii* female after copulating with (A) no mates, (B) one mate, (C) two mates, and (D) three mates. Above the seminal receptacles is the slit-like gonopore through which sperm were transferred by the male.

Sperm Mixing

Of the eggs carried by females who had only mated with healthy males, a mean of 0.6% were unfertilised, ranging from 0.12% to 0.99% (Table 4.1). This indicated that the number of unfertilised eggs generally carried by females is negligible.

Table 4.1. Results of the sterile male experiment. I and N refer to irradiated and normal males respectively.



Group	Mating sequence	Number of females	Fertility of eggs laid
A	I-I	10	No broods of fertile eggs laid
B	I-N	10	80% of females produced normal first broods and 20% produced smaller broods. Subsequent broods either aborted or were left with very few eggs.
C	N-I	10	70% lost entire first and second broods and 30% had a few fertile eggs. Increased number of fertile eggs in third brood.
Control	N	10	0.6% of eggs infertile

Females in group A (those who had mated only with irradiated males) produced completely unfertilised broods. These eggs were often coloured a much duller orange when compared to fertilized eggs, and were lost within four days. This indicated that the radiation to which the males were exposed resulted in complete sterilisation of the males.

Eight females in group B (those who had mated with an irradiated and then a healthy male) initially produced what seemed to be (not counted so as to avoid damage to the female) a normal sized, fertilised brood. The other two produced a smaller than usual, but fertilised brood (see Fig. 4.4). Six of the group B females produced a second brood during the experiment, all produced a normal sized brood initially, but within four days, the brood had reduced to approximately half its original size, but the eggs remaining were successfully fertilized. Two crabs were left with only about 20 fertilized eggs and one crab lost the entire brood. Three crabs produced a third brood during the experiment, but almost all eggs were lost save a few (50, 5 and 3 fertilized eggs in the three females).

Seven of the group C females (those who had mated with a healthy then an irradiated male) lost their entire first brood. Only three females had fertilised eggs in their first brood but only 13, 10 and 67 eggs were fertilized and remained attached to the pleopods in these females. Four group C females produced a second brood, two completely lost their eggs within four days, and the remaining two carried 30 and 10 fertilized eggs to hatching. Two group C females produced a third brood, both of normal size. After four days, one was left with only one fertilized egg, and the other was left with approximately 50% of the original brood (see Fig. 4.4). The probability of females producing broods of mixed paternity is low, although we cannot make a precise estimate of what exactly it might be.

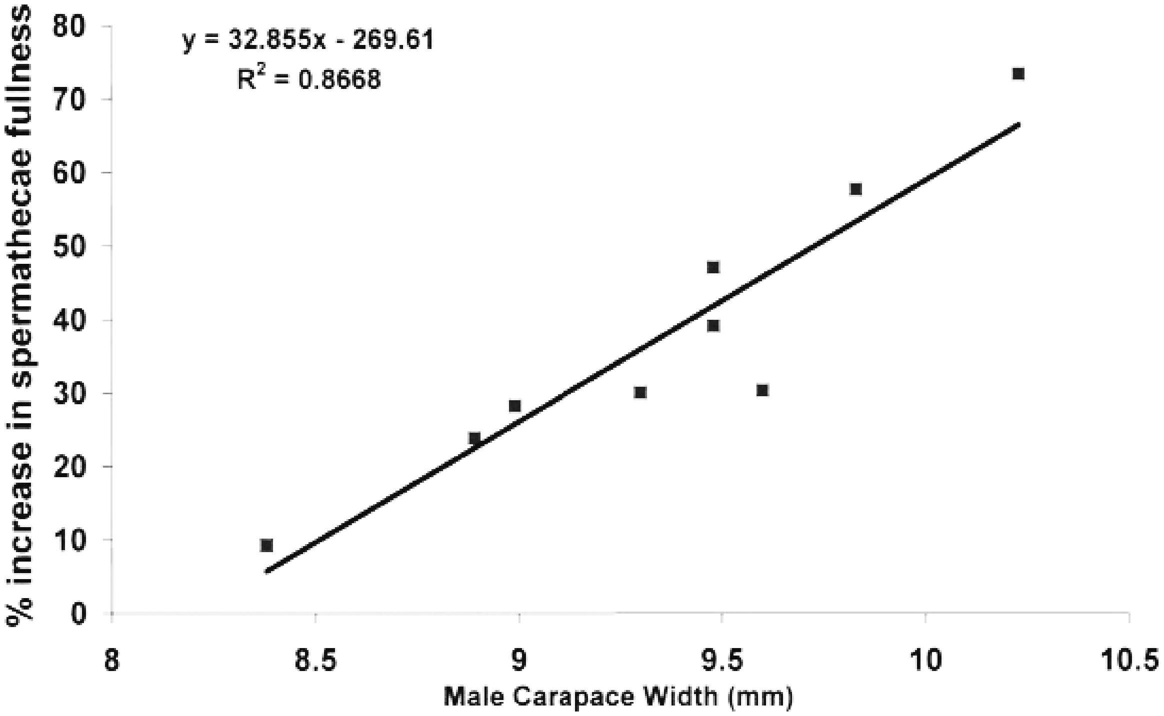


Fig. 4.3. Relationship between male *H. cookii* carapace width and the percentage increase in the fullness of the female's seminal receptacles (N = 9).

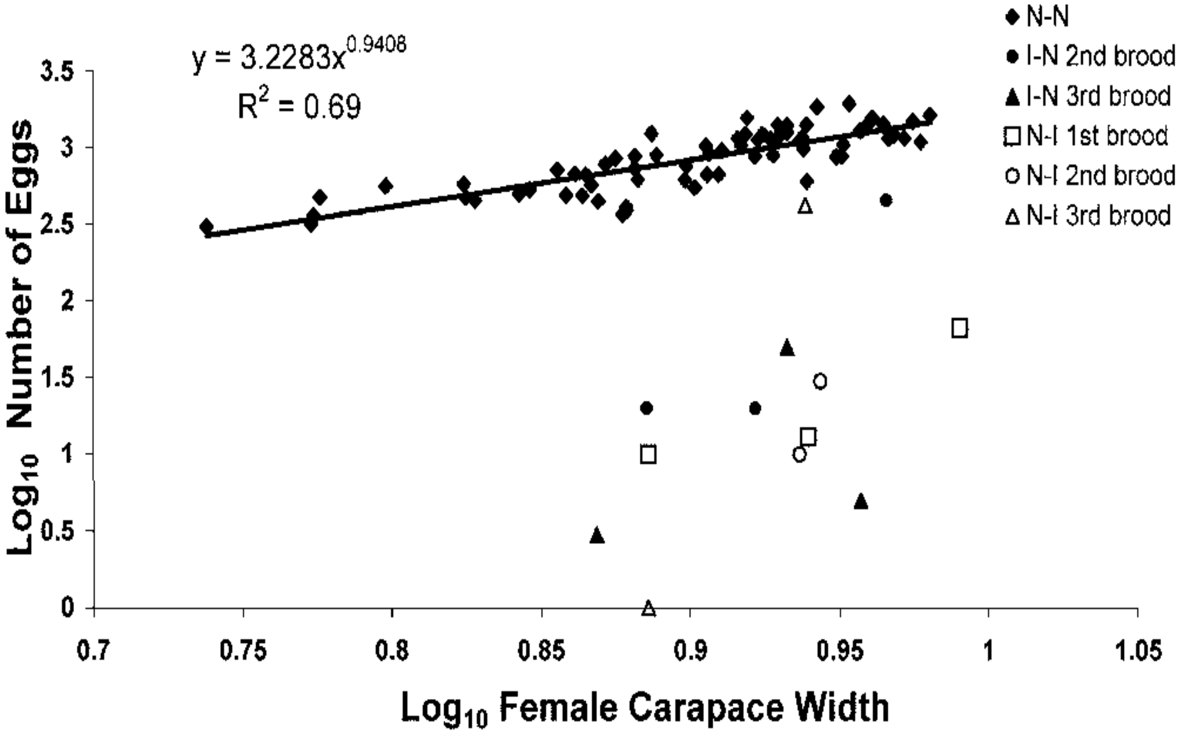


Fig. 4.4. Relationship between log10 number of eggs and log10 female carapace width (n = 86) for field collected crabs (N-N). Regression equation and R^2 value are shown. Other brood size values are for females who were mated sequentially with either Normal (N) or Irradiated (I) *H. cookii* males. Females that had no measurable brood are not shown.

Sperm Supply

Figure 4.5 shows that females carried sperm stores year round. Seminal receptacles were fullest (up to 75%) during autumn - winter months and again during the summer. Although there were some differences between years (2004-2005) the pattern was similar. In terms of our simple model, this pattern can be explained by changes in the rates of copulation and brood production. Superimposed on variations in seminal receptacle fullness are changes in abundance of mature females (Fig. 4.5). During the colder months brood cycles are longer so that at average summer (December to February) temperatures (18.8°C) the incubation period is around 15 d, but at average winter (June to August) temperatures (8.8°C) the incubation period is around 50 d, more than 3 times longer (Van den Brink, et al. 2012). Thus there are two factors at work (or in combination): in winter there are fewer broods produced and therefore less sperm used, but there are also fewer mature females to be mated by the males. There was no seasonal pattern of changes to monthly mean CW for either males or females, ranging from 6.5 mm - 9.5 mm, with population size structure changing little month by month. Thus we cannot say that male size had any effects on seminal receptacle fullness that might result from the presence of larger males transferring larger ejaculates (see Fig. 4.3). The operational sex ratio (mature males/mature female) averages 0.3 in spring-summer (Nov-Feb), but in autumn-winter (March-July) it is 0.8, almost 3 times higher. Therefore we have two competing explanations for seasonal changes in seminal receptacle fullness: length of the brood cycle and female abundance. At the moment we cannot say which (or both) of these explanations would suffice.

In terms of our model of seminal receptacle fullness, we argue that mean EJ (ejaculate volume) does not change with season and we assume that mean SP (fertilization volume) is also constant, so this leaves NC (number of copulations) and NB (number of broods) as the major variables that change seasonally. More elaborate experiments are obviously needed to test our model.

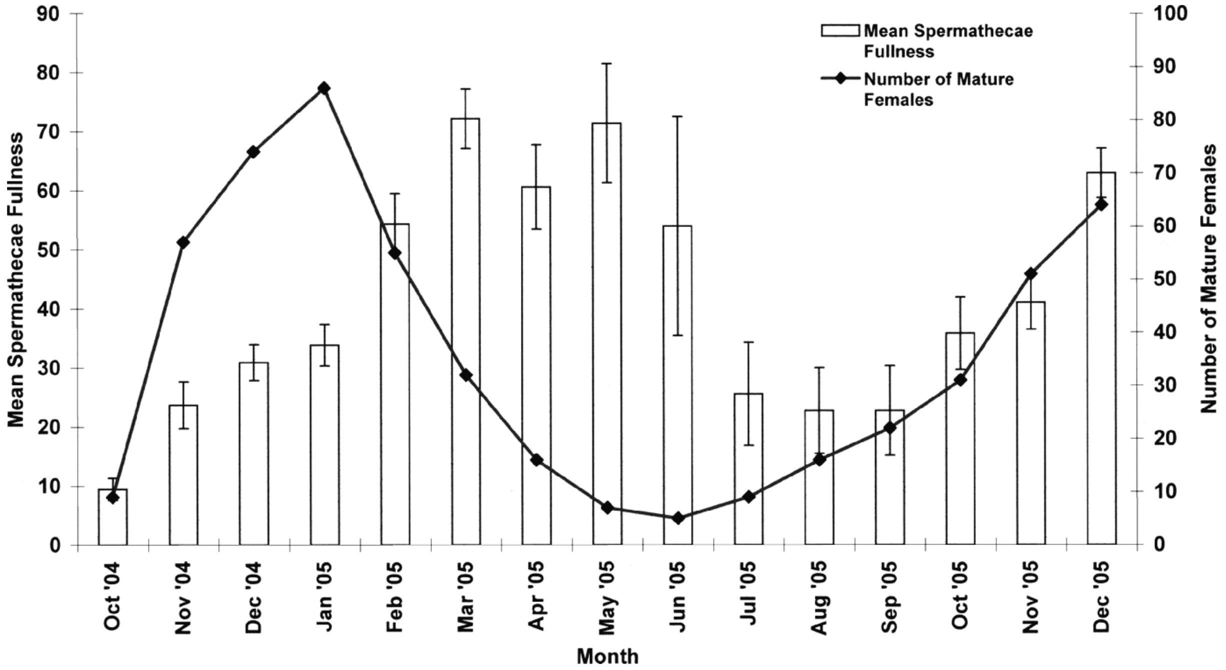


Fig. 4.5. Comparison of the number of mature *H. cookii* females found throughout the year and the estimated percentage seminal receptacle fullness from each monthly population survey sample (October 2004-December 2005).

DISCUSSION

Sperm storage allows females to produce fertilised broods even if they do not encounter a male during the inter-brood period prior to oviposition (Hartnoll, 1985). Because sperm are stored near the oviduct, fertilisation in crabs is internal. From visual estimates, female *H. cookii* could fill their seminal receptacle to 100% after three or four copulations, but only used approximately 15% of the ejaculate of a single male to fertilise one brood. Paul (1984) reported that wastage of sperm cells during fertilization is common in majids. Female *Chionoecetes opilio* used approximately 19% of the sperm cells in the seminal receptacles to fertilise a single brood and would not oviposit eggs with a sperm cell to oocyte ratio of less than ; 7:1 (Sainte-Marie and Lovrich, 1994). Hybrid females of the freshwater hymenosomatids, *Amarinus lacustris* (Chilton, 1882) and *A. paralacustris* (Lucas, 1970) produced 15 egg masses (of declining fertility) without further impregnation after one post-puberty impregnation (Lucas, 1980). With full seminal receptacles, a female *H. cookii* could potentially fertilise about 6 broods, without re-mating, before sperm ran out, assuming that the entire contents of the seminal receptacles was useable.

Halicarcinus cookii females that had mated with a sterile then healthy male produced a normal sized, fertilised brood initially, but in their second and third broods the eggs were lost leaving only some fertilised eggs attached to the pleopods. This indicates that there is some, albeit small, degree of sperm mixing in *H. cookii*. For the first brood, *H. cookii* appears to show little sperm mixing. For the second and third broods, however, females that had mated with a healthy then a sterile male seemed to show a small degree of sperm mixing as females that laid a normal to small sized brood lost all but a few eggs which remained attached to the pleopods and developed, indicating they were fertilised by healthy sperm. The time required for sperm to mix inside the seminal receptacles may also be a factor resulting in mixed paternity of the second and third broods. Females generally laid their first brood after mating within 24 hours, while their second brood was laid an entire incubation period after the female's copulation. Therefore during the incubation of the first brood, the sperm in the seminal receptacles may have had time to mix leading to the observed mixed paternity in the second brood. From this study, the occurrence of sperm mixing in *H. cookii* can be established, but the degree of sperm mixing and the exact proportion of paternity is still uncertain. Unlike most other crabs, hymenosomatids have very soft flexible exoskeletons (due to the low level of calcification) so the shape of the walls of the seminal receptacles could be changed by the female, thereby causing sperm mixing. Jensen et al. (1996) suggest that in *Cancer magister* movements of the second walking leg (adjacent to the seminal receptacles) could cause sperm mixing, although this crab is not likely to be storing the sperm of as many males as *H. cookii*. Koga et al. (1993) used sterilised male *Scopimera globosa* to investigate sperm precedence and to estimate the proportion of each female's brood fertilised by the last male to mate. Females were introduced into the burrows of irradiated and control males and they found that 87% of the eggs were fertilized by normal male sperm when they were the last male to mate (Irradiated-Normal, I-N), while 100% were fertilized by treated males when they were the last to mate (N-I). Overall the last male to mate fertilised an average 94.1% of eggs extruded by females in the burrows, which is significantly more than the expected 50% if sperm of the last two males were thoroughly mixed. Thus capturing females, sealing them inside their burrow and mating underground, ensured a high level of paternity. Apparently, no correction was made for possible loss of eggs from the pleopods (as seen in *H. cookii*), which could have been much higher for those eggs fertilised by irradiated male sperm. For example the brood size carried by females whose last mate had been irradiated (N-I) was only 63.2% of the control (N-N) and when the irradiated male was penultimate (I-N) the brood size was 91.4% of the control. Thus it may well be that some fertilised eggs were lost when irradiated sperm was involved, and therefore the degree of sperm mixing was underestimated in this experiment. Similarly in the freshwater crayfish

Orconectes rusticus, which stores sperm in an annulus ventralis (an external seminal receptacle), for a long period prior to egg extrusion and fertilisation, use of the sterile male technique showed that the last male to mate fertilized 92% of the eggs although there was a wide range of success, especially for I-N mating (Snedden, 1990). Brood sizes were about the same for all females and only the percentage of fertility varied between broods. This result is surprising because fertilization is external, which should result in substantial mixing of sperm from multiple males. Eggs and sperm meet in the glair chamber, formed beneath the abdomen prior to fertilization, so that it might be expected that last male paternity would be much smaller, perhaps even the same as earlier mates. But the fertilisation process in decapod Crustacea in general is poorly understood; in particular the signal from the eggs that causes spermatophores to burst open and release sperm is unknown. In *O. rusticus*, it may be that the spermatophores from earlier mates were not equally sensitive to the dehiscence stimulus.

Dunnington (1999) investigated sperm layering within the seminal receptacles in *H. innominatus* through histological analysis, but the evidence was inconclusive. Although dense sperm packets could be seen the layers were not obviously separated, and sperm mixing may have occurred. Separate layers of ejaculates can form within the seminal receptacles in species where males inject spermatophores surrounded by seminal plasma that hardens after transfer into the female's seminal receptacles, such as the spider crab, *Inachus phalangium* (Diesel, 1988, 1990, 1991) and the snow crab, *Chionoecetes opilio* (Sainte-Marie and Sainte-Marie, 1999). In such species, the sperm that fertilizes the eggs are likely to come almost exclusively from one male. However, in *C. opilio*, there is evidence for multiple paternity of broods when several ejaculates co-occur close to the oviduct opening (Sainte-Marie et al., 2000).

In *H. cookii* there was a positive correlation between the amount of ejaculate transferred to the female and male size. However, when females were given the choice between mating with a male larger than or smaller than themselves, they did not show any preference. Even though both females and males have a terminal moult, there is still a range of different-sized mature crabs in the population because the pubertal moult can occur over a range of pre-pubertal sizes (McLay and van den Brink, 2010). Female choice has yet to be convincingly demonstrated in brachyurans. In many crustaceans males tend to be larger than females, suggesting that there is selection for larger male size, which leads to sexual dimorphism. The selective force behind larger male size is likely to be primarily male-male competition, of which the transfer of more sperm is likely to be included. By transferring more sperm, a male is indirectly competing with rivals by increasing his potential to fertilise more eggs. Larger male *Gammarus pulex* (Linnaeus, 1758) were more successful at mating than smaller males (Bollache and Cezilly, 2004). Similarly, large male fiddler crabs, *Uca paradussumieri* Bott, 1973, had greater success in mating and longer copulation times than smaller males, suggesting more sperm was transferred, than by their smaller counter-parts (Jaroensutasinee and Jaroensutasinee, 2003). After mating with larger male spiny lobsters, *Jasus edwardsii* (Hutton, 1875), females produced significantly larger broods than after mating with smaller males (MacDiarmid and Butler, 1999).

Our model of seminal receptacle fullness in *H. cookii* only included number of copulations and the number of broods as the major variables and assumed that ejaculate volume and fertilization volume remained constant. This is only a first approximation of what is going on and other factors are likely to be important. Future experiments on sperm transfer need to control for both male and female size, space available in the seminal receptacles and stage of brood development. Larger females produce larger broods and so must use more sperm/brood. Males may transfer more sperm when a female is closer to fertilising a new brood, thereby diluting the sperm of other males.

The reproductive characteristics of *H. cookii* are typical of the Hymenosomatidae (Lucas, 1980). Characteristic of brachyurans, sperm storage in *H. cookii* allows females to produce multiple fertilised broods without re-mating, thus ensuring a high reproductive output. Sperm mixing inside the seminal receptacles appears to occur to some degree indicating that sperm competition occurs in this species. However, the last male *H. cookii* to mate achieves the greatest paternity so we would expect males to show post-copulatory guarding if the female is close to releasing her previous brood. This prediction is tested and the mating behaviour of this species is described in Chapter 3.

ACKNOWLEDGEMENTS

I would like to thank Mathis Hasler and Jenny Lydon, Oncology Department, Christchurch Hospital, for help with sterilising the male crabs. Kimberley Seaward provided valuable social assistance. Jack van Berkel, Edward Percival Field Station, provided moral support, copious, occasionally relevent advice.

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Chapter 5:

The effect of temperature on brood duration in three *Halicarcinus* species (Crustacea: Brachyura: Hymenosomatidae)

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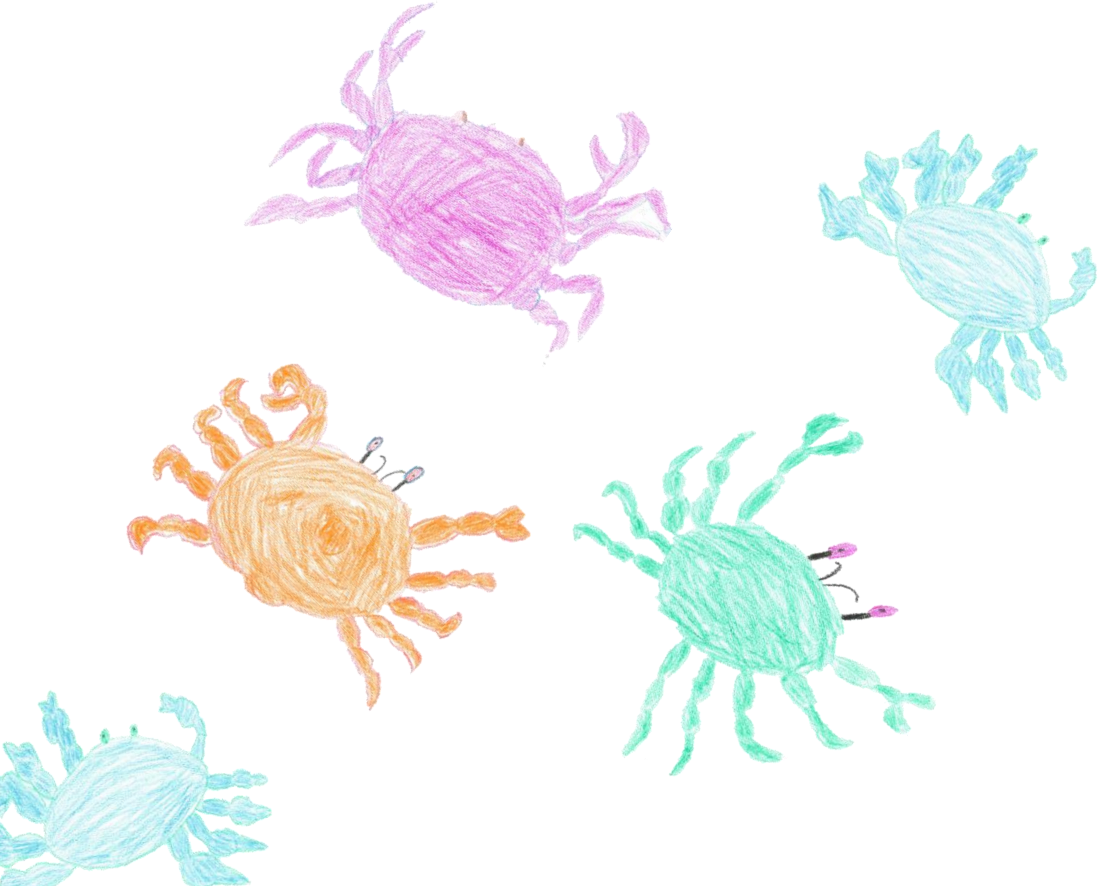
Journal of the Marine Biological Association of the United Kingdom 2012, 92(3): 515–520



ABSTRACT

The effect of temperature on brood development was investigated for three intertidal hymenosomatid crabs: *Halicarcinus cookii*, *H. varius* and *H. innominatus* in Kaikoura, New Zealand. The duration of brood incubation decreased as temperature increased, as did the interbrood period. The duration of each stage of brood development also decreased with increased temperature, but the proportion of total incubation time for each stage remained relatively similar at different temperatures. Hymenosomatid crabs have determinate growth, but moult to maturity at different sizes, thereafter devoting most of their energy to reproduction. The number of broods a female could carry in her lifetime was estimated for each species. *Halicarcinus cookii* was estimated to be able to produce 8 complete broods of 1146 eggs per lifetime, *H. varius* was estimated to be able to produce 7 complete broods of 1051 eggs per lifetime, and *H. innominatus* was estimated to be able to produce 6 complete broods of 1081 eggs per life time. With the predicted global temperature rise of 2°C in the next 50 years, the authors estimate that, for all three species, a female could produce one extra brood per lifetime (a 10–15% increase in fecundity depending on species), even more if crabs reach maturity faster, potentially leading to a significant population increase.

INTRODUCTION

Growth and reproduction can be considered competing processes in terms of the allocation of energy. If less energy is allocated to growth, more energy can be invested into greater reproductive output (Hines, 1982; Hartnoll, 1985). Reproductive output is determined by the number of offspring produced over a lifetime (Shields, 1991). Hymenosomatid crabs of the genus *Halicarcinus* have a reproductive strategy involving a terminal, pubertal moult where reproduction begins only when growth has ceased (Melrose, 1975; McLay & Van den Brink, 2009). The terminal moult in *Halicarcinus* species allows females to maximise their reproductive output during a comparatively short (approximately six month) adult life span by producing broods continuously and successively, without the need for the female to suspend reproduction for moulting (Van den Brink & McLay, 2009; Van den Brink & McLay, 2010). However, as body size is the primary determinant of brood size, and a terminal moult prevents further growth, the number of eggs per brood remains small (Hartnoll, 1969). Due to their small size, hymenosomatids have some of the lowest fecundity levels among the Brachyura (Lucas, 1980; McLay & Van den Brink, 2009).

A female's reproductive output is influenced by the number of eggs per brood, the number of broods produced, their incubation time and survival rates. Being ovigerous throughout the year exposes *Halicarcinus* females and their externally carried eggs to various environmental factors that can affect their reproductive output. During different seasons, the growing embryos in the eggs are exposed to a range of temperatures that may affect their incubation time or survival that a discrete breeding season would avoid (Jansen, 1971). If different temperatures cause significant differences in incubation time then it is reasonable to assume that temperature can determine the number of broods produced in a lifetime and has the potential to influence local recruitment and population dynamics.

In this study the effect of temperature change on brood duration, comparing three species of hymenosomatid crabs, *Halicarcinus cookii*, *H. varius* and *H. innominatus*, living in the same intertidal habitat were measured.

MATERIALS AND METHODS

Crabs were collected from locations around the Kaikoura Peninsula in New Zealand (see Dunnington, 1999; Hosie, 2004). Three temperatures that encompassed the annual mean sea water temperature of 12.9°C were chosen. It would have been useful to include a temperature the same as the mean, but facilities did not allow that. However, our intermediate temperature of ~15°C was close to the mean. For each temperature, 25 females for *Halicarcinus cookii* and *H. varius*, and 30 for *H. innominatus*, each marked with a commercial bee tag bearing a different number, were placed in a 50 × 20 × 5 cm tray with 3 L water and an air pump. For *H. cookii* and *H. varius*, temperature control rooms were set up but for *H. innominatus*, females were monitored in holding tanks during different seasons and ambient water temperatures were recorded (Dunnington, 1999; Hosie, 2004). The mean temperatures for *H. cookii* were 10.31°C ± 0.21°C, 15.35°C ± 0.17°C and 20.28°C ± 0.096°C respectively, while for *H. varius* the mean temperatures were 10.04 ± 0.67°C, 14.35 ± 0.19°C and 20.73 ± 0.23°C. For *H. innominatus*, Summer (Dec-Feb) temperatures averaged 18.7°C, Winter (Jun-Aug) temperatures averaged 10.45°C and Autumn (Mar-May) and Spring (Sept-Nov) temperatures averaged 14.8°C and 14.2°C respectively. For ease of comparison between species, the temperatures are rounded and referred to as 10°C (Winter), 15°C (Autumn/Spring) and 20°C (Summer). Note that the latter two temperatures were higher than the annual mean of 12.9°C.

Females were monitored through one complete brood cycle. The five stages of brood development were: stage 1 eggs have 100% bright yolk (orange in *H. cookii*, olive green/yellowish in *H. varius* and olive green in *H. innominatus*) with little or no embryo cleavage; stage 2 eggs show 75% orange yolk and obvious embryo cleavage; stage 3 eggs show 50% yolk, more cleavage and the development of chromatophores; stage 4 eggs have chromatophores, 25% yolk and an embryo with developing eyespots; stage 5 eggs have less than 10% yolk, prominent eyespots on a fully developed zoea ready to hatch. The interbrood period was the time between a brood hatching and the oviposition of a new brood (Dunnington, 1999; Hosie, 2004).

Records of the brood cycle began from the first change in brood stage observed and ended when that same stage was reached in the following brood to ensure an accurate record of the beginning of a stage. For example, if the female was initially observed to carry a brood at stage 2, the recorded brood cycle began only when the brood first developed into stage 3, and ended the day before the first observation of stage 3 of the following brood. In this way we ensured that the entire duration of each stage for each species was measured and so we did not have to make any assumptions about how much of a stage might already have been completed when the crab was added to the experiment, and also allowed time for acclimatisation to the temperature. The mean duration of each brood stage at each temperature was compared using one way ANOVA. The interbrood period was also recorded and compared between temperatures to investigate the influence of temperature on the time it takes for a female to lay a new brood (Dunnington, 1999; Hosie, 2004). This is a reflection of the effects of temperature on the duration of the ovarian cycle.

RESULTS

Brood cycle

Incubation time for all three species was negatively correlated with temperature (Figure 5.1). Mean incubation period for all species ranged from 43.8–56.8 days at 10°C, 22.8–32.4 days at 15°C and 14.7–18.9 days at 20°C. [The mean incubation period for *Halicarcinus cookii* at 10°C was 43.8 days, at 15°C: 22.8 days ± 1.0 days and at 20°C: 14.7 days ± 1.2 days. For *H. varius* the incubation duration at 10°C was 53.3 days, at 15°C: 25.7 ± 0.7 days and at 20°C 16.9 ± 0.4 days. For *H. innominatus* the mean incubation duration at 10°C was 56.8 ± 0.5 days, at 15°C: 32.4 ± 0.3 days and at 20°C: 18.9 ± 0.5 days. (Figure 5.1). There was a significant difference in total incubation duration according to temperature between 15°C and 20°C for *H. cookii* and *H. varius* (as 10°C had no variation) (ANOVA: *H. cookii*: $F_{1, 14} = 51.04$, $p < 0.0001$; *H. varius*: $F_{1,21}=99.38$, $p < 0.001$) and for all temperatures for *H. innominatus* ($F_{3, 116} = 1575.60$, $p<0.001$).

Mortality was high during the experiment at 20°C. Therefore sample sizes for *H. cookii* and *H. varius* were smaller (n= 7 and 8 in 20°C respectively) compared with the other temperatures where sample size remained at 25 for *H. cookii* and *H. varius*, and 30 for *H. innominatus* due to negligible mortality or successful replacement of females. Although additional females were added to compensate for the loss, this proved unsuccessful due to high mortality of 76% for *H. cookii* and 73% for *H. varius*. Females entering the experiment with broods at stages 4 or 5 tended to lay another brood successfully, but females entering the experiment carrying broods at stages 1 or 2 often died before laying their next brood, or simply failed to lay a second brood. As no females of either *H. cookii* or *H. varius* completed an entire brood cycle in

10°C, the total brood duration was calculated as the sum of the average duration for each individual brood stage, and therefore show no overall estimate of variation.

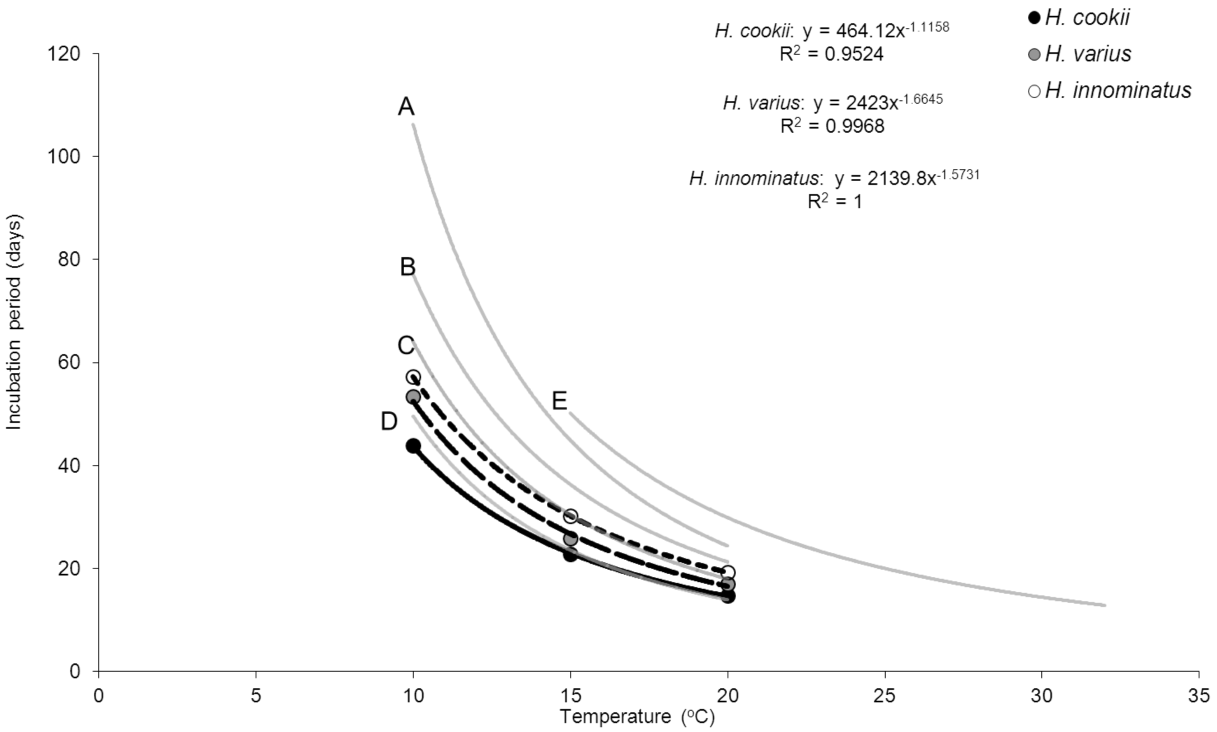


Fig. 5.1. Mean incubation time (days) according to temperature for *Halicarcinus cookii*, *H. varius* and *H. innominatus* (black lines). Symbols represent experimental results. Regression equations and R^2 values are also shown. Grey lines indicate incubation time for other crab species: A=*Palaemon serratus*, B=*Carcinus maenas*, C=*Inachus dorsettensis*, D= *Macropipus depurator*, E= *Elamenopsis kempi* (from Ali et al. 1995).

Interbrood period

The interval between the hatching and oviposition of a new brood was generally shorter at higher temperatures for all three species (Figure 5.2). Note that in Figure 5.2, the minimum interbrood period is shown as one day; although results for *Halicarcinus cookii* and *H. varius* include proportions of a single day where a brood was laid within one day, *H. innominatus* was recorded with a minimum one day interbrood period. Therefore 1 was added to the results for *H. cookii* and *H. varius* in Figure 5.2 for ease of comparison (for *H. cookii* actual average interbrood periods in days were: 10°C = 0.92, 15°C = 0.28, 20°C = 0.25; and for *H. varius*: 10°C = 0.74, 15°C = 0.30, 20°C = 0.17).

For *H. cookii*, there was a significant difference in interbrood duration between temperatures ($F_{3,\,34} = 9.45$, $p < 0.001$) (ANOVA) and interbrood durations were significantly longer in 10°C than both 15°C and 20°C ($p < 0.05$ in all cases) while interbrood period at 15°C and 20°C were not significantly different ($p = 0.894$) (Tukey's HSD test). Similarly, for *H. varius* the interbrood period was significantly longer at 10°C than in 15°C and 20°C ($F_{2,55}$:3.687,$p < 0.05$), but there was no significant difference between 15°C and 20°C ($p > 0.05$). For *H. innominatus*, there was no significant difference between interbrood periods at different temperatures ($F_{3,\,116} = 3.23$, $p > 0.01$, higher p value used because variances were heterogeneous).

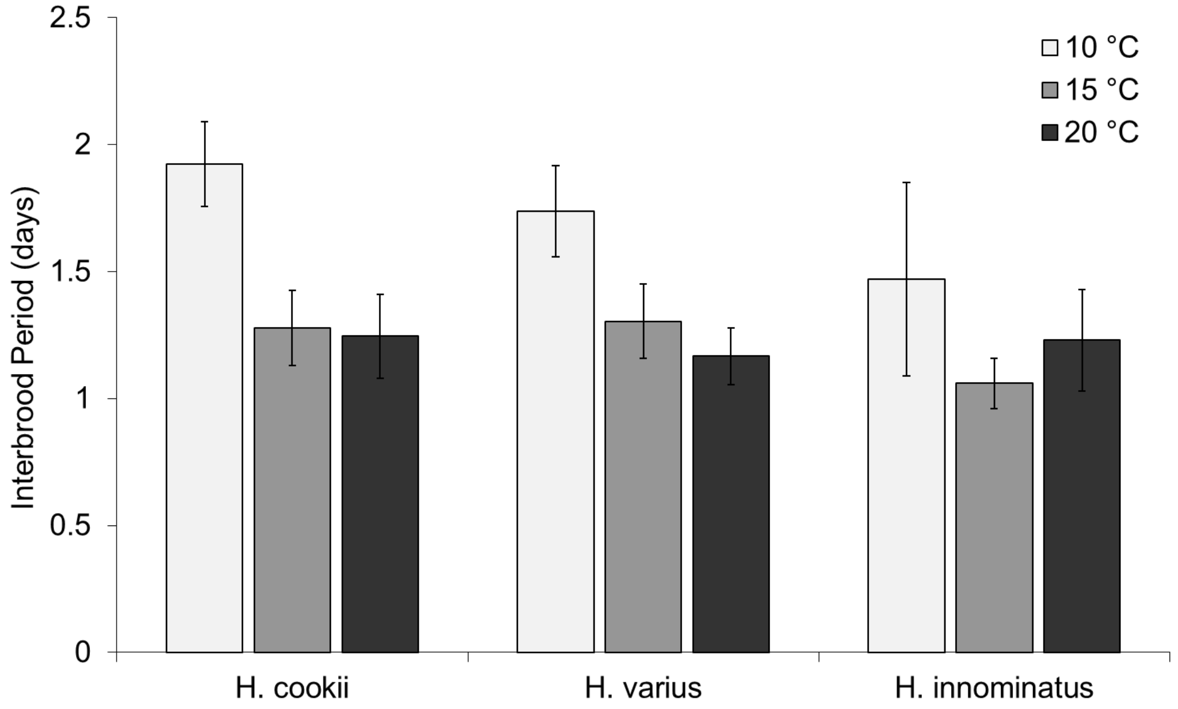


Fig. 5.2. Mean duration (days) of the interbrood period between larval release and oviposition of the following brood (stage 5 to stage 1) at temperatures of 10, 15 and 20°C for *Halicarcinus cookii* (n=13, 19 and 13 respectively), *H. varius* (n= 17, 15 and 8 respectively) and *H. innominatus* (n= 30 for all temperatures). Error bars are ± 1 SE.

Brood stages

There were significant differences in incubation period at different temperatures for all stages for all three species (Table 5.1). The mean percentage of total incubation time for each brood stage was calculated for all three species. The proportions of incubation time for each stage were relatively similar between species. For the three species at all three temperatures, stage 1 was the longest in duration of all brood stages while stages 3 and 5 were the shortest brood stages (Figure 5.3).

Table 5.1. Results of the ANOVA for the duration of each brood stage at different temperatures.

Brood stage	*H. cookii*	*H. varius*	*H. innominatus*
1	$F_{2.46} = 119.0$, $p<0.001$	$F_{2.27} = 23.8$, $p<0.001$	$F_{3.116} = 159.01$, $p<0.001$
2	$F_{2.28} = 29.6$, $p<0.001$	$F_{2.27} = 87.4$, $p<0.001$	$F_{3,116} = 118.13$, $p<0.001$
3	$F_{2.36} = 60.6$, $p< 0.001$	$F_{2.27} = 14.4$, $p< 0.001$	$F_{3, 116} = 67.10$, $p< 0.001$
4	$F_{2.45} = 66.3$, $p<0.001$	$F_{2.27} = 19.0$, $p<0.001$	$F_{3,116} = 216.71$, $p<0.001$
5	$F_{2.39} = 50.8$, $p<0.001$	$F_{2.27} = 71.4$, $p<0.001$	$F_{3, 116} = 4.46$, $p<0.01$

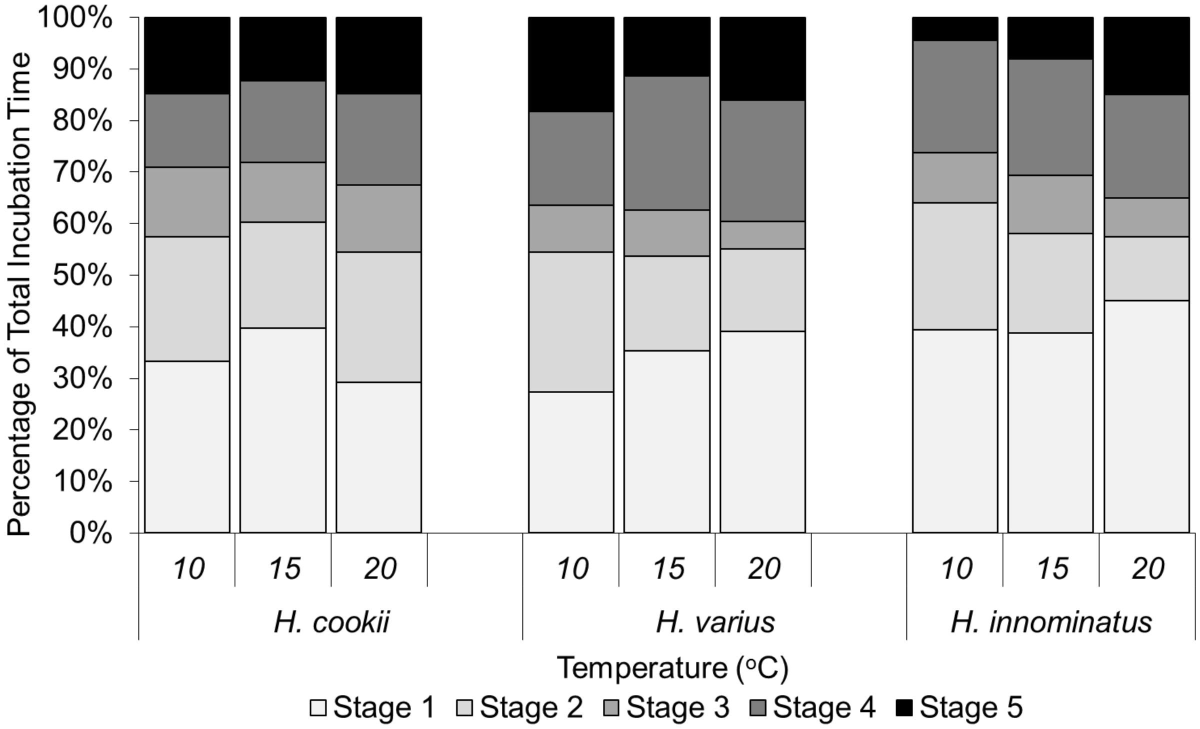


Fig. 5.3. Each brood stage as a percentage of the entire brood cycle for *Halicarcinus cookii*, *H. varius* and *H. innominatus* at different termperatures

For *Halicarcinus cookii* data for duration of each stage and for each temperature were normalised with a square root transformation (Cochran $p = 0.053$). For *H. varius* variances for brood stage duration at 15°C were heterogeneous (Cochran's test $p < 0.05$) and all attempts to make them homogenous failed. The criteria for significance was then lowered to $p = 0.001$. For *H. innominatus* variances for the ANOVA were heterogeneous for all stages. Data were log transformed for brood stage 1, 3 and 5 to stabilise the variances. For brood stages 2 and 4, all attempts to make the variances homogeneous failed and the significance levels for these two analyses were raised to <0.01.

DISCUSSION

Of the three species, *Halicarcinus cookii* has the shorter incubation time at any given temperature than *H. innominatus* and *H. varius*. While interbrood periods were similar, at a mean temperature of 15°C, *H. cookii* incubation took 22.8 days, whereas *H. varius* took 25.7 days (Hosie, 2004) and *H. innominatus* 30.2 days (Dunnington, 1999). At maximum mean temperatures of around 20°C *H. cookii* incubated their eggs in 14.67 days, while *H. varius* took 16.88 days (Hosie, 2004) and *H. innominatus* took 22.3 days (with a mean temperature of 18.7°C) (Dunnington, 1999). These are all relatively short incubation periods when compared with other hymenosomatid species at similar temperatures such as *H. ovatus* (29 days), *Amarinus paralacustris* (25.5 days) (Lucas, 1980) and *A. laevis* (29 days) (Lucas & Hodgkin, 1970). The three study species also have relatively short incubation periods when compared with various other crab species at a range of temperatures (Figure 5.1).

At temperatures around 15–20°C incubation in *Elamenopsis kempi* took 48 days (Ali et al., 1995). However *E. kempi* occurs in sub-tropical waters and is the only hymenosomatid species, for which data are available, to incubate its eggs in a mean water temperature greater than 20°C (ranging from 25–32°C). At these temperatures incubation was completed in about 23 days, suggesting that relative to their respective temperature regimes, *E. kempi* has a faster incubation time. Similarly, brood incubation in the sub-Antarctic species *Halicarcinus planatus* took 60 days at 6–8° C at the Kerguelen Islands (Richer de Forges, 1977). This may simply be an indication that temperature related differences in incubation rates between species are based on adaptations to their environments with their optimal incubation times in synchrony with the temperature regime they experience in the field.

Although there were significant differences in the duration of each individual brood stage, their proportions of the total incubation time were similar. These results are reflected in the field. As the three *Halicarcinus* species all live in similar habitats on the same rocky shore and cannot burrow or hide, any bias in sampling can be eliminated. As they can store sperm to reduce sperm limitation, they have consistent brood production, and as females appeared to be completely passive participants during mating and mate-guarding, behavioural effects on selection can also be ruled out. Therefore, for a random sample of crabs from the shore, the proportion of females with eggs at different stages should be a reflection of the duration of each stage.

Van den Brink & McLay (2010) found that in the population of *H. cookii* sampled from the field, most ovigerous females were found carrying stage 1 eggs and fewest carrying stage 4 eggs., they estimated that the brood stage of longest duration was likely to be stage 1, followed by stages 2, 5, 3 and the shortest, stage 4. The present results differ slightly from those estimates, but follow a similar trend for all three species (see Table 5.2).

Table 5.2. Comparisons of the proportion (%) of each brood stage during brood development in the Laboratory and observed in the field.

Brood stage	*H. cookii*		*H. varius*		*H. innominatus*	
	Field	Lab	Field	Lab	Field	Lab
1	44	34	36	34	39	41
2	16	22	18	20	20	19
3	12	13	11	8	9	9
4	10	17	20	23	24	22
5	18	14	15	15	8	9

Once mature, *Halicarcinus* species can mate at any time, and with short interbrood intervals, have the potential to have a high reproductive output and produce recruits year-round (Van den Brink & McLay, 2010). This is in contrast with species such as *Hemigrapsus sexdentatus* from the same habitat, which have a very restricted breeding season and only produce one brood per year (Brockerhoff & McLay, 2005). With this continuous brood production regardless of season, water temperature directly affects the duration of brood incubation with incubation time decreasing in increasing temperatures. Temperature therefore influences the number of broods a female can produce per lifetime.

The mean annual seawater temperature in Kaikoura from December 2003 through to November 2005 was 12.9°C ranging from 8.77°C (Aug) to 17.86°C (Feb) (Figure 5.4). Over the summer months from November to April (peak breeding period, due to the increased numbers of adult females and therefore more net reproduction in the population, see Van den Brink and McLay, 2010) the mean temperature was 15.36°C, while over the winter months from May to October the mean temperature was 10.5°C. Assuming an adult life span of about 6 months then during the peak breeding period an *H. cookii* female could produce a maximum of approximately eight broods in a lifetime, *H. varius* about seven broods, and *H. innominatus*, about six broods. Given the mean fecundities of 8 complete broods 1146 eggs for *H. cookii*, 7 complete broods of 1051 eggs for *H. varius* (Hosie, 2004), and 6 complete broods of 1081 eggs per brood for *H. innominatus* (Dunnington, 1999), an average sized female *H. cookii* could be expected to produce 9168 larvae in a lifetime, *H. varius* 7357 and *H. innominatus* 6486 offspring in a lifetime (Table 5.3).

In the temperature regime experienced by these three *Halicarcinus* species in the field, successive brood production over a female's adult life exposes broods to a range of temperatures according to different seasonal changes in climate. Incubation times of decapod eggs are closely linked to the temperature of the water they are incubated in (Wear, 1974). At Kaikoura the water temperature varies throughout the year due to seasonal changes, and as the three *Halicarcinus* species produce eggs for most of the year, not just in optimal conditions, their eggs experience a range of temperatures.

The incubation times of the study species were typical of decapods in that incubation time decreased as temperature increased, probably due to an increase in the speed of metabolic processes with increased temperature (Leffler, 1972). However, in the 20°C temperature control room *Halicarcinus cookii* and *H. varius* did not survive. At this temperature it is possible that heart failure, which affects oxygen uptake, oxygen delivery and oxygen utilisation caused the observed high mortalities (Stillman, 2002). Stillman (2002) reported that at the thermal limits of *Petrolisthes* species, heart function was damaged irrecoverably. This was due to either the molecular properties of the heart muscle being damaged, or that the nerves innervating the heart were damaged. The high mortality may also have been a result of oxygen depletion. Walther, et al. (2010) suggested that mortality at higher temperatures in the spider crab, *Hyas araneus* could be explained from the principles of oxygen- and capacity-limited thermal tolerance; that the brooding of the crustacean eggs enhances the oxygen demand of the female at constant oxygen supply capacity and, thereby, exacerbates any oxygen limitation. Despite the presence of an air pump during the experiment, the oxygen levels were likely to be lower at 20°C than at the other temperatures, and may, therefore, have been below the tolerance level of the crabs.

In contrast, *H. innominatus*, kept in ambient temperatures where the highest mean temperature was 18.9°C, suffered negligible mortality. As ambient temperature fluctuates, *H. innominatus* was not exposed to the consistently high temperatures experienced by the other two species in the temperature control rooms, and therefore experienced less physiological stress. The ambient temperature experienced by *H. innominatus* is obviously more accurate to the natural habitat as temperatures fluctuate daily, whereas the temperature control rooms were kept relatively constant. When considering the observed incubation times and potential levels of mortality observed in *H. cookii* and *H. varius* in the temperature control rooms, it can be assumed that an optimal consistent temperature for these species to incubate eggs is about, and perhaps slightly less than 15°C.

Sea temperatures from December 2003 through to November 2005 in Kaikoura ranged from 8.77°C to 17.86°C, reaching over 15°C in only 8 of the 24 months recorded (one third of the year) with an average of 15.36°C in the summer months and 10.5°C in the winter months. The mean sea water temperature during this time was 12.9°C, suggesting that these *Halicarcinus* species are well adapted to the temperature of their environment.

Table 5.3. Estimated number of broods produced by H. *cookii*, H. varius and H. innominatus per month during November 2004 – April 2005 and the same months in approximately 2050 after a 2°C sea temperature rise using regression equations (Figure 5.1). This period is both peak breeding season and an average female adult life span. Incubation period includes interbrood interval.

Month	Average Temperature		Days per month	Estimated Broods per Month					
				H. cookii		*H. varius*		*H. innominatus*	
	2005	2050		2005	2050	2005	2050	2005	2050
Nov-04	13.8	15.8	30	1.2	1.4	1	1.2	0.9	1.1
Dec-04	14.1	16.1	31	1.3	1.5	1	1.3	0.9	1.1
Jan-05	16.11	18.11	31	1.5	1.7	1.3	1.6	1.1	1.4
Feb-05	17.86	19.86	28	1.5	1.7	1.4	1.7	1.2	1.4
Mar-05	16.21	18.21	31	1.5	1.7	1.3	1.6	1.2	1.4
Apr-05	14.11	16.11	30	1.2	1.4	1	1.3	0.9	1.1
Total broods per lifetime				8.2	9.4	7.1	8.7	6.2	7.5
Complete broods per lifetime				8	9	7	8	6	7

However, a change in water temperature may affect the generation time of the species and therefore impact the population. Global temperatures are predicted to rise by at least 2°C in the next 50 years (Hoegh-Guldberg et al., 2007). Such a change may result in sea temperatures in Kaikoura reaching over 15°C for half or more of the year. Although at higher temperatures, there is the possibility of increased mortality in the three *Halicarcinus* species (in cases of prolonged periods with water temperatures over 20°C where mortalities above 70% were observed), the fact that the natural environment fluctuates in temperature rather than remains at a consistent temperature suggest that it is more likely that broods would develop faster, allowing each female to produce more offspring per lifetime and resulting in possible population increase and a change in geographical boundary limits.

If temperatures rise 2°C as predicted, each of the three species could produce one extra brood per female lifetime (Table 5.3). This would result in the production of over 1000 extra larvae per female resulting in a 10–15% increase in fecundity. Assuming a larval survival rate of about 1–5%, a 2°C sea temperature rise could result in a single female producing 10–50 extra surviving offspring per lifetime.

Additionally, an increase in temperature is also likely to increase larval growth rates and therefore generation time, adding to the potential population growth. Larval development in crabs is temperature related, with an increase in temperature resulting in shorter development times (Nakanishi, 1981; Vinuesa et al., 1985; Okamoto, 1993; Anger *et. al.*, 2003; Walther et al., 2010). Less time spent in the plankton as vulnerable larvae may also increase survival rates to final instars and eventually adults, thus potentially increasing the size of the population.

Furthermore, the current six month peak breeding season in the three *Halicarcinus* species may increase as temperatures rise. Walther et al. (2010) found the larval release of the spider crab *Hyas araneus* occurred almost a month earlier than it did 30 years ago, correlating with a recorded increase in water temperature of 1.1°C. An increased temperature and extension of the peak breeding time in Kaikoura may allow the three *Halicarcinus* crabs more time to carry eggs and therefore produce even more offspring per lifetime (provided they live long enough).

Although the exact geographical ranges of these three *Halicarcinus* species is not known, an increase in temperature may shift or extend their natural distributional boundaries that are currently limited by temperature. Walther et al*.* (2010) suggested that with a 1.1°C temperature rise since 1969, the southernmost limit of the geographical range of *H. araneus* may have moved north from the English Channel, and that continual rise in water temperature may result in further northward shift of the geographical range.

ACKNOWLEDGEMENTS

Many thanks to Jack van Berkel at the Edward Percival Field Station, Kaikoura for all his help, and to all those who experienced Kaikoura with us. Also, special thanks to Jonathon Hutchens for his motivation to complete this part of the project.

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Chapter 6:

Some like it hot: The effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi*, (Decapoda: Brachyura: Varunidae).

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Journal of the Marine Biological Association of the United Kingdom, Published online 2012

ABSTRACT

The duration of brood development in the introduced crab, *Hemigrapsus takanoi* in the Oosterschelde, the Netherlands, was compared at three different water temperatures. At 12, 18 and 24°C the females took an average of 32, 11 and 8 days respectively to lay eggs, which took 86, 28 and 18 days respectively to complete development. Five stages of development were identified, with each brood stage comprising a similar proportion of the duration time at different temperatures. The duration of each brood stage was also somewhat proportional to the number of females found carrying each brood stage in the field at the beginning of the breeding season. There appears to be a trigger for the breeding season in *H. takanoi* in the field at around 15°C above which ovary development begins. The results suggest that an increase in water temperature as a result of climate change may result in an increased Net Reproductive Rate in *H. takanoi* due to earlier onset of the breeding season and increased number of broods per inter-moult period resulting in population growth. Increased temperatures may therefore lead to increased invasiveness of *H. takanoi* where it is already present, and range extension into locations where its establishment is currently excluded by unsuitable temperature.

INTRODUCTION

The environmental envelope of a species is the set of environmental conditions within which the species can persist. These environmental ranges can determine the potential geographical range of a species with boundaries where the environmental requirements of the species are no longer met (Walker & Cocks, 1991). For marine crustaceans temperature and salinity are two important elements of a species' environmental envelope that together can be used to predict its range (Summerson et al., 2007). This is of particular use with the prediction of the potential range expansion of introduced species (Kaustuv et al., 2001, Peterson & Vieglais, 2001, Ba et al., 2010, Herborg et al., 2009, Oliveira et al., 2010, Sorte et al., 2010).

The predicted global temperature rise may shift geographic boundaries of a species and open new environments to the possibility of invasive species successfully establishing new populations (Aronson et al., 2007). An increase in temperature may also result in environments currently at a suboptimal for invasive marine crustacean populations becoming more suitable. This could result in increased growth and development of individuals and potentially lead to higher reproductive output, more surviving offspring as they spend less time in the vulnerable planktonic stages, and considerable population growth (Van den Brink et al., 2011).

The most successful invasive species have a wide range of environmental tolerances (Zerebecki & Sorte, 2011). While crustaceans are known to adapt to different temperature regimes (Truchot, 1973, Burggren & McMahon, 1981, Anger, 1991, Cuculescu et al., 1995), invasive species populations will establish and grow most rapidly and successfully in environments with the optimal temperature range for their growth and reproduction.

The effect of temperature on brood incubation and larval development in crabs is well documented (Leffler, 1972, Wear, 1974, Anger, 1991, Nagaraj, 1993, Wiess et al., 2009, Van den Brink et al., 2011). Higher temperatures increase physiological processes and therefore decrease the duration of embryonic and larval development (Lleffler 1972). With increasing environmental temperatures, individuals would therefore be expected to mature earlier than they would in colder temperatures, until temperatures become too high resulting in mortality caused by developmental disruption.

Hemigrapsus takanoi (Asakura & Watanabe, 2005) was first reported in the Oosterschelde, the Netherlands by Nijland (2000) as *H. penicillatus* (de Haan, 1835). *Hemigrapsus takanoi* was probably introduced into Europe via ballast water or hull fouling, and spread to the Oosterschelde with the transport of the Pacific Oyster *Crassostrea gigas* (Thunberg, 1793) from France (Nijland, 2000, Nijland & Beekman, 2000, Faasse et al., 2002). The introduction of *H. takanoi* presented the ecosystem with a new competitor for the native crabs, particularly the European green crab *Carcinus maenas* (Linnaeus 1758), itself an exceptionally successful invader elsewhere (Cohen et al., 1995, Grosholz & Ruiz, 1995, Jensen et al., 2002, Thresher et al., 2003): and a predator of various commercially important species including mussels (Dauvin et al., 2009).

In an investigation of the life history of *H. takanoi* from the Oosterschelde, the effect of temperature on the Net Reproductive Rate (R_0) of the embryonic stage of *H. takanoi* was studied. Net Reproductive Rate estimates the number of females expected to reach reproductive age by considering the number of offspring per female and the mortality rate at each stage of development. While the R_0 is dependent on growth, mortality and reproduction, here we consider only reproduction and the effect of temperature thereon. By looking specifically at the time it takes for females to lay eggs, and the incubation time of eggs carried by females we test the hypothesis that with an increase in temperature, the speed of reproduction will increase significantly. In combination with growth and mortality, an increased reproductive rate may lead to an increase in the R_0 for *H. takanoi* which may result in population growth and increased success and distributional range as an invasive species.

MATERIALS AND METHODS

Mature female *Hemigrapsus takanoi* were collected from the intertidal zone at Sas van Goes, in the Oosterschelde estuary in early May 2011. Mature females were identified by broad abdomens that cover the entire ventral side of the body as described in McLay & van den Brink (2009) for *Halicarcinus cookii* (Filhol, 1885). The crabs were brought to the lab where each was measured and labelled with a commercial bee tag, each tag bearing a different number. A total of 81 female crabs were collected and divided equally into three 60×40 cm tanks (27 crabs in each) containing unfiltered Oosterschelde seawater. The crabs ranged from 12.26 mm to 20.84 mm in carapace wdith. One tank was kept at a constant temperature of 12°C (the current long term mean temperature of the Oosterschelde at Goes and one at 18°C (the current long term maximum temperature of the Oosterschelde at Goes (both temperatures according to http://www.sea-temperature.com/water/goes/411 accessed 06/07/2011) and one at 24°C (the rounded maximum temperature of the Oosterschelde at Marollegat in 2010 according to the Dutch meteorological website of Rijkswaterstaat (http://www.hmcz.nl/ accessed 13/07/2011) The temperature of the water in the tanks was measured daily to monitor the temperature variations. The mean temperatures of the water in the three tanks were 12.4°C (±1), 18.5°C (±0.4) and 24.03°C (±1.1) [these will be referred to as 12, 18 and 24°C respectively].

An air bubbler in each tank provided oxygen and the salinity of the water was kept consistent with the water from the Oosterschelde (33-35 ‰). Crabs were given a sandy substrate with rocks for shelter and were fed crushed local mussels (*Mytilus edulis*) once a week. Three males were kept in each tank to guarantee a sperm supply. If females died during the experiment they were immediately replaced with a new female bearing a new number.

Females were monitored over a complete brood cycle to determine the duration of brood development at each temperature. Once a female laid a brood, a sample of around 10 embryos were removed and photographed so that the stages of development could be determined. Females were monitored daily so that the duration of each brood stage could be accurately recorded. The mean development time for each brood stage at each temperature was compared using a one-way ANOVA and with a power trend line (Van den Brink et al*.,* 2011). The interbrood interval (time from hatching of the first brood to first appearance of the next) was measured to determine whether gonad development occurred simultaneously with brood development or whether it occurred sequentially with brood development.

Field surveys in different locations in the Oosterschelde were conducted weekly from 10 April 2011 to 20 June 2011 to determine whether and how many crabs were carrying eggs. For one hour the same two people haphazardly collected as many crabs as possible. This usually entailed one lifting a rock while the other collected crabs. During these surveys crabs found with eggs were recorded along with the stage of brood development. From this it was possible to identify the beginning of the egg-laying season in the wild. Surveys beyond June 20 were unfortunately unfeasible, so only data for the beginning of the breeding season are presented, however, anecdotal and personal observations indicate that a high proportion of ovigerous crabs were found again in August.

RESULTS

Of the 27 crabs, nine crabs laid eggs in the 12°C tank; 18 crabs laid eggs in the 18°C tank; and 21 crabs laid eggs in the 24°C tank. The time it took before eggs were laid varied between temperatures and individuals, but there was no relationship between the size of the crab and the time it took to lay eggs. In the 12°C tank crabs took an average of 31.6 ±15 days to lay eggs (n=9), in the 18°C tank they took an average of 10.7 ± 8 days (n=18) and in the 24°C tank they took an average of 7.6 ±5 days to lay eggs (n=21). Some females lost part or all of their broods before the broods fully developed. As these eggs were fertilised (indicated by the length of time they were carried and that there were signs of development), the loss of eggs is attributed to the artificial environment rather than lack of fertilisation.

Mating was observed in the 18 and 24°C tanks on two separate occasions. During the experiment mortality was similar in all temperatures; six crabs died in the 12°C tank, eight died in the 18°C tank and nine died in the 24°C tank.

Five stages of brood development were identified visually by the amount of yolk present and the observable level of development of the eyespot of the larvae inside (Figure 6.1). Stage 1 was completely filled with orange yolk and no other decipherable structures (100% yolk). Stage 2 was identified when the yolk was pulled from the outside membrane leaving a translucent space (70% yolk). At stage 3, small chromatophores are visible (30-50% yolk). Stage 4 had obvious chromatophores and the beginning of an eyespot (25% yolk). By stage 5 the fully developed larva is visible with a complete eyespot (10% yolk).

In the field survey the number of ovigerous females of *H. takanoi* increased rapidly at the end of May (Figures 6.2 & 6.4). A total of 1543 adult, female crabs were surveyed rangingeach week from 56–352 crabs. The first ovigerous crab was found on 4 May and was carrying stage 1 eggs. Comparing these results to the mean water temperatures in the Oosterschelde, it appears that broods were not laid until the water temperature had been about 15°C for almost 20 days (Figure 6.2).

Brood cycle

The incubation period in the experimental crabs differed significantly between the different treatment temperatures ($F_{2,26}$:3202.946, P<0.001). Incubation was shortest at 24°C, lasting a mean of 17.6 days (± 3 days). At 18°C incubation lasted a mean of 27.9 days (± 3 days). At 12°C a mean of 86.2 days (± 3 days) (Figure 6.3).

There were significant differences in incubation period at different temperatures for all brood stages (Table 6.1). The mean percentage of total incubation time for each stage was relatively similar between temperatures (Table 6.2; Figure 6.4).

During the field survey at the beginning of the breeding season females carrying stage 1 broods were always most numerous, even with a sharp decline in numbers found on 14 June (Figure 6.5). Females carrying stage 2 broods were the second most numerous at all times. Females carrying stages 4 and 5 were the next most numerous, generally found in similar numbers, while females carrying stage 3 broods were the rarest.

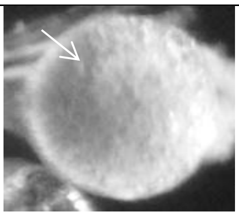
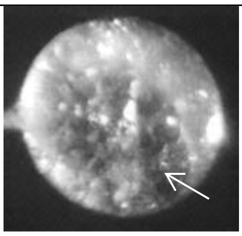
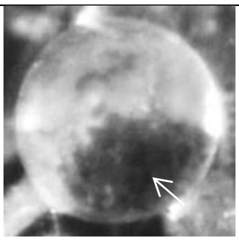
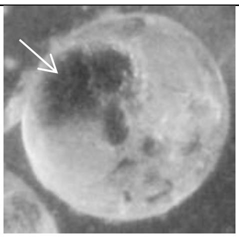
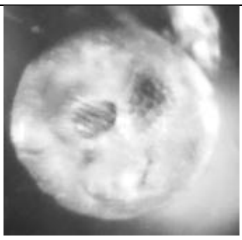
Stage	Description	Image
1	100% bright yellow-green yolk, no other structures obvious	
2	75% yolk	
3	30-50% yolk with development of chromatophores	
4	25% yolk with more obvious chromatophores and developing eye spots	
5	10% yolk with prominent eye spots – a fully developed zoea ready to hatch.	

Fig. 6.1. Description and illustration of the five stages of egg development in *H. takanoi*. Arrows indicate the egg yolk.

Interbrood interval

In the experiment five females (19%) were observed to lay a second brood in the 24°C tank, and only two (7%) laid a second brood in the 18°C tank, while none produced a second brood in the 12°C tank. The interbrood interval in the 24°C tank lasted an average of five days, while in the 18°C tank both observed interbrood intervals lasted six days. The second broods were not monitored until hatching, but the data suggests similar development time as the first brood.

Following the hatching of the first brood ecdysis occurred in eight females in the 24°C tank after an average of 22 days while in the 18°C tank ecdysis occurred in four females after an average of 34.5 days. The average moult increment was 12.61% but sample sizes were too small to discern any effect of temperature on moult increment. Two crabs in the 24°C tank moulted following the second brood after 21 and 19 days. During the three month experiment, no second moult was observed, and no brood was laid after ecydsis at any temperature.

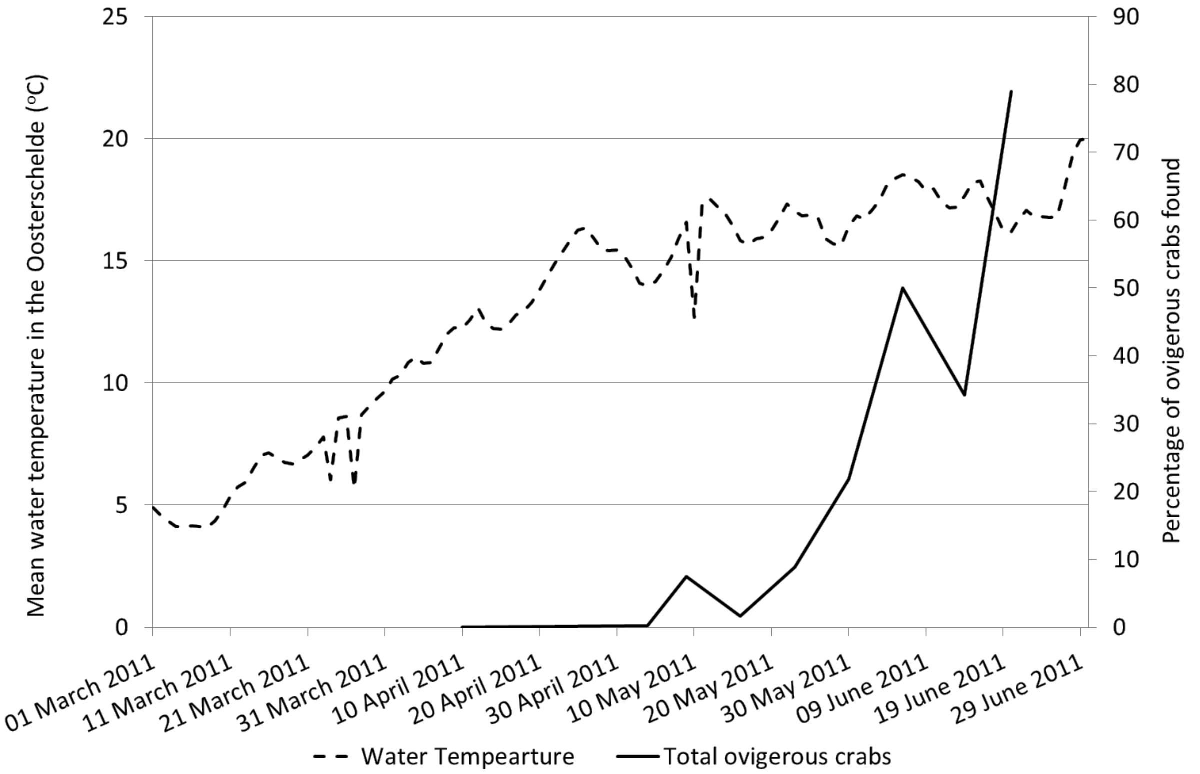


Fig. 6.2. The percentage of females that were ovigerous found in the field between 10 April and 20 June (right y-axis) and the water temperature taken at Marollegat in the Oosterschelde between 1 March and 30 Jun 2011 (left y-axis, data provided by the Rijkswaterstaat: Hydro Meteo Centrum; http://www.hmcz.nl/).

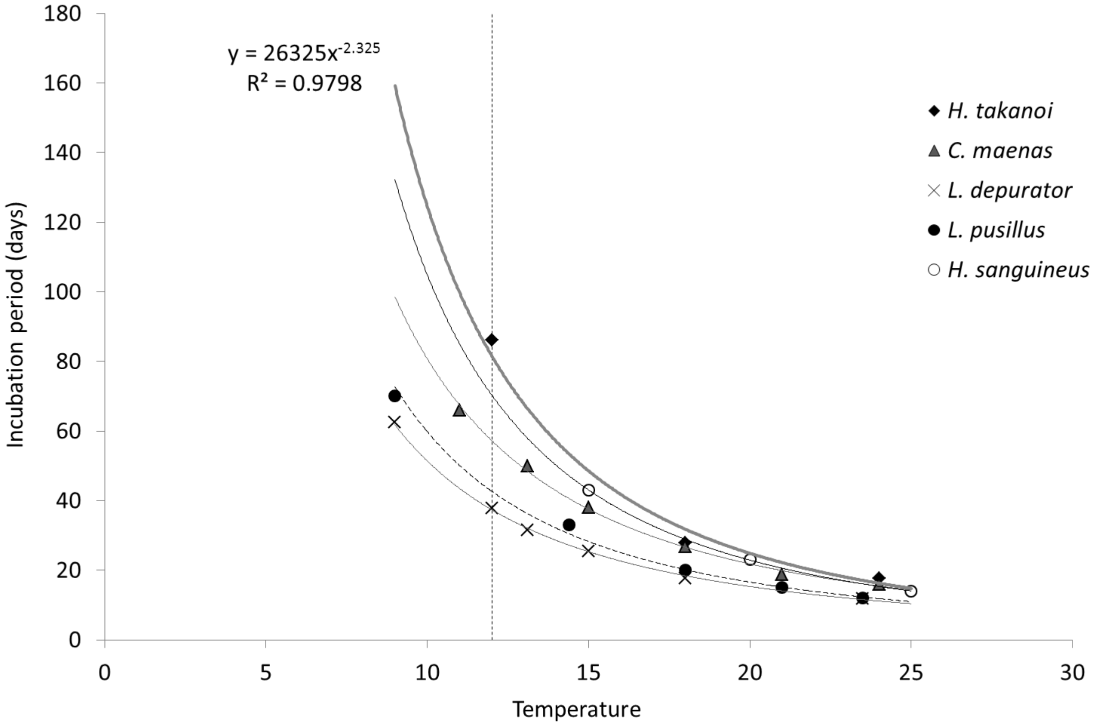


Fig. 6.3. Mean incubation time (days) at different temperatures from the current study for *Hemigrapsus takanoi* compared with data from Wear (1974) for *Carcinus maenas*, *Liocarcinus depurator* (as *Macropipus depurator*) and *L. pusillus* and from Fukui (1988) for *H. sanguineus*. The equation shown is for *H. takanoi*.

There were significant differences in incubation period at different temperatures for all brood stages (Table 6.1). The mean percentage of total incubation time for each stage was relatively similar between temperatures (Table 6.2, Figure 6.4).

During the field survey at the beginning of the breeding season females carrying stage 1 broods were always most numerous, even with a sharp decline in numbers found on 14/06 (Figure 6.5). Females carrying stage 2 broods were the second most numerous at all times. Females carrying stages 4 and 5 were the next most numerous, generally found in similar numbers, while females carrying stage 3 broods were the rarest.

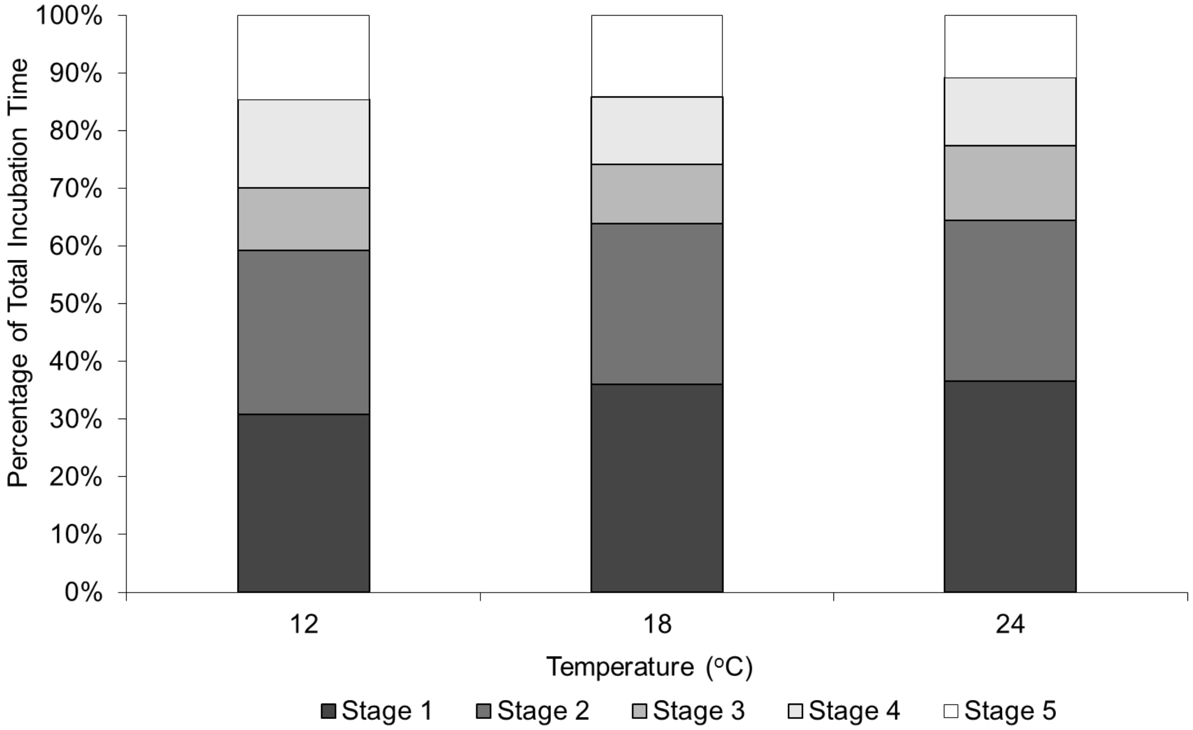


Fig. 6.4. Each brood stage as a percentage of the entire brood cycle for *Hemigrapsus takanoi* at different temperatures.

Table 6.1. Results of the one-way ANOVA for the duration of each brood stage at different temperatures *for Hemigrapsus takanoi.*

Brood stage	**F**	**P**
1	$F_{2,31}$=131.203	<0.001
2	$F_{2,31}$=19.036	<0.001
3	$F_{2,30}$=40.309	<0.001
4	$F_{2,29}$=229.075	<0.001
5	$F_{2,27}$=55.379	<0.001

Table 6.2. The proportion of total embryonic development time for *Hemigrapsus takanoi* at different water temperatures. Numbers in brackets are the average number of days).

Brood stage	**12°C**	**18°C**	**24°C**
1	30% (26.8)	37% (11.75)	37% (7.2)
2	28% (24.8)	28% (9.1)	28% (5.5)
3	10.7% (9.3)	10.2% (3.3)	13% (2.6)
4	15.3% (13.3)	11.7% (3.8)	11.7% (2.3)
5	14.6% (12.7)	14.1% (4.6)	10.8% (2.1)

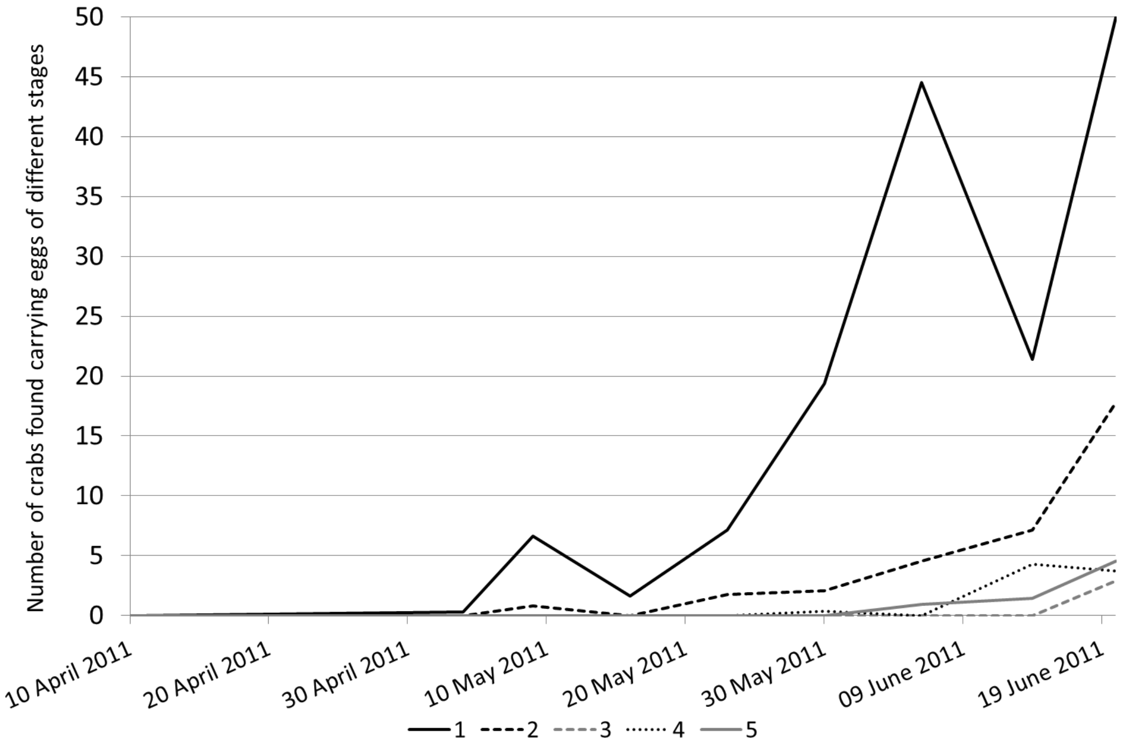


Fig. 6.5. The percentage of female *Hemigrapsus takanoi* found carrying eggs of different stages between 10 April and 20 June 2011.

DISCUSSION

Water temperature had an obvious effect on both the time to lay and the duration of brood development in *Hemigrapsus takanoi*. At 12°C females took three times longer to lay eggs (almost 32 days) than at 18°C (11 days) and five times longer than at 24°C (8 days). The total incubation time at 12°C (86 days) was also almost three times longer than at 18°C (28 days) and almost five times longer than at 24°C (18 days). The longer delay in laying eggs in the 12°C tank may have been a result of temperature shock after the crabs were removed from the field where temperatures were warmer (ranging between 14-16°C at the time of collection), and placed in cooler water in the lab. This difference in time to lay eggs and decrease in incubation time with increased temperature is due to an increase in the speed of metabolic processes with increased temperature (Leffler, 1972). These results are comparable to the similar species *H. sanguineus* (Fukui, 1988) (Figure 6.3). Obviously a controlled temperature and emersion is not the same as the natural environment, which fluctuates in temperature and tidal heights, however it does provide a basis for comparison of the effect of different temperatures on the Net Reproductive Rate (R_0) for the embryonic life stage of this species.

Comparing the brood development time in *H. takanoi* with those of other crab species present in the Oosterschelde, *Carcinus maenas, Liocarcinus depurator* (Linnaeus, 1758) and *L. pusillus* (Leach, 1816) (as *Macropipus*) (see Wear (1974) and *H. sanguineus* (De Haan, 1835) (see Fukui, (1988) showed that *H. takanoi* and *H. sanguineus* had similar incubation periods at different temperatures, but at temperatures below 20°C the incubation periods were particularly longer than the other three species (Figure 6.3). The incubation period at 12°C for *H. takanoi* was considerably longer than the extrapolated incubation period of the other species at 86.2 days compared to 70 days for *H. sanguineus*, 57 days for *C. maenas*, 37 days for *L. depurator* and 43 days for *L. pusillus*.

Each of the five stages of brood development also decreased in duration with increased temperature, but remained similar in terms of proportion of total development time at all temperatures, suggesting that the duration of each stage of development was affected by temperature in the same way. This is consistent with the effect of temperature on the five brood stages of three hymenosomatid species: *Halicarcinus cookii*, *H. varius* (Dana, 1851) and *H. innominatus* Richardson, 1949 (see Van den Brink et al., 2011).

The length of each stage of brood development is somewhat comparable to the number of crabs found carrying each brood stage in the field at the beginning of the breeding season. Stage 1 was always the longest in duration and females carrying stage 1 broods were most commonly found. Likewise stage 2 was the next longest stage at all temperatures and was also the second most commonly found in the field. Stages 3, 4 and 5 differed in their proportion of total incubation period with different temperatures, and were found in similar numbers in the field. Although stage 3 was the rarest found in the field, in the lab it was the shortest brood stage only in the 12°C tank.

In the field survey crabs were not found carrying eggs until the water temperature had risen to about 15°C for almost 20 days. This temperature of about 15°C may therefore be a trigger for the seasonal ovary development after which eggs are laid and the reproductive season begins. This is also supported by the much smaller number of crabs (nine, 33%) to have laid eggs in the 12°C tank compared to the 18°C (18, 66%) and 24°C tank (21, 77%). As the water temperature in the Oosterschelde had reached 15°C almost 20 days prior to collection, the nine that did lay eggs in the 12°C tank may have already begun ovary development while still in the field.

In 2010 the water in the Oosterschelde reached temperatures above 15°C from late April to mid-October; approximately five and a half months according to the data from Rijkswaterstaat. If 15°C is the temperature at which *H. takanoi* can successfully reproduce, the current breeding period of *H. takanoi* in the Oosterschelde is therefore likely to be about five months long; from May to September. This is consistent with the breeding season of the similar species, *H. sanguineus* whose breeding season covers the same months (Pillay & Ono, 1978, McDermott, 1991, Epifanio et al., 1998, McDermott, 1998, Dauvin et al., 2009).

A rise in average water temperatures may result in the reproductive season of *H. takanoi* beginning earlier in the year and lasting longer than they currently do. McDermott (1998) found that the breeding period of *H. sanguineus* along the Atlantic coast of the United States is shorter in the northern (cooler) parts of its range than in the southern (warmer) parts where temperatures increase earlier and decrease later in the year. Fukui (1988) reported that the water temperature in Tanabe Bay, Wakayama Prefecture, Japan, which is almost always above the proposed breeding trigger of 15°C, allows the breeding season to continue between February and November for both *H. sanguineus* and *H. penicillatus* (Fukui, 1988).

In the 24°C tank, five females (19%) laid a second brood compared to two (7%) in the 18°C tank and none in the 12°C tank, showing that at higher temperatures crabs are more likely to produce a second brood prior to ecdysis, but that even at 24°C the majority of crabs did not lay a second brood. Whether this surprisingly small proportion of second broods is due to insufficient sperm supply, captivity, temperature or something else cannot be answered in the current study and requires further investigation. Nevertheless, it appears that at higher temperatures not only do broods develop faster, but there is a greater chance that more broods are laid per intermoult period. Fukui (1988) calculated that *H. sanguineus* and *H. penicillatus* had 5 - 6 broods per year in the warmer waters (15 - 30°C) of Tanabe Bay, Japan. In New Jersey, USA, which experiences similar water temperatures to the Oosterschelde, *H. sanguenius* produced ‘at least two broods per breeding season’ (McDermott, 1998). On the Opal coast in France, less than 200 km from the Oosterschelde, Dauvin et al*.* (2009) reported that mature *H. sanguineus* females could produce 3 - 4 broods per breeding season. Depending on size, female *H. sanguineus* can produce 40 000 eggs per brood (McDermott, 1998, Dauvin et al., 2009). Given the similarities of the species, it is reasonable to assume that in the Oosterschelde *H. takanoi* similarly capable of producing four broods of 40 000 eggs each per breeding season if conditions allow.

These results are important when considering the Net Reproductive Rate (R_0) of the species, and the effect of temperature thereon. However, other factors must also be taken into account with respect to the R_0 of a crab species, specifically growth and mortality. Further research would shed more light onto these aspects for *H. takanoi*.

Growth affects Net Reproductive Rate in crabs because the lifecycle of crabs is divided between growth and reproduction. Hartnoll (1985) described how some crabs grow until a pubertal moult after which they continuously reproduce, but no longer grow (determinate growth) (e.g. Van den Brink & McLay, 2010), while others interchange growth and reproduction, producing offspring between each moult (indeterminate growth) (e.g. Berrill, 1982). *Hemigrapsus takanoi* has indeterminate growth and must stop reproducing to moult and grow. During the experiment 10 females were observed to moult (with an increase in size of about 13%) after an average of 22 days in the 24°C tank compared to four females in the 18°C tank after an average of 34.5 days, suggesting that the speed of growth increases with increased temperature. In increased water temperatures, it is possible that the crabs will also grow faster, experiencing the moult–reproduction cycle more times per year, and therefore be able to reproduce more often in a summer season and increase their R_0.

Mortality is also a major influence on the R_0 of a species since whether an individual survives to reproductive age, and how long an individual survives at reproductive age obviously has an effect on the number of offspring that individual can produce during its lifetime. The planktonic stages of crustaceans are particularly vulnerable to predation and changes in environmental conditions. The longer larvae remain in the plankton, the higher chance of mortality. As temperature influences development time, larval mortality can therefore also be affected by temperature and influence the R_0. Larval development of the xanthid crab, *Panopeus herbstiil* Milne Edwards, 1834, in colder waters in spring was prolonged until the water warmed to accommodate the megalopa stage, while in the colder waters in autumn the gradually cooling water temperatures would not favour newly hatched larvae and mortality in the late zoeal and megalopa stages would be extremely high (Costlow et al., 1962). Similarly, mortality of Zoea 1 stages of *Cyrtograpsus altimanus* Rathbun, 1914 and *C. angulatus* Dana, 1851 was significantly lower at 20°C than at 15°C (Moresino & Helbling, 2010).

Mortality of juvenile and adult crabs can also be influenced by temperature. Leffler (1972) found that mortality in juvenile blue crabs, *Calinectes sapidus* Rathbun, 1896, was directly proportional to temperature between 13 and 34°C and was very high during ecdysis at elevated temperatures. He suggested that this is due to the elevated metabolic rate increasing O_2 consumption in higher temperatures where O_2 is more limited. Mortality during ecdysis is especially high because O_2 consumption increases greatly during moulting, even doubling in *Pachygrapsus crassipes* Randall, 1839, compared to normal rates (Roberts, 1957). Death usually results from asphyxiation, because the respiratory surfaces do not function properly during the moult (Passano, 1960). The effect of temperature on the metabolic rate and mortality of *Hemigrapsus takanoi* at different life stages requires further investigation before conclusions about its effect can be made.

The results suggest that the current temperature regime of the Oosterschelde is perhaps slightly below the optimal range of the Net Reproductive Rate (R_0) for *H. takanoi*. An increase in water temperature may shift the temperature regime closer to the optimal range for *H. takanoi* and result in increased R_0 and consequently increased population growth. Furthermore, if temperatures rise, locations where *H. takanoi* breeding is currently inhibited due to low temperatures may eventually become more vulnerable to an invasion and establishment of the species. The current distribution of *H. takanoi* on the Atlantic coast of Europe is not precisely known, but according to Dauvin (2010) the species has been reported from the northern coast of Spain to the southern coasts of Denmark. In his study Dauvin (2010) presented the distribution of *H. takanoi* as that of *H. pencillatus*, most likely because the two were not known as separate species until 2005 (Asakura & Watanabe, 2005), and recent research has suggested that only *H. takanoi* is present in Western Europe (Asakura and Watanabe, 2005, Yamasaki et al., 2011). Assuming that this distribution is limited by temperature and if global temperatures rise, *H. takanoi* may spread to the northern coasts of Denmark and southern coasts of Norway and Sweden and perhaps even further north.

ACKNOWLEDGEMENTS

Many thanks to IMARES for providing the means to conduct the experiment. Thanks to Martine van den Heuvel-Greve, Bart van den Heuvel and Mare and Nanne van den Heuvel for their help collecting crabs for the pilot stage of the experiment, and special thanks to Jonathon Hutchens for his 'motivational encouragement' and help in the lab during weekends and holidays.

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Chapter 7:

Competition and niche segregation following the arrival of *Hemigrapsus takanoi* in the formerly *Carcinus maenas* dominated Dutch delta.

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Journal of Sea Research 2012, 73:126-136

ABSTRACT

In a combined study including a 20 year monitoring programme of the benthic communities of four Dutch delta waters and a snapshot survey conducted in the Oosterschelde tidal bay in 2011, the populations of the native portunid European shore crab *Carcinus maenas* and the introduced varunid crabs *Hemigrapsus takanoi* and *Hemigrapsus sanguineus* were investigated. Whereas *C. maenas* was the most common shore crab in these waters, its numbers have declined on the soft sediment substrates during the last 20 years. As the two exotic crab species were first recorded in the Dutch delta in 1999, they could not have initiated the decline of the native *C. maenas*. However, within a few years *H. takanoi* completely dominated the intertidal hard substrate environments; the same environments on which juvenile *C. maenas* depend. On soft sediment substrate the native and exotic shore crab species are presently more or less equally abundant. *H. takanoi* might initially have taken advantage of the fact that *C. maenas* numbers were declining. Additionally *H. takanoi* are thriving in expanding oyster reefs of *Crassostrea gigas* (Pacific oyster) in the Dutch delta waters, which provide new habitat. Nowadays *H. takanoi* appears to be a fierce interference competitor or predator for small *C. maenas* specimens by expelling them from their shelters. These interactions have led to increased mortality of juvenile *C. maenas*. At present the *C. maenas* populations seem to be maintained by crabs that survive and reproduce on available soft sediment habitats where H. takanoi densities are low.

INTRODUCTION

The introduction of nonindigenous marine organisms can alter an environment and the communities of species therein (Carlton and Geller, 1993; Cohen and Carlton, 1998; Jensen et al., 2002). Introduced species may have an impact on native species through predation, associated parasites or diseases, as fouling organisms or as competitors for food or space (Jensen et al., 2002). While direct predation is likely to have the most obvious impact, increased predation risks due to the presence of nonindigenous species can also be important. Competition for space may result in the displacement of a native species to areas with less shelter and protection from predators, and thus indirectly increase the risk of predation (Jensen et al., 2002).

Several studies have shown that spatial heterogeneity of habitats with rocks, shells and vegetation provide protective refuges from aquatic predators for many species of decapod crustaceans (Fernandez et al., 1993; Heck and Thoman, 1981; Moksnes et al., 1998; Navarrete and Castilla, 1990; Pillay and Ono, 1978). When shelter opportunities are not sufficient, the population growth for these decapods can be limited by predation (Wahle and Steneck, 1991) or regulated by cannibalism (Moksnes, 2004). These spatial refuges are of particular importance in the intertidal zone where shorebirds and other terrestrial predators pose an added predation pressure along with environmental stressors such as extreme temperatures (Abele et al., 1986; Taylor, 1981), desiccation (Grant and McDonald, 1979) and strong water currents or wave action (O'Neill and Cobb, 1979).

Shelter is of such importance in the intertidal zone that its increase can greatly influence the resident crustacean communities. An experiment in Japan where the number of rocks was doubled a given intertidal area resulted in a four-fold increase in the density of the shore crab *Hemigrapsus sanguineus* (Lohrer et al., 2000). The number of juvenile Dungeness crabs, Cancer magister, occupying the intertidal area was successfully tripled at some locations due to the addition of extra oyster shells to areas of sand and mud in Grays Harbour, Washington State, the United States (Dumbauld et al., 1993; Wainwright et al., 1992). However, the introduction of *Hemigrapsus oregonensis* to that area and the resulting competition for space caused the displacement of many *C. magister* to more exposed habitats, and consequently their numbers greatly decreased (Jensen et al., 2002). Introduced ecosystem engineers can provide novel spatial heterogeneity, which may facilitate the establishment and success of other introduced species (Floerl et al., 2004; Wallentinus and Nyberg, 2007). The invasive Pacific oyster, *Crassostrea gigas* has been shown to increase spatial heterogeneity and consequently its presence in the form of beds results in higher biodiversity compared to areas where *C. gigas* is absent (Troost, 2010). *Crassostrea gigas* is now common throughout NW Europe and has established many oyster beds on the Dutch coast, providing a new habitat for species to occupy (Troost, 2010).

The benthic habitats of delta waters in the Netherlands are predominantly soft bottom sand/mud flats with sporadic areas of mussel and beds of the introduced oyster C. gigas. The waters are also edged by dykes reinforced with rocks and other hard substrata (Bouma et al., 2005). Prior to the 1990s the native European shore crab or green crab, *Carinus maenas*, was the dominant crab species in the Dutch delta. In the Oosterschelde tidal bay (one of the large Dutch delta waters) it was very abundant on hard substrates (De Kluijver and Leewis, 1994) and also the most abundant crab species on soft substrates (Hostens and Hamerlynck, 1994). In the Westerschelde estuary (also situated in the Dutch delta) only *Liocarinus holsatus* was more abundant, but no other decapod crabs reached the densities of *C. maenas* (Hostens and Hamerlynck, 1994). In the 1990s two *Hemigrapsus* species; *H. sanguineus* and *H. takanoi* (originally identified as *H. penicillatus* as it was not classified as a separate species until 2005; Asakura and Watanabe, 2005) were introduced into European waters. *Hemigrapsus takanoi* most likely arrived in the Netherlands via oysters transported from France (Faasse et al., 2002; Nijland, 2000; Nijland and Beekman, 2000) while *H. sanguineus* may have arrived as larvae from established French colonies via ballastwater resulting in secondary colonization (J.-C. Dauvin, personal communication). The first specimens of *Hemigrapsus* (most likely *H. takanoi*) were observed in the Oosterschelde tidal bay in the year 2000, but probably arrived earlier in 1999. A year later the species was also present in the Westerschelde estuary and within the following few years *H. takanoi* could be found throughout the Dutch delta (Wolff, 2005). *Hemigrapsus sanguineus* was also first observed in the Oosterschelde in 1999 (D'Udekem d'Acoz and Faasse, 2002), but after that this species seems to have spread less abundantly at least in the Dutch delta waters as recordings are restricted to the western shores. On the outer shores; the North Sea coasts, the species is more successful (Dauvin, 2009; D'Udekem d'Acoz, 2006).

The current study investigates the distribution, abundance and population development of *H. takanoi* after its arrival in the Dutch delta waters and the Oosterschelde in particular. By comparing distribution data of surveys conducted on the soft sediment prior to and following the introduction of *H. takanoi*, and using the results of a ‘snapshot’ survey of the hard substrate, potential impacts on the *Carcinus maenas* populations in the Dutch delta waters are investigated. By way of explanation for the long-term changes we focus on competition, replacement and changes in habitat use for the species.

MATERIALS AND METHODS

The Delta waters

The region of the Dutch delta waters is located where the rivers Scheldt, Rhine and Meuse reach the North Sea (Fig. 7.1). The Dutch delta consists of five water bodies, from south to north: the Westerschelde estuary, Lake Veerse Meer, the Oosterschelde tidal bay, Lake Grevelingen and Lake Haringvliet. The Westerschelde estuary is the mouth of the river Scheldt and is still in open connection with the North Sea. The other water bodies used to drain the Rhine, Meuse and also the Scheldt, but have been partially closed off from river inputs and exchange with the North Sea by a coastal engineering project; the ‘Delta project’, which was initiated in the 1950s (Troost, 2009; Wijnhoven et al., 2008). Since it was dammed off from the Oosterschelde estuary in the east and the North Sea in the west, Lake Veerse Meer used to be a brackish lake with a differing summer and winter water level. Recently (in 2006) the connection with the Oosterschelde has been restored which made the lake saline again (Wijnhoven et al., 2010). The Oosterschelde estuary was largely closed off from river inputs by compartmentalisation dams and locks. A storm surge barrier, which can be closed in times of dangerously high water levels and surges, was built in the mouth of the estuary creating a tidal bay. This has reduced the tidal amplitude and current velocities in the system (Troost, 2009). Lake Grevelingen used to be an estuary, but since it was dammed off from the North Sea and from river inputs, it is now a brackish lake and later gradually a saline lake (Troost, 2009). Although the Haringvliet still discharges water from the rivers Rhine and Meuse, it is now a freshwater lake after it was closed off from the North Sea by a dam and sluices (Wijnhoven et al., 2008), and can therefore be excluded from the current study as the species under investigation are not present there.

All the delta waters have predominantly soft sediment substrate in the form of sand and mud. However, due to the building of dykes, their reinforcement with rocks (Bouma et al., 2005) and the introduction of the pacific oyster, *Crassostrea gigas*, in the 1960s (Troost, 2009) rocks, shells and other hard substrata provide substantial areas with shelter refuges in specific areas for otherwise vulnerable species.

Life histories

Hemigrapsus takanoi

Although there is limited information about the life history of *H. takanoi*, and larval phases have not yet been described (Yamasaki et al., 2011), the similarities between the species and the more commonly documented *H. penicillatus* suggest that their life histories are probably similar. Assuming this; *H. takanoi* females can become ovigerous at about 6–7 mm CW and can lay broods several times per year during the summer months (Pillay and Ono, 1978; Van den Brink et al., 2012). The duration of brood and larval development is highly dependent on water temperature, but the crabs can go through all larval and juvenile instar stages until they reach maturity after several months (J.-C. Dauvin, personal communication). The species predominantly inhabits intertidal areas of mudflats, estuaries and lagoons with sufficient shelter opportunities, typically among rocks and boulders, but can also be found in soft sediment and occasionally in subtidal regions (Asakura and Watanabe, 2005). They have a preference for low hydrodynamic muddy habitats (Dauvin, 2009).

Hemigrapsus sanguineus

The females of *H. sanguineus* can reach maturity at sizes as small as 10.6 mm CW (Dauvin and Dufossé, 2011) and also breed several times a year during the summer months (Epifanio et al., 1998). The larvae hatch and develop in the plankton until they reach the megalopa stage and settle. They then develop through five juvenile instar stages (Epifanio et al., 1998) and reach maturity at about 7.5 months old (Epifanio et al., 1998). *Hemigrapsus sanguineus* inhabits shallow hard-bottom intertidal and sometimes subtidal habitats where they live on artificial structures, on mussel beds and oyster reefs and any habitat with shelter opportunities (McDermott, 1998). They typically can be found in high hydrodynamic habitats with fine and medium coarse sands (Dauvin, 2009).

Carcinus maenas

Female European green crabs, *Carcinus maenas,* can reproduce after their pubertal moult at a minimum size of about 34 mm carapace width (CW) (Berrill, 1982). They lay their eggs in spring and after an interval depending on water temperature (Wear, 1974) the larvae hatch into the water column where they develop through four zoeal stages and one megalopa stage before settling as a first instar juvenile crab (Dawirs, 1985). After about seven instars the juveniles mature at about 2–3 years old depending on location and temperature range (Berrill, 1982). The species inhabits coastal waters and intertidal environments, where juveniles can typically be found and adults also inhabit shallow subtidal waters up to 30 m deep. They occupy a variety of habitats from rocky shore, areas with boulders beds or oyster reefs to sand- or mudflats (Almeida et al., 2011; Amaral et al., 2009).

Soft substrate monitoring

The present study uses the results of a large scale long term monitoring of the macrobenthic communities of the Dutch delta waters executed within the frame of the Dutch water systems monitoring program ‘MWTL’. Sampling has taken place twice a year, in spring and autumn, according to a standardized methodology from 1990 to 2010 using ‘Reineck’ box-corers (from which cores are taken manually) for subtidal areas in the Oosterschelde and the Westerschelde and at depths below−2 m(relative to the Dutch

Ordnance Level; NAP) in Lake Grevelingen and Lake Veerse Meer, 'flushing samplers' for the 0–2 m stratum in the two lakes, and using hand corers in the intertidal areas (Oosterschelde and Westerschelde). The dataset consists of 13,600 samples with a sampled surface of 0.020 m2 for the 'flushing' samples and 0.015 m2 for the box- and hand corer samples, sampled to an approximate depth of 30 cm in the sediment and sieved over a 1 mm mesh to collect the organisms. Ten samples were taken in each year × season × region × stratum combination in each water body during the period 1999–2010. During the period 1992–1998, 20 samples were taken for each combination, and an additional 5 (Western Scheldt) or 15 (Eastern Scheldt) samples were taken during the period 1990–1991 for each combination (see distribution of sample sites in Fig. 7.1). Lake Grevelingen and Lake Veerse Meer were sampled for the first time in autumn 1990 while the Oosterschelde and the Westerschelde were first sampled in spring 1990, with data available up to, and including autumn 2010. All water bodies were sampled according to a stratified random sampling scheme till 1995 when the random sample sites became fixed sites sampled every year, except for the Westerschelde where samples are still taken randomly in every campaign. During the surveys all *C. maenas* and *Hemigrapsus* sp. individuals collected were measured for their carapace width (CW) to the nearest mm. Additionally the sediment type at each sample location was recorded so that it could be determined whether hard elements (i.e. shells, stones) were present. As the monitoring programme focuses on soft sediment communities, oyster reefs and commercial mussel plots were not sampled. Due to the relatively small areas sampled, the densities of larger crab specimens might be somewhat underestimated by this methodology.

Observed numbers of crabs were initially calculated into average densities on soft sediment substrates for entire water bodies (i.e. Grevelingen, Oosterschelde, Veerse Meer and Westerschelde). As this study focuses on potential *C. maenas–H. takanoi* interactions, the size frequency distributions of the two species (on the basis of the total numbers per 5 mm size classes) were compared between water bodies and periods. The entire research period was therefore divided into three, taking the settlement history of *H. takanoi* into account. The years 1990–1998 without *H. takanoi* in the Dutch delta, the years 1999–2004 during which *H. takanoi* was present particularly on hard substrates, but densities were very low or the species was lacking on soft substrates (given that the species was not found during the soft sediment monitoring programme), and the years 2005–2010 during which *H. takanoi* was frequently observed on soft sediment, were distinguished. Due to the very low numbers of *C. maenas* (and the absence of *H. takanoi*) during the first two periods in Lake Veerse Meer, which was brackish at that time, the results of those periods were combined. Potential significant differences (at pb0.05) in relative abundance of size classes between periods were tested by using t-tests (taking variance into account; i.e. by F-testing). To compare the number and distribution of locations where *C. maenas* was found before and after the arrival of *H. takanoi* in the soft sediment samples, periods of the same length with similar numbers of samples taken were distinguished; i.e. 1997–2003 and 2004–2010.

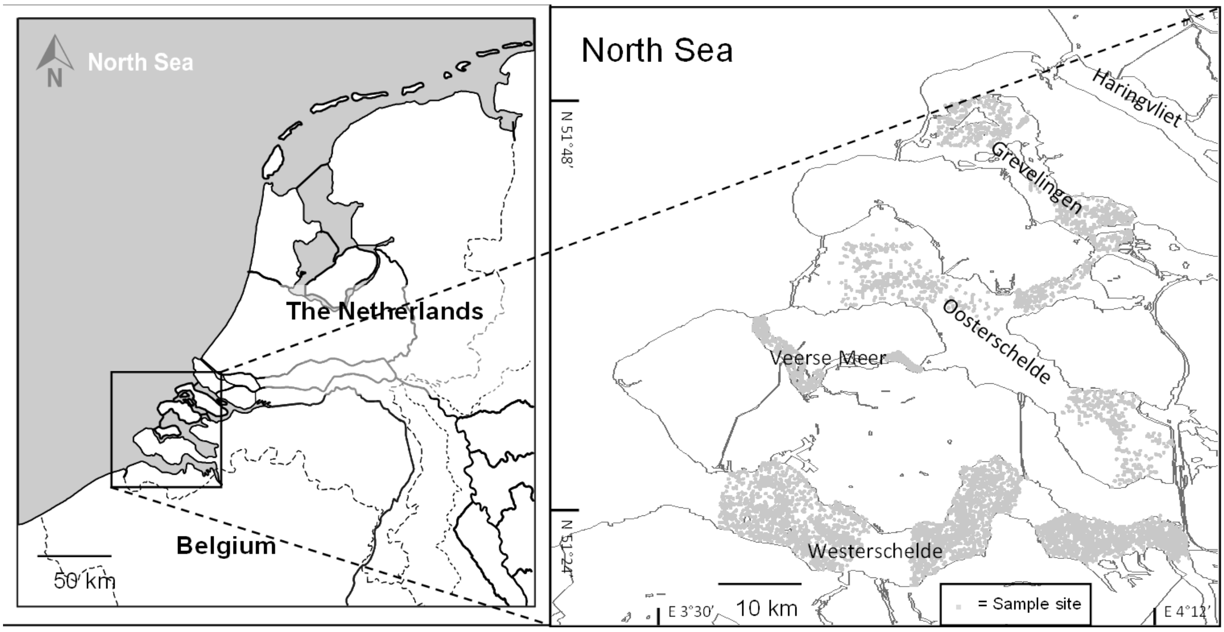


Fig. 7.1. Location of the Dutch delta and the various water bodies in the Netherlands with indication of the sample sites on soft sediment during the 1990-2010 research period.

Hard substrate survey

In May–June 2011 the walls of dykes (foreshore reinforcements) at ten locations in the Oosterschelde were sampled (see Fig. 7.6). While the walls of dykes always contained hard substrata, the substrate types differed between locations, generally with the presence or absence of oyster shells (*Crassostrea gigas*) (Table 7.1). At each location two people collected as many crabs of all species as possible by hand over 1 h (this generally entailed one person lifting rocks and the other collecting crabs as quickly as possible before they escaped). This provided a 'snapshot' of the crab community at a particular point in time. This method may underestimate small individuals as they were harder to catch, particularly in oyster beds.

All crabs were brought to the laboratory where they were determined to species level and their CW was measured. The size and the species proportion of the total number of crabs found was then compared between locations and combined to produce a generalized representation of the crab communities on the hard substrate in the Oosterschelde.

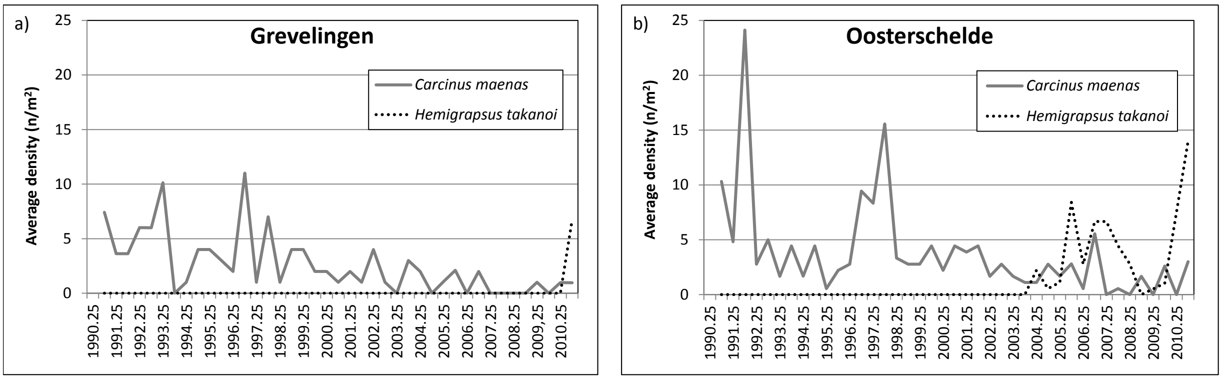
Table 7.1. Substrate types at the ten locations sampled in the Oosterschelde indicated in Figure 7.6 during the hard substrate survey.

Location	**Substrate**
Outside barrier	Rocks, seaweed
Inside barrier	Rocks, oyster shells
Schelphoek	Rocks, oyster shells, debris
Colijnsplaat	Rocks, sand
Viane	Rocks, oyster shells, mud
Goese Sas	Rocks, mud
Gorishoek	Rocks, oyster shells
Yerseke	Rocks, debris, mud
Noordtak	Rocks, sand
Oesterdam	Rocks, sand

RESULTS

Abundance over time

In Lake Grevelingen densities of *C. maenas* showed a significant decrease throughout the 20 year sampling period (Figure 7.2; Table 7.2) although numbers fluctuated. It is only recently (2010) that *H. takanoi* was first observed in Lake Grevelingen, where it was immediately more abundant on soft sediment substrate than *C. maenas*. Whereas *C. maenas* used to be by far the most abundant crab species in the Oosterschelde, from spring 2004 on *H. takanoi* showed up in the samples. From that time on *H. takanoi* was often more abundant than *C. maenas*, although in 2009 it was temporarily the reverse. Taking the whole 20 year period into account, a significant decrease in *C. maenas* densities was found (Table 7.2). In Lake Veerse Meer, *C. maenas* used to be present in low densities as indicated by the occasional presence in the samples (Figure 7.2c). After the opening of the connection with the Oosterschelde, by which salinity increased and *H. takanoi* appeared. Initially, *H. takanoi* densities remained around 5 m−2, but in 2010 they reached almost 80 m−2. In the Westerschelde *C. maenas* used to be and still is the most abundant crab species on soft sediment (Figure 7.2d). *Hemigrapsus takanoi* appeared in the samples for the first time in spring 2007, but was only occasionally found.



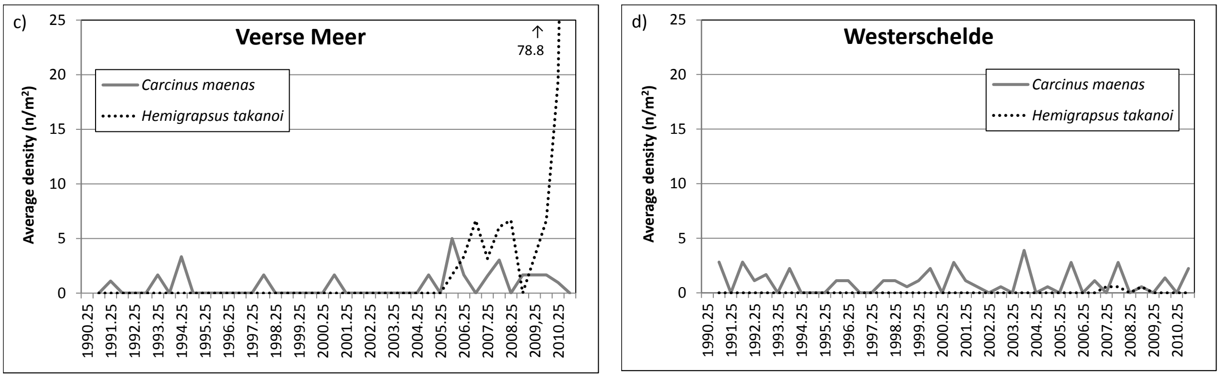


Fig. 7.2. Developments in average densities (n/m2) of *Carcinus maenas* and *Hemigrapsus takanoi* on soft sediment substrates during the period 1990 – 2010 in Lake Grevelingen (a), the Oosterschelde (b), Lake Veerse Meer (c) and the Westerschelde (d)

Table 7.2. Trends in *Carcinus maenas* densities during the research period. Indicated are significant regressions according to; Density = a * Year + b, with R^2-value and significance level p; ns = not significant

Water body	A	B	R^2	p
Grevelingen	-0.026	52.3	0.376	0.000
Oosterschelde	-0.055	110.6	0.210	0.003
Veerse Meer				ns
Westerschelde				ns

Distribution patterns

The presence of the species in samples gives a good view of the distribution of the species over Delta waters during a period before *H. takanoi* colonized soft substrates. *Carcinus maenas* appeared to be widely spread over the entire Grevelingen, Oosterschelde and large parts of the western and central parts of the Westerschelde (Figure 7.3a). It is noteworthy that densities of crabs on soft sediment substrates are quite low, i.e. they are not present in most of the samples. *Carcinus maenas* is for instance found in 2.9% of the samples taken during the period 1997–2003.

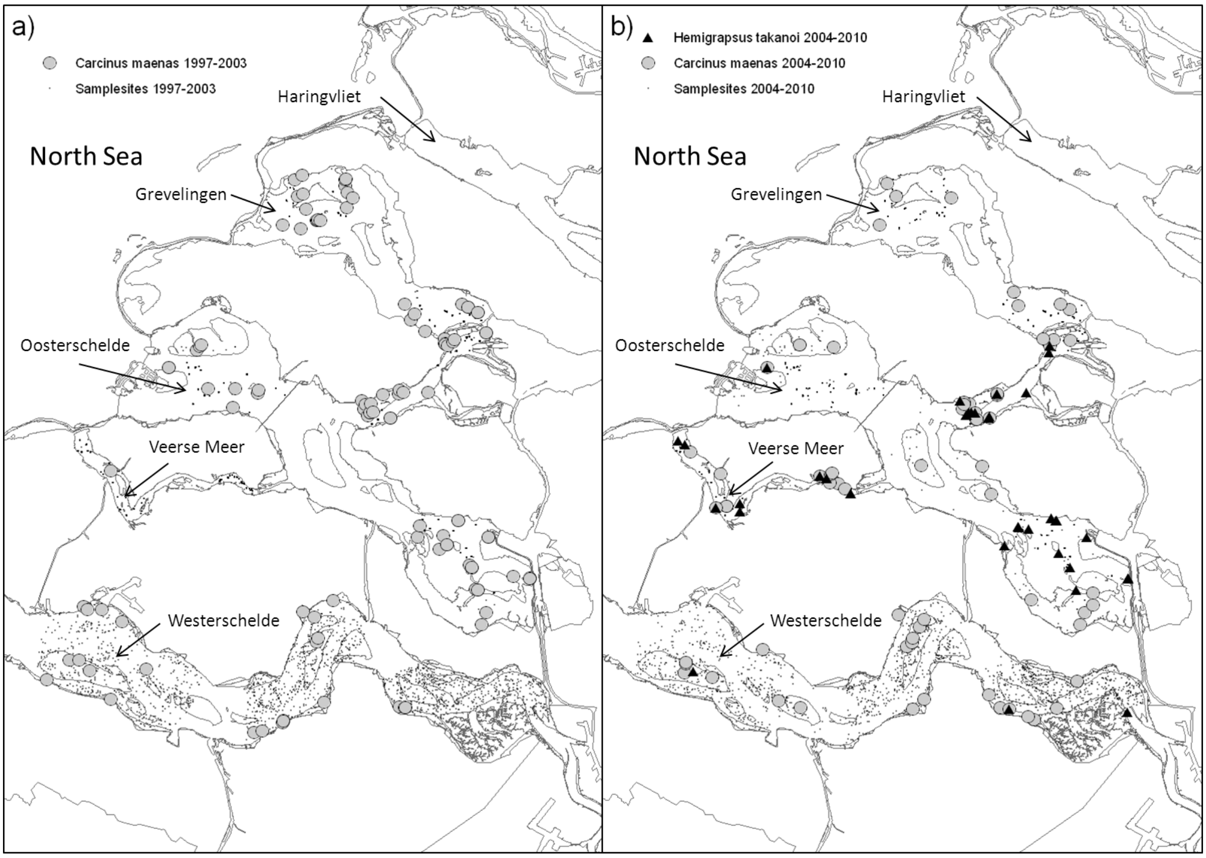


Fig. 7.3. Sites of observation of *Carcinus maenas* and *Hemigrapsus takanoi* for the periods 1997-2003 (a) and 2004-2010 (b) during the monitoring of the soft sediment communities.

Particularly for the Oosterschelde and Grevelingen the chances of observing *C. maenas* in soft sediment samples are about the same for the entire water bodies. For the next 7 years (2004–2010) with about the same sampling intensity, and an even larger area with potentially suitable conditions for *C. maenas* as the Veerse Meer became a salt water lake, *C. maenas* was present in only 1.8% of the samples. In some regions the chances of finding the species are about the same (e.g. most of the Westerschelde area) or larger due to improved conditions (central and eastern parts of Lake Veerse Meer). It is however clear that *C. maenas* is less widespread in large parts of the Grevelingen and the Oosterschelde. During the period 2004–2010, the chance of finding *H. takanoi* in the samples is about 1.5%. The species was particularly found in the Oosterschelde and recently in Lake Veerse Meer. Whereas there is some overlap in the distribution of the two species (*C. maenas* and *H. takanoi*), they were only found together in the same sample twice (once in the Oosterschelde and once in the Westerschelde), which is however just a factor of two lower than can be expected from the encounter rate of the species on soft sediment if the two do not influence each other. For the Oosterschelde alone, observing the two species together only once, is about a factor of 3 lower than can be expected from their separate encounter rates. It is particularly striking that the large decrease of the densities of *C. maenas* in a large part of the eastern Oosterschelde (to levels where the species was not found during 7 years of monitoring) coincides with the appearance of *H. takanoi* in reasonable numbers.

Although with the MWTL programme the soft sediment substrate communities are monitored, the substrate can also contain hard elements like (pieces of) shells and stones. About 11% of the soft sediment substrates of the Dutch delta waters can be characterized as being a substrate containing hard elements. When hard elements are present in the sediment, the chance of encountering *C. maenas* is significantly (t-test after F-test, pb0.05) more than two times higher than on soft sediment without hard elements (Figure 7.4). This pattern was observed during the years without *H. takanoi* in the samples (1990–2003). Now that *H. takanoi* can also be found (2004–2010), *C. maenas* can still be significantly more often encountered on soft sediments with hard elements, where the encounter rate on both classes of substrate has decreased significantly with a factor of 2. In the meantime the encounter rate for *H. takanoi* is significantly higher (t-test after F-test, pb0.05) on soft sediment with hard elements than on soft sediment without hard elements; about a factor of 4. This leads to a pattern that currently in the Dutch delta waters the chance of finding *H. takanoi* on soft sediment with hard elements is about the same as for *C. maenas* (slight differences observed are not significant, see Fig. 7.4).

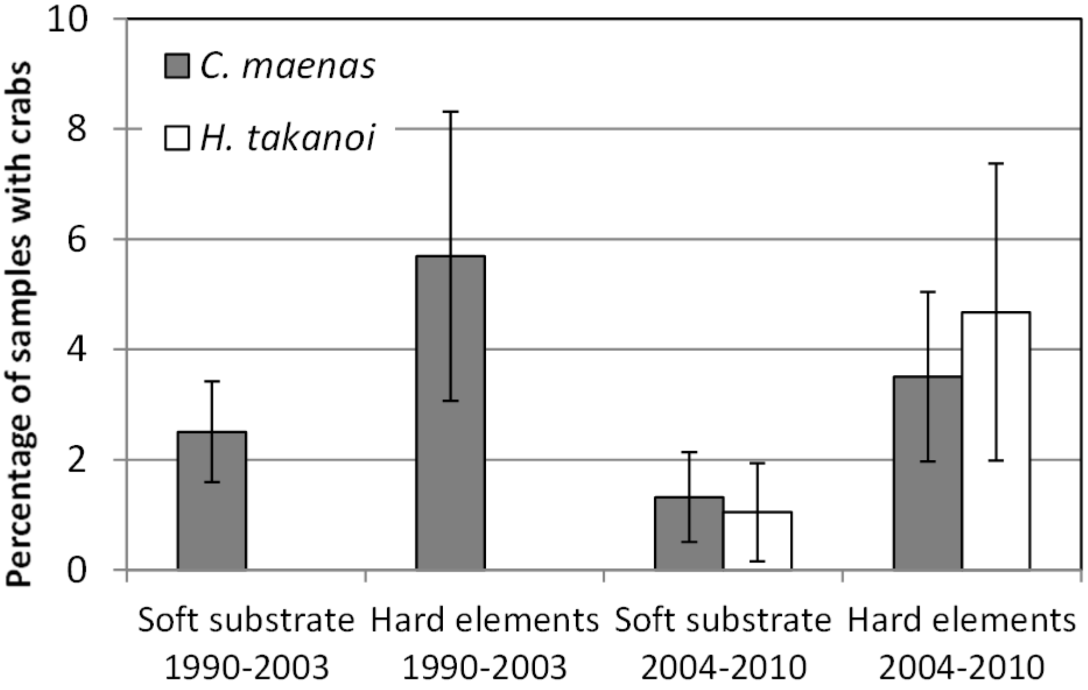


Fig. 7.4. The encounter rate (in%) for *Carcinus maenas* and *Hemigrapsus takanoi* on soft substrate without shells and stones (soft substrate) or on soft substrate with shells or stones (hard elements) during the periods 1990-2003 and 2004-2010 for the entire Dutch delta

Throughout the recent snapshot survey five species of crabs were collected from the hard substrate at the base of the dykes: *C. maenas*, *H. takanoi*, *H. sanguineus*, *Porcellana platycheles* and a single *Liocarinus navigator* (Herbst, 1794) (as these are not relevant to the current study, *P. platycheles* and *L. navigator* are not mentioned further). There were significantly more *C. maenas* found in locations where oysters were present compared to those without oysters, while significantly more *H. takanoi* and *H. sanguineus* were found in locations without oysters compared to those with oysters (K–W test, α is 0.001). Ovigerous *H. takanoi* were found at all locations. Ovigerous *H. sanguineus* were found at all locations in which the species was present, and no ovigerous *C. maenas* were found at any location. At all locations except around the storm-surge barrier *H. takanoi* was by far the dominant species ranging from 81% of the total number of crabs sampled at Schelphoek to 98% of the total number of crabs sampled at Goese Sas (Figure 7.5).

Outside the barrier *H. sanguineus* was the most dominant crab species, comprising 91.5% of the total number sampled. Just inside the barrier the proportions of *H. takanoi* and *H. sanguineus* found were almost equal with 45% and 43% of the total numbers respectively. *Carcinus maenas* was present at all locations in varying proportions of the total number of crabs collected, but was never close to being in the majority. The proportion of *C. maenas* ranged from 0.7% at the Goese Sas to 17.8% at Viane.

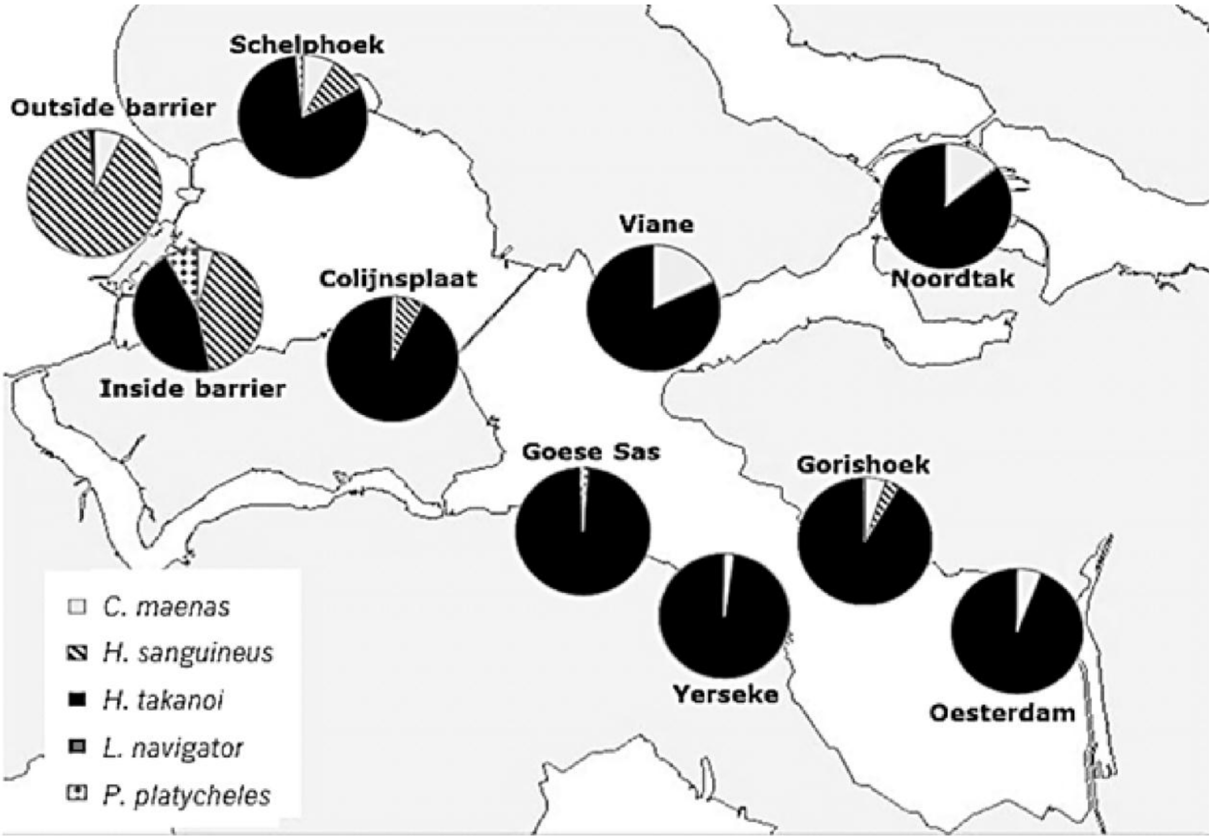
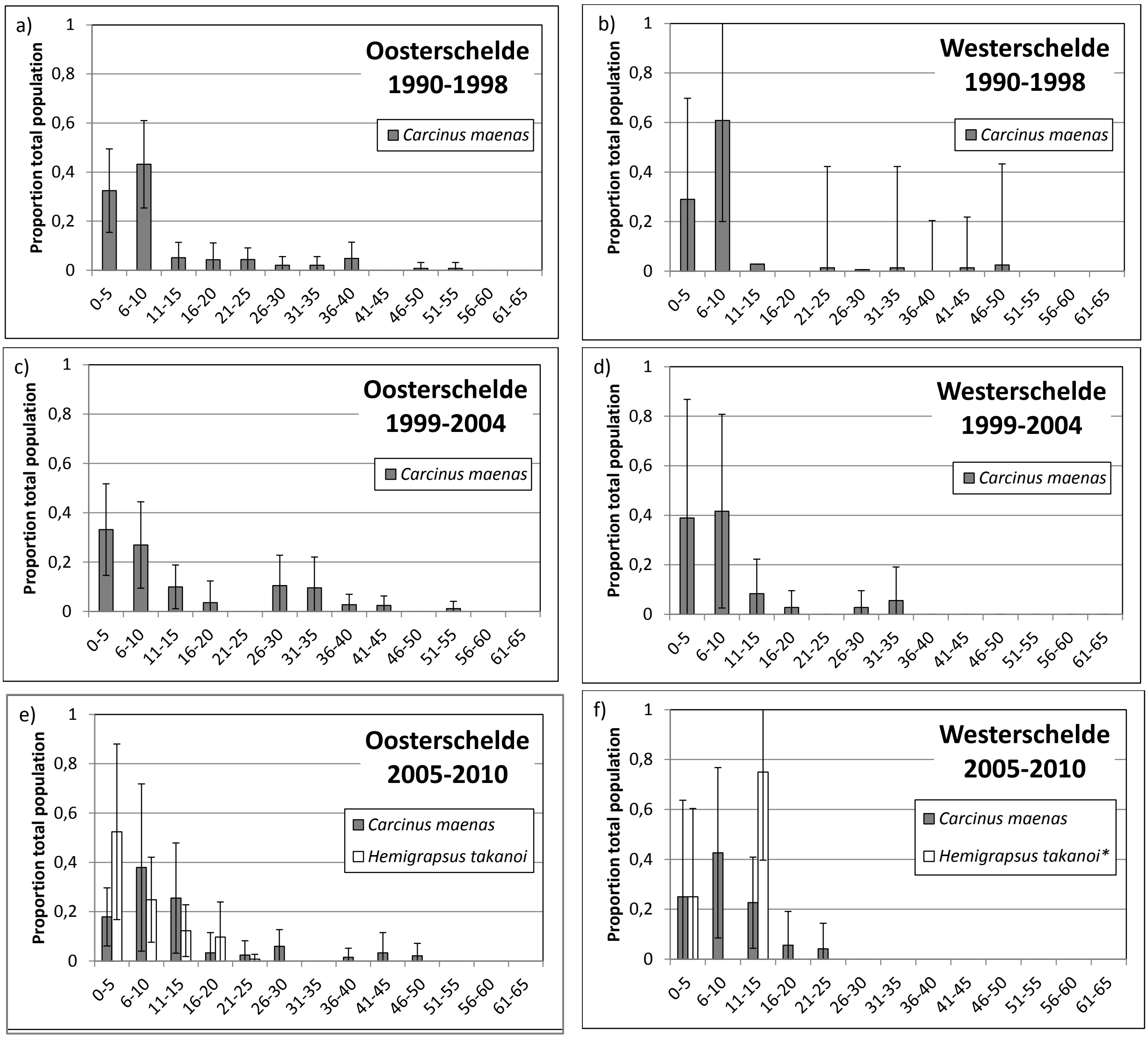


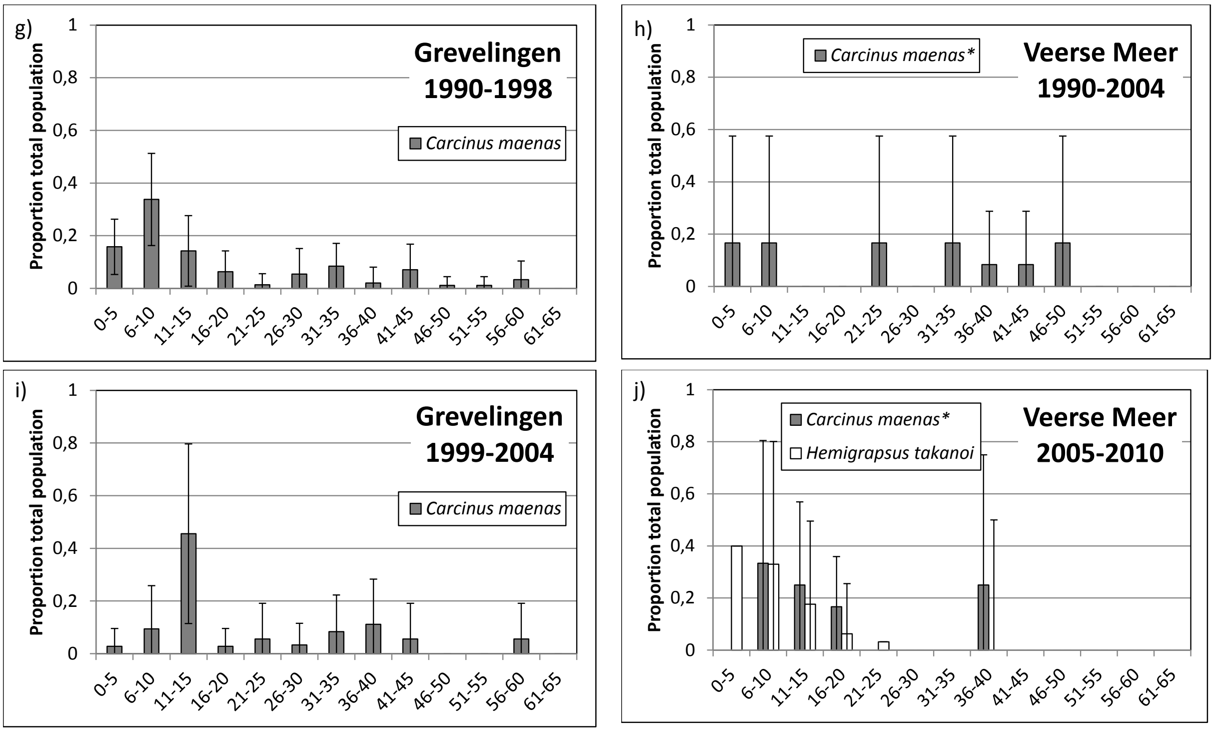
Fig. 7.5. Sampling sites of the hard substrate survey in the Oosterschelde tidal bay in 2011 indicating the species proportions of total number of crabs found.

Size structure

Roughly dividing the research period for the Oosterschelde into three, distinguishing a period without *H. takanoi* (1990–1998), a period with known presence of *H. takanoi* at least on hard substrate, but low densities or absence of the species on soft substrate (1999–2004), and a period with *H. takanoi* also abundant on soft sediment (2005–2010), the size distribution graphs for *C. maenas* only show minor changes (Figure 7.6a,c,e). The relative share of the smaller sized specimens in the total population seems to have decreased (significantly for the 0–5 mm size class between the first and last periods) where a tendency in this direction seems to be apparent when *H. takanoi* was not yet present in the samples from soft sediment. It is particularly this size class that makes up the largest part of the *H. takanoi* populations at present. In recent years the share of the 10–15 mm size class for *C. maenas* has increased (significant difference between the first and last periods). In the Westerschelde, with a similar history concerning the arrival of *H. takanoi*, although densities are much lower so far, a similar pattern seems apparent (Figure

7.6b,d,f) although only the increase in the 11–15 mm size class from the first to the last period is significant. In Lake Grevelingen, where at least *H. takanoi* seems to be not very abundant or was even lacking till 2010, comparison of the same periods also indicates a significant decrease of the smaller sized *C. maenas* individuals from 1990–1998 to 1999–2004 in favour of the 11–15 mm size class (i.e. a significant increase) (Figure 7.6g,i,k). During the last 6 years only a handful of specimens were observed making the relative comparison of size classes uncertain. But it should be noted that the smaller specimens of *C. maenas* were already less abundant in the *C. maenas* populations in Lake Grevelingen than in the Oosterschelde and the Westerschelde during the 90s, and that the species as a whole was only occasionally found in the samples of Lake Grevelingen after 2004. Due to the low densities, size distribution patterns for Lake Veerse Meer are not very clear for the period 1990–2004 (Figure 7.3h). After the opening, both *C. maenas* and *H. takanoi* entered the lake, where clearly smaller sized C. *maenas* specimens are relatively less abundant than one would expect under circumstances with local reproduction; moreover the size class 0–5 mm is completely lacking (Figure 7.6j). This is not the case for *H. takanoi*, for which more than 70% of the population exists of the 0–10 mm specimens.





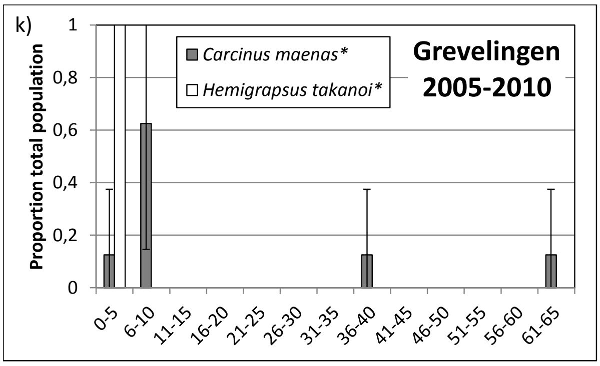


Fig. 7.6 Proportional distribution of the total number of observed specimens per species (*Carcinus maenas*, *Hemigrapsus takanoi*) per period and water body over 5 mm carapace (CW) size classes; a) Oosterschelde from 1990 to 1998; b) Westerschelde from 1990 to 1998; c) Oosterschelde from 1999 to 2004; d) Westerschelde from 1999 to 2004; e) Oosterschelde from 2005 to 2010; f) Westerschelde from 2005 to 2010; g) Grevelingen from 1990 to 1998; h) Veerse Meer from 1990 to 2004; i) Grevelingen from 1999 to 2004; j) Veerse Meer from 2005 to 2010; k) Grevelingen from 2005 to 2010.

*Total number of specimens observed is rather small (<8)

In the hard substrate snapshot survey measurements of carapace width (CW) were combined for all locations to produce an overall representation of the size distribution per species on the hard substrate in the Oosterschelde. *Hemigrapsus takanoi* and *H. sanguineus* were abundant in sizes less than 20 mm CW with the greatest number of both species measuring between 10 and 15 mm CW (Figure 7.7). No *C. maenas* smaller than 10 mm CW were observed and an obvious peak in size for *C. maenas* specimens was lacking. *Carcinus maenas* was found in similar numbers per millimetre class with an average of 6 (±4) crabs at sizes between 15 mm CW and 40 mm CW. Out of a total of 201 *C. maenas* specimens found, 35 (17%) measured more than 40 mm CW. The largest C. *maenas* found has 60.6 mm CW and was found just inside the barrier.

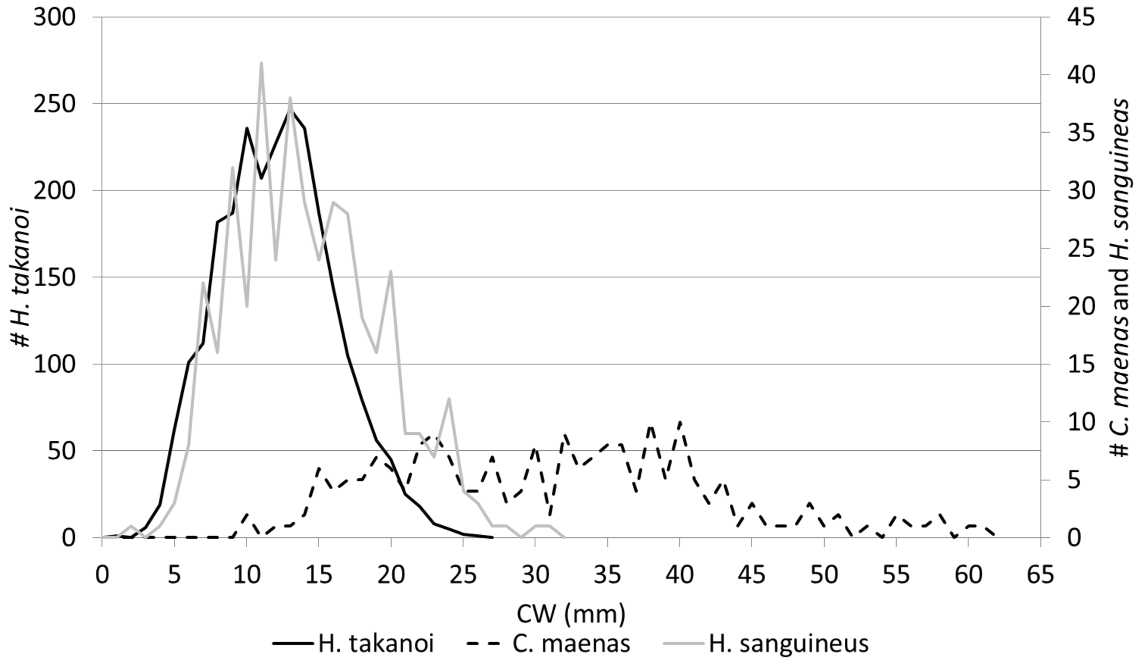


Fig. 7.7 Size distribution of three crab species for the combined hard substrate survey sites in the Oosterschelde for *H. takanoi, C. maenas* and *H. sanguineus* in 2011

DISCUSSION

Over the last 20 years, the *C. maenas* populations show a clear decrease on the soft sediment substrates in the Oosterschelde (Figure 7.2b). *Carcinus maenas* also used to be very abundant on hard substrates (e.g. De Kluijver and Leewis, 1994), but at present numbers are reduced and outnumbered by the *Hemigrapsus* species (Figures 7.5 and 7.7). However, the question of whether *Hemigrapsus* caused or contributed to the observed decrease in *C. maenas* is not a straight forward one.

Both introduced *Hemigrapsus* species occupy *C. maenas* habitats in the delta waters. Compared with other locations in the 2011 snapshot survey of the Oosterschelde, *H. sanguineus* was found in large numbers around the storm-surge barrier and particularly just outside the barrier, while *H. takanoi* was particularly abundant within the barrier. This is likely to be due to the different hydrodynamic properties of the habitats sampled as the storm-surge barrier has reduced the current velocities inside the barrier considerably (Escaravage et al., 2006). In general, the further east in the Oosterschelde, the lower the energy of the habitat, which can explain the reduced numbers of *H. sanguineus* going further east into the tidal bay. This is consistent with the observations by Dauvin et al. (2009) who found *H. takanoi* predominantly in low hydrodynamic muddy habitats, while *H. sanguineus* was found predominantly in high hydrodynamic habitats on the Opal Coast in France. Both species were found to live sympatrically in harbours (Dauvin et al., 2009). This is probably the result of developed habitat segregation in the region of origin (e.g. Japan) (Asakura and Watanabe, 2005; Asakura et al., 2008).

Although *C. maenas* prefers hard to soft sediment substrates, they are habitat generalists (Griffen, 2011). Twenty years of intensive monitoring of soft sediment habitats provides a detailed picture of the population developments in the Dutch delta, showing changes in densities and spatial distribution. Furthermore, evaluating size frequency distributions in these habitats is of value as changes in the population can be observed even when considering that different life stages may utilise different habitats.

The interaction between *Hemigrapsus* and *C. maenas* in the Dutch delta network is complicated by the spread of larvae by water currents. Broadcasting of recruits can prop the population up in a particular water body, but low reproduction in 1 year could result in the population quickly declining. This means that variation in population changes is expected between each body of water in the delta. *Hemigrapsus takanoi*, which was clearly much more abundant on soft sediment in the Oosterschelde than in the other delta waters, seems to reproduce considerably in this water body. This is also indicated by the size-frequency distribution showing smaller sized individuals being most abundant. In the Westerschelde the densities of *H. takanoi* on soft sediment remain low thus far, indicating a rather low reproductive success or populations dependent on recruitment from the Oosterschelde or the North Sea on a yearly basis. The population developments of *H. takanoi* in Lake Grevelingen are highly uncertain. The species has not been recorded for the water body as far as we know, although it might be expected that at least on hard substrates the species might have been present before the first recordings of the present study on soft sediment in 2010. Being all smaller sized individuals, it seems that if populations of *H. takanoi* occurred in Lake Grevelingen, they probably depend on recruitment from elsewhere, likely the Oosterschelde, and that such an inflow of new recruits occurred in 2010. Also for Lake Veerse Meer this must have been the case until the year 2010. Densities were fluctuating and, as for the Oosterschelde, recruitment of the soft sediment environments appeared to be low, for instance in the year 2008. In 2010 the *H. takanoi* populations seem to have exploded in Lake Veerse Meer. Still these specimens might be recruits coming from the Oosterschelde, as also there an increase in the densities was observed. But as the increase in the Oosterschelde is not that spectacular, it might be that 2010 is the first year with successful reproduction of *H. takanoi* in Lake Veerse Meer.

Several studies on the Atlantic coast of the USA have reported a replacement of *C. maenas* by *H. sanguineus* as a direct result of competition (Jensen et al., 2002), predation on juveniles (Lohrer and Whitlatch, 2002) and forced dietary shifts leading to reduced reproduction in *C. maenas* (Griffen et al., 2011). This appears not to be the case in Dutch delta waters because, at least on soft sediment, the decrease in *C. maenas* densities in the Oosterschelde had already started before *Hemigrapsus* appeared in the samples. Moreover, the decrease of *C. maenas* does not seem to be a gradual one, but rather one with fluctuations with a period of lower densities during 1992–1995, which cannot be explained by the presence of *Hemigrapsus*. Additionally, in the Westerschelde where *Hemigrapsus* was present since the year 2000, such an impact on *C. maenas* cannot be observed. *Hemigrapsus* densities are lower, as indicated by the first occurrences in soft sediment samples not before 2007, but it does not strengthen the hypothesis of *Hemigrapsus* causing the collapse of the *C. maenas* populations in the Dutch delta and the Oosterschelde in particular. In Lake Grevelingen a decrease in *C. maenas* populations was also observed although no *H. takanoi* were found (Figure 7.2, Table 7.2). It should be noted that general deterioration of the system is taking place here and that several other species show declining trends (Wetsteijn, 2011). In Lake Veerse Meer the conditions significantly improved for *C. maenas* with the increase in salinity in 2004. For this water body it can be observed that the *C. maenas* populations responded and increased. However after 1 to 2 years, *Hemigrapsus* outnumbered *C. maenas* also in Lake Veerse Meer, but it is not evident that *C. maenas* populations suffered from the increase of *Hemigrapsus* because *C. maenas* is still present.

However, the *Hemigrapsus* species prefer hard substrate habitats, and it is very likely that their appearance on soft sediment in reasonable numbers was delayed compared to hard substrates. We know that this is the case because the species were first observed in the year 2000 in the Oosterschelde (and probably arrived in 1999), and several recordings from hard substrates occurred during the following years (e.g. Breton et al. 2002; Wolff, 2005). Griffen et al. (2011) showed a difference in impact due to competition rates and predation of *H. sanguineus* on *C. maenas* between habitats dominated by hard or soft sediment. In the Gulf of Maine, where the intertidal area is particularly rocky, there was a high density of *H. sanguineus* which resulted in high levels of competition and predation on *C. maenas*, while at Long Island, where there is predominantly soft bottom habitats, there was a much lower density of *H. sanguineus* and less competition and predation. Therefore, the pressure of *Hemigrapsus* on the *C. maenas* population is likely to be relatively isolated to the hard substrate areas.

It cannot be neglected that *Hemigrapsus* succeeds in dominating habitats and entire regions where *C. maenas* used to flourish (Figs. 7.4 and 7.5). It seems that the *Hemigrapsus* species may be profiting from decreasing *C. maenas* populations. Competition between the two species probably also plays a role and leads to the dominance of *Hemigrapsus* particularly on hard substrates, but also a further decrease of the *C. maenas* populations to some extent.

Although *C. maenas* can grow much larger than either *H. sanguineus* or *H. takanoi* (Jensen et al., 2002; Pillay and Ono, 1978), there is likely to be predation of juvenile *C. maenas* by *Hemigrapsus* individuals (Griffen et al., 2011) on top of the cannibalism already seen in *C. maenas* (Moksnes, 2004) and interspecific competition between full grown *Hemigrapsus* individuals and juvenile *C. maenas* of the same size (Jensen et al., 2002). Furthermore, larger *C. maenas* tend to move to other environments (i.e. deeper waters) (Thiel and Dernedde, 1994) and consequently these interactions are less important due to size and niche segregation. However reproduction of *C. maenas* and particularly the settlement of the *C. maenas* larvae seem to be largely restricted to intertidal habitats (Thiel and Dernedde, 1994; Zeng et al., 1999). This is also indicated by the size–distribution graphs (Fig. 7.6) showing large numbers of smaller sized *C. maenas* specimens in the intertidal Oosterschelde and Westerschelde; as indicated before, particularly before the decrease of the *C. maenas* populations, but the smallest size-classes (0–5 and 5–10 mm CW) are largely lacking in the non-tidal Lake Grevelingen and Lake Veerse Meer. It is likely that almost no reproduction of C. *maenas* takes place in these non-tidal waters, as in the deeper parts of the tidal waters. This implies that the populations of the species in subtidal waters depend on colonization of sub-adult and adult specimens from intertidal zones, and, for the two non-tidal lakes of surrounding tidal waters, the Oosterschelde and the North Sea pre-delta region.

Small *C. maenas* are more likely to be found in high abundance on a substrate providing spatial refuges, and to avoid predation juvenile *C. maenas* stay as long as possible in the intertidal area before emigrating to deeper water for the winter (Griffen, 2011; Thiel and Dernedde, 1994). This is in contrast to larger conspecifics, which are more abundant in deeper waters throughout the year. Although Thiel and Dernedde (1994) did not present data for April–June, it can be assumed that smaller crabs are the first to recolonize the intertidal area at the end of winter as they are still more vulnerable to predation than larger conspecifics. As we found *C. maenas* in the intertidal region at all ten snapshot survey locations and with sizes ranging from 10 mm to 61 mm CW, the recolonization of the intertidal area by *C. maenas* is likely to have occurred prior to the hard substrate survey which began in May. Berrill (1982) reported that on the central coast of Maine in the United States *C. maenas* recruits measured 16–30 mm in carapace width (CW) after the end of their first winter. The two areas (the coast of Maine and the Dutch delta) experience similar water temperatures, suggesting that *C. maenas* would follow a similar growth rate in both locations. This is supported by the results of the present study as no *C. maenas* measuring less than 10 mm were found in May–June.

Small crabs use rocks and shells in the intertidal zone for shelter and protection against predators (Berrill, 1982) and populations on these substrates are often regulated by cannibalism (Moksnes, 2004). The expansion of the Pacific oyster, *Crassostrea gigas*, in the delta waters has provided plenty of new hard substrates with spatial refuges in the last 20 years (Troost, 2010). These new niches are perfect protective nurseries for small *Carcinus maenas*, yet *Hemigrapsus* species still outnumbered them by far even in areas containing oysters. While it cannot be said that the Pacific oyster facilitated the introduction of *Hemigrapsus* because it was present long before the crabs were introduced and because more *Hemigrapsus* crabs were found in locations without oysters than with them, the introduced oysters certainly have provided more heterogeneous habitats and hard substrate for the crabs to utilise making it possible to compete successfully and dominate over the native *C. maenas*.

If juvenile *C. maenas* are likely to be found in higher and more heterogeneous substrates (Pillay and Ono, 1978) and if the previous year's recruits measure between 16 and 30 mm CW (Berrill, 1982), one would expect a peak in the number of smaller *C. maenas* found on the hard substrate at the base of the dykes in May. Additionally, in any reproducing populations one would expect more young cohorts than older ones due to mortality of older individuals. However, this was not the case; the numbers of small *C. maenas* found did not exceed that of larger and therefore older *C. maenas* specimens.

Figure 7.6 indicates that a decline in especially the smaller size-classes is particularly evident in the Oosterschelde and was already initiated in the beginning of this century. In the Westerschelde, where *H. takanoi* is less abundant, the pattern is less pronounced and started to develop around 2005 (Figures 7.6b,d,f). The results for Lake Grevelingen (Figures 7.6g,i,k) indicate that in the early 1990s the size distribution was already not as one would expect for a water body where reproduction was occurring. As juvenile specimens of *C. maenas* occur mostly in the intertidal zone (Berrill, 1982), which is lacking in Lake Grevelingen, this is not surprising. The observed collapse of *C. maenas* in Lake Grevelingen is most likely due to deterioration of the lake itself, although a reduced influx from other environments (i.e. the Oosterschelde) might also play a role. The same applies to Lake Veerse Meer, currently suitable for *C. maenas*, but without an important habitat for juveniles due to the lack of intertidal zones.

Unfortunately there are no data available for the crab communities at the same hard substrate locations sampled in this study prior to the arrival of *H. takanoi*. However, given the lack of an expected peak in *C. maenas* numbers smaller than 30 mm CW, and that at these smaller sizes *C. maenas* was overwhelmingly outnumbered by *H. takanoi*, it is likely that there is size-dependent interspecific competition and/or predation occurring on hard substrates between the two species, and that *H. takanoi* is the more successful species. Various studies with *H. sanguineus* support this suggestion (Griffen et al., 2011; Jensen et al., 2002; Lohrer and Whitlatch, 2002).

This study shows that while the invading *Hemigrapsus* species were not the initial cause of the reduction of the *C. maenas* populations, they successfully colonized the Dutch delta region due to minimal competition from other crab species. The ‘new’ hard substrate environments of the expanding *C. gigas* oyster reefs have provided more suitable substrates to promote this colonization. Once settled and numerous the two *Hemigrapsus* species appeared to be predators on and/or superior competitors for the space occupied by juvenile *Carcinus maenas* shore crabs. However, while increased predation and competition pressure in the preferred habitat for *C. maenas* recruits may contribute to a decrease in population size, as *C. maenas* is more of a habitat generalist than the *Hemigrapsus* species, it is unlikely that *Hemigrapsus* will cause the complete elimination of *C. maenas* from the area (Griffen, 2011).

ACKNOWLEDGEMENTS

Thanks to Mandy Godschalk for her efforts in the field and in the lab for the snapshot survey. Thanks also to the research assistants of the Monitor Taskforce of the NIOZ—Yerseke (formerly NIOO-CEME) for performing the long term monitoring of soft sediment communities in the Dutch delta waters, and Rijkswaterstaat (RWS) for funding the MWTL monitoring programme. Thanks to J.-C. Dauvin and an anonymous reviewer for their useful comments on an earlier version of the paper and to Jon Hutchens for his general support. This is Monitor Taskforce Publication Series 2012-04.

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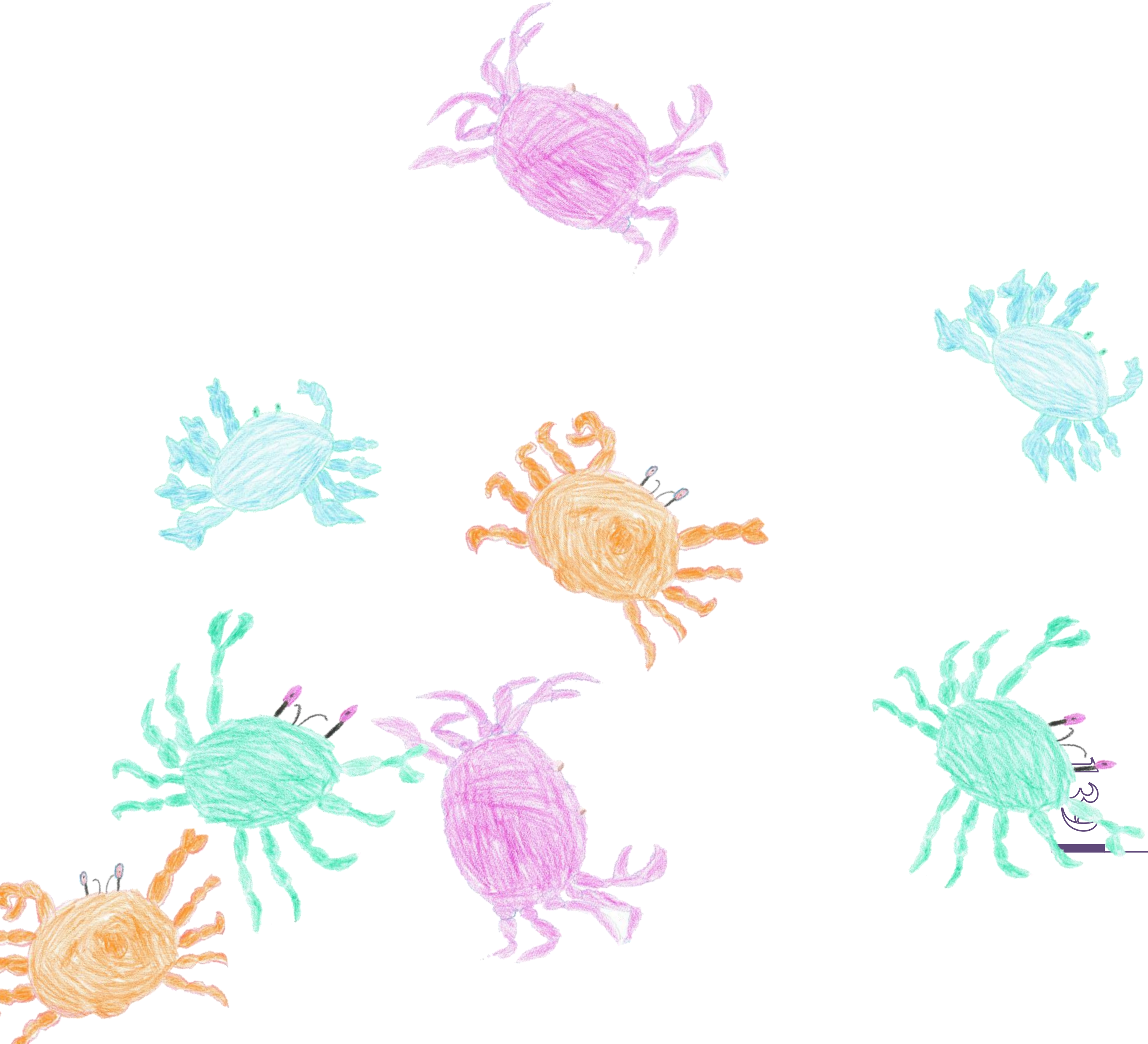
Chapter 8:

General Discussion



Reproduction in crabs: strategies, invasiveness and environmental influences thereon

This thesis explores the traits and characteristics of primarily two crab species of similar size, and habitat preferences (intertidal, often among shellfish and/or macroalgae), but representing different reproductive strategies, environments and statuses as native and invasive. The effect of temperature on reproductive rates was investigated for both species and extrapolated to make predictions about the effect a climate change induced temperature rise may have on the population dynamics and distribution of the species.

This first species investigated, *Halicarcinus cookii*, is endemic to New Zealand and has never been known to spread to new locations. This species represents a rarely studied reproductive strategy with determinate growth (growth phase finishes before reproductive phase begins), hard-shell mating and ventral seminal receptacles. Adult females continuously carry fertilised broods their entire adult life, with very little interbrood time. This thesis includes an in-depth novel investigation of this particular species and presents the most complete overview of the reproductive biology of the species currently available.

The second species, *Hemigrapsus takanoi*, originally from the north west Pacific coasts, from Northern Japan to China, is a successful invader of the Atlantic coasts of Europe, having been presumably been introduced via ballast water (Dauvin et al., 2009). This species represents a different reproductive strategy to *Halicarcinus cookii*. As it has indeterminate growth (growth and reproductive phases alternate), hard-shell mating and ventral seminal receptacles. Females carry one or two consecutive fertilised broods and then stop reproducing for a period to moult and grow. This species has only relatively recently been described as a separate species (Asakura & Watanabe, 2005) yet it is a successful invader in north-westen Europe including Spain, France, Belgium, the Netherlands and Germany (Shinji et al. 2009, Dauvin et al. 2009; Doi et al., 2009). This thesis investigates *H. takanoi* as an invasive species as well as the effect its arrival has had on the native green crab *Carcinus maenas*. As a case study these processes have been studied in the delta waters of SW Netherlands.

This thesis is intended to address one objective and two hypotheses outlined in Chapter 1:

1. To provide new information on the reproductive strategy, growth patterns, sexual selection, behaviour and population dynamics of the rarely studied crab species, *Halicarcinus cookii*.

2. H_0: Temperature has no effect on reproductive rates in crabs and global temperature rises will not influence reproductive rates to a point where population changes and invasive success of non-native species are affected.

3. H_0: The arrival, presence and effect of *Hemigrapsus takanoi* in the Dutch delta waters has had no effect on the native green crab *Carcinus maenas*.

1. Objective: Reproduction in *Halicarcinus cookii*

Growth and reproduction

Growth and reproduction in the Brachyura are competing processes resulting in the different reproductive strategies defined in McLay and Lopez Greco (2011). Chapter 2 described how *Halicarcinus cookii* showed determinate growth, which terminates with the pubertal moult when individuals become reproductively active. With the ability to reproduce continuously, recruitment in *H. cookii* would be expected to occur relatively consistently throughout the year, resulting in a stable population structure (Begon & Mortimer, 1986; Southwood, 1977). With constant recruitment, the population structure is largely attributable to the pattern of mortality and seasonal changes in climate. In the *H. cookii* population on the Kaikoura Peninsula in New Zealand there was an obvious annual cycle of a somatic growth phase followed by a reproductive phase. The growth phase occurred primarily in the colder autumn and winter months (April-July), during which the majority of new recruits were settling out of the plankton and growing through their immature instars and mature individuals were at a minimum (Figure 3.1). The majority of the population then entered the reproductive phase in summer (November to March) as immature individuals developed through their pubertal moult resulting in the population being dominated by mature individuals (Figure 3.1). The numbers of mature individuals decreased in autumn (April-May) as the individuals came to the end of their approximately 12-18 month life span, 6 months of which are spent as a mature adult.

As suggested in Chapter 2, *H. cookii* females mature at larger sizes in warmer temperatures than in the colder temperatures. Females were recorded to mature over a wide size range of approximately 5-9 mm Carapace width (CW) (Figure 2.6), suggesting that environmental influences such as temperature may influence the number of juvenile instars and/or the percentage moult increment. The exact number of juvenile instars, or even if there is a fixed number of juvenile instars, in *H. cookii* is currently unknown. Assuming the percentage moult increment (PMI) remains constant throughout the growth phase it is estimated that with a mean PMI of 18%, a female with 2 mm CW would moult approximately 10 times before reaching the mean adult female CW of 8.4 mm. However, PMI is likely to decrease as size increases (Richer de Forges, 1977).

Temperature can have a parabolic effect on growth in the geographical distribution of a species, resulting in smaller individuals at both geographical (and therefore temperature) extremes and the largest individuals in the middle of the geographical distribution where temperatures are intermediate (Hartnoll, 1982; Hartnoll & Bryant, 2001) as seen in the blue crab, *Callinectes sapidus* (Leffler, 1972).This effect would require the temperatures at the latitudinal extremes of the species distribution to exceed the optimal temperatures for the species. *Halicarcinus cookii* has a relatively small temperature tolerance range and high mortality was observed at just 20°C (Chapter 5) and almost no activity was observed at 5°C (pers. obs). This suggests that water temperatures exceed the optimum at both latitudinal extremes of the distribution of *H. cookii* in New Zealand. Therefore such a parabolic size range on a spatial scale would be expected for *H. cookii*, where the lowest PMI, resulting in the smallest mature individuals would be expected in the northern-most and southern-most populations, and the highest PMI, resulting in the largest individuals would be found in the populations in between.

However, this hypothesis may not apply to all crab species. *Hemigrapsus takanoi* has a temperature tolerance range exceeding what the species is most likely to encounter in its latitudinal extremes in the wild (see Chapter 6). Personal observations during experimental work for Chapter 6 suggest that *H. takanoi* can survive in constant temperatures over 30°C, an unlikely natural environment. Nevertheless this hypothesis relates specifically to growth and has not been investigated for either *H. cookii* or *H. takanoi*.

Sexual selection and reproductive behaviour

Sexual selection is a driving force in the development of sexual dimorphism, intrasexual competition and reproductive behaviour. Sexual selection occurs when there are differences in reproductive priorities between males and females. This is usually a conflict between males attempting to produce as many offspring as possible (which often leads to male-male competition) while females attempt to produce the best quality offspring, with the most preferable genetic make-up as possible (e.g. female choice or sperm storage).

The differences in growth observed between males and females in Chapter 2 show that *H. cookii* is typical of brachyurans in that males grow larger and develop much larger chelipeds in relation to body size than do females. In the Kaikoura population, males dominated the larger size classes above 12 mm CW while females were more common in the intermediate size classes. The maximum size recorded for a female was 11.51 mm, while the largest male was 12.92 mm CW. Males also showed a much stronger positive allometry in cheliped growth of both propodus length and height than did females (Table 2.1). This obvious sexual dimorphism therefore, suggests that sexual selection has been an influencing factor in *H. cookii*.

The lack of female choice for males to mate with (as observed in Chapter 3, where females appeared to make no distinction between males of different sizes) suggests that male-male competition for females is the primary driving force of the sexual selection in *H. cookii*. Male-male competition occurs when mature females are limited (Zimmer, 2001). However, in Chapter 3 (Figure 3.1) the *H. cookii* population showed that females almost consistently outnumbered males throughout the year, and particularly in the summer months of November to April. It therefore appears that the overall sex ratio is strongly biased toward females and that males need not compete for females as there are many receptive females per male.

Although adult females are not limiting to males, Chapter 3 shows that males have a clear preference for mating with females close to spawning (Figure 3.7). Eggs are fertilised just prior to deposition on the abdomen of the female where they are carried until they hatch into planktonic larvae. Ovary development was shown in this chapter to coincide with the development of the fertilised brood already laid in *H. cookii* (Figure 3.4). This implies that these ‘attractive’ females that are about to spawn are closer to laying a new brood than females carrying newly fertilized eggs.

In Chapter 3 ‘attractive’ females were shown to make up to 20% of the mature female population, making them limited to males and suggesting that the sex ratio of adult males:‘attractive’ females, as opposed to the total adult population sex ratio, was indeed biased towards males. The adult sex ratio was generally less than 1.0 males per female, especially during the summer months, while the ratio of males per ‘attractive’ female ranged between 1.4 and 6.8. This ratio explains the presence of intrasexual competition between males.

Not only did males prefer to mate with females about to spawn, in Chapter 3 they also showed more antagonistic behaviour towards other males, and more post-copulatory mate guarding in the presence of females close to spawning compared to other females. This indicates that with the basic need to maximise paternity, it is worthwhile for males to invest energy in being the last male to mate with a female before she lays a new brood.

Chapter 4 revealed that this competition to be the last male to mate with a female before she lays a new brood is based on sperm competition inside the female seminal receptacles. Females store sperm to ensure a supply is available even when the encounter rate with males is low in the female biased sex ratio. The chapter shows that *H. cookii* has ventral-type seminal receptacles (Figure 4.1) and due to this structure, the sperm located closest to the entrance of the seminal receptacles (the last sperm transferred) and close to where the oviduct delivers the ova is the first used to fertilise the new brood of eggs.

Furthermore, in Chapter 4 (Table 1.1) there appeared to be some, although little sperm mixing in *H. cookii*. If sperm layering does occur, the layers were shown to be not completely isolated from each other. Consequently, by increasing the amount of sperm transferred during a single copulation, a male's ejaculate can occupy more space inside the seminal receptacles, diluting that of rival males, so that his sperm have a higher chance of fertilising more eggs. Females cannot decrease the amount of sperm they carry, as they are sperm-accumulators because they cannot moult and shed the contents of their seminal receptacles. Larger males were shown in Chapter 4 (Figure 4.3) to transfer more sperm to the females than smaller males. Therefore, larger males have a reproductive advantage over smaller males in terms of sperm competition, resulting in selection favouring the increased male size shown in Chapter 2.

Although *Halicarcinus cookii* is not currently known as an invasive species anywhere, its life history and evidence from similar species of the same genus suggest that it may successfully establish populations in non-native areas if given the chance. Hymenosomatids are the only crabs known to lack a megalopa stage (Melrose, 1975, Lucas, 1980). The megalopa stage is the final planktonic stage prior to settling and metamorphosing into the first juvenile instar. This stage can be delayed according to conditions such as temperature and available suitable habitat (Wear and Fielder, 1985). Lacking this stage, and the consequent short planktonic phase, along with the continual reproduction seen in Chapters 3-5, would favour rapid local recruitment and establishment of a population in a new area, especially where temperatures are optimal (see Chapter 5). However, a short planktonic phase does not favour widespread larval dispersal. Long distance dispersal would depend on the live transport of reproducing adults. The only New Zealand hymenosomatid to have a trans-Tasman distribution is *Halicarcinus innominatus*, which established in Hobart, Tasmania, Australia after being shipped with New Zealand oysters (*Tiostrea chilensis*) in the 1920s. However, it has not spread far from the point of introduction (Lucas, 1980). Tavares (2003) reported that *Halicarcinus planatus* was unintentionally transported live from Chile to Rio de Janeiro, Brazil with the Pacific oyster, *Crassostrea gigas*. However, he suggested that an established population would be restricted to the south coast of Brazil due to warm temperatures as far north as Rio. There are no known records of an introduced hymenosomatid species causing noticeable changes to the local environment. Therefore, if successfully transported as adults, *H. cookii* may establish a population in a non-native area, but its distribution and impact is likely to be limited.

2. H_0: The effects of temperature on the reproduction and invasiveness of crabs

The rate of physiological processes such as reproduction is influenced by abiotic factors. The effect of temperature on brood incubation and larval development in crabs is well documented (Leffler, 1972; Nagaraj, 1993; Nijland & Beekman; 2000; Wear, 1974; Wiess et al., 2009). Higher temperatures increase physiological processes and therefore decrease the duration of embryonic and larval development (Lleffler 1972). While representing two very different environmental envelopes and reproductive strategies (*Halicarcinus cookii* has determinate growth and constant brood production while *Hemigrapsus takanoi* has indeterminate growth and does not produce broods constantly) the brood development of *H. cookii* and *H. takanoi* both showed a decrease in duration with increasing temperature. This is consistent with the various studies of other species presented in the literature (Leffler, 1972; Nagaraj, 1993; Nijland & Beekman, 2000; Wear, 1974; Wiess et al., 2009) and shows the linear relationship in reproductive rates of crabs as a response to environmental temperature.

If average global temperatures were to increase, the subsequent changes in reproductive rates are likely to increase the Net Reproductive Rate (R_0) of individuals currently in lower than optimal temperatures as mentioned in Chapters 5 and 6. The R_0 estimates the number of females expected to reach reproductive age by considering the number of offspring per female and the mortality rate at each stage of development and is dependent on growth, mortality and reproduction. An increased R_0 (greater than 1.0) will consequently influence the population growth and distribution of a crab species in a given area primarily leading to shorter brood maturation periods and possibly more broods per female lifetime.

In Chapter 5, (Table 5.3) the three *Halicarcinus* species were estimated to be able to potentially produce an extra brood of eggs per female life if temperatures were to increase by an average of 2°C. The increased rate of development of embryos, and probably larvae as well, along with the extended high reproduction season would increase overall reproductive rate of the population substantially and lead to population growth. This is possible due to the reproductive strategy of these species including a terminal moult, hard shell mating and continuous brood production so that no reproductive time is lost during the female's adult life.

In Chapter 6 (Figure 6.3) the same response to increased temperatures was seen in *Hemigrapsus takanoi* as was seen in the *Halicarcinus* species in Chapter 5 (Figure 5.1.). However, predicting what may occur on a population level was more complicated due to the different reproductive strategy. Although *H. takanoi* also has hard shell mating, it does not show continuous brood production and has a defined reproductive season. However, it was suggested in Chapter 6 that a threshold water temperature of about 15°C may trigger the start of the reproductive season, and if temperatures rise, this threshold may be reached earlier in the year, thereby extending the reproductive season. This is seen in other *Hemigrapsus* species such as *H. sanguineus* (Fukui, 1988; McDermott, 1998). In the current study the effect of temperature on the number of broods produced could not be determined, but it is apparent from the literature on *H. sanguineus* that more broods per female per year are likely to be produced in warmer temperatures (Dauvin et al., 2009; Fukui, 1988; McDermott, 1998). As *H. takanoi* has been recorded as far south in Europe as Spain (Shinji et al. 2009), it is possible that the species has increased rates of reproduction there compared with cooler areas further north such as the Netherlands, however, little comparative information is available to establish whether or not this is the case.

An increase in temperature may extend the geographic range of a species to areas that would otherwise be limited by cold temperatures. However, *H. cookii* is endemic to New Zealand and is known from both the North and South Islands and Stewart Island as well as the Chatham Islands (Melrose, 1975). Therefore the native habitat of the species occupies the majority of the entire habitat available. The only habitat possibly currently restricted by cold temperatures further south would include the sub-Antarctic Snares Islands, Auckland Islands and Campbell Islands, but to reach these habitats *H. cookii* would have to cross a water barrier against the flow of the cold Antarctic current moving north. Therefore expansion of the distribution of *H. cookii* would initially depend on human assisted transport to new locations rather than natural dispersal.

An increase in temperature, leading to a potential increase in R_0, may increase the success of *Hemigrapsus takanoi* as an invasive species. Two of the five consequences of climate change for invasive species suggested by Hellmann et al. (2008) were the establishment of new invasive species and the altered distribution of present invasive species. Therefore environments currently suboptimal for the species may become more suitable, thereby increasing population growth, and in a shift of geographic boundaries, thus opening up new areas to colonise, increasing its distribution in its non-native environment (Aronson et al., 2007; Hellmann et al., 2008). Theoretically global temperature rise could also result in a restriction of species distributions in areas where temperatures were suitable, but have become too warm. In Chapter 5, high mortality was observed in *Halicarcinus cookii* at 20°C. This species is therefore clearly a temperate species with a relatively small range of temperature tolerance. If temperatures rise too high, the species may be severely negatively affected.

The limited temperature tolerance in *Halicarcinus* species may be a factor restricting the invasiveness of these species in new environments unless they fall in the specific appropriate temperature range. The restricted dispersal of *H. planatus* in Brazil attributed to high temperatures (Tavares, 2003) suggests that the invasiveness of the species is limited by high temperatures. Conversely, in its native range *Hemigrapsus takanoi* experiences temperatures from -2 to 29°C (Summerson et al. 2007), which demonstrates that the species has a wide natural temperature tolerance range that would support their establishment over a wide geographical range. Personal observations during experimental work for Chapter 6 also suggest that *H. takanoi* can survive in temperatures over 30°C. Such a wide range of temperature tolerance favour successful establishment in a range of new environments (Hellmann et al., 2008). Therefore, range restriction due to temperature is dependent on the tolerance range of the individual species.

Other factors are important to the successful establishment of a non-indigenous species and temperature is only one factor affected by climate change. Abiotic factors such as salinity, sediment type and exposure time, as well as biotic factors such as food availability, competition and predation all influence the ability of a species to establish in a new environment. These must be taken into account when investigating the potential invasiveness of a species in a new environment.

3. H_0: *Hemigrapsus takanoi* as an invasive species in the Dutch delta

The colonisation of a new habitat will involve new interactions, such as predation and competition, with species not previously encountered. The interactions of the invasive *H. takanoi* with the native *C. maenas* in the delta waters of SW Netherlands were investigated in Chapter 7 of this thesis. Over a 20-year period it appeared that the initial introduction of *H. takanoi* led to a widely distributed and abundant population of the species in the delta. While it appears *C. maenas* was already declining prior to the introduction of *H. takanoi*, albeit fluctuatingly (Figure 7.2), it seems that *H. takanoi* took advantage of the declining *C. maenas* numbers and established itself in the same habitats frequented by juvenile *C. maenas*. In these habitats *H. takanoi* reached very high densities and is likely to compete with the juvenile *C. maenas*. Although not specifically detected in the current study, the arrival, establishment and consequent competition of *H. takanoi* is likely to impact the population of *C. maenas* in the delta. Indeed anecdotal evidence suggests that *H. takanoi* now dominates the once *C. maenas* dominated mussel beds in the Oosterschelde tidal bay (one of the delta waters of SW Netherlands) (Jacob Capelle pers. comm.).

Anderson and Epifanio (2010) suggested that the high fecundity of *Hemigrapsus sanguineus* (also present in the Dutch delta waters) may be a factor contributing to its success as an invasive species. Typical broods in *H. sanguineus* can contain between 15 000-50 000 fertilised eggs, and individual females typically produce 3-4 broods per breeding season (Gerard et al., 1999; McDermott, 1998). Like *H. sanguineus*, female *H. takanoi* can also produce up to 50 000 eggs per brood (Gough, 2009). The high numbers of *H. takanoi* present in the delta, especially in the Oosterschelde, suggest that millions of larvae are produced each breeding season.

While fecundity is important to the success of *H. takanoi* in the delta and as a competitor for *C. maenas*, other factors such as interspecific interactions must also play a role. *Carcinus maenas* is a soft shell mater, where males must wait until the female has just moulted in order to mate with her (Berrill, 1982). Although mature females mate and produce a brood only about once a year (Vinuesa, 2007), they still have enormous breeding potential in favourable habitats, with mature females producing 185 000 to 200 000 eggs per year (Broekhuysen, 1936). High fecundity is a probable factor contributing to the success of *C. maenas* as an invasive species in other locations such as the east (Jensen et al., 2002; DeGraaf & Tyrrell, 2004) and west coasts of North America (Cohen et al., 1995; DeGraaf and Tyrrell, 2004; Grosholz & Ruiz, 1995; Jensen et al., 2002), and Australia (Thresher et al., 2003). Even if a female *H. takanoi* produced four broods of 40 000 eggs per year (160 000) it would still be less than an average female *C. maenas*. While larval survival rates are variable and depend on many factors such as temperature (Mohamedeen & Hartnoll, 1990), salinity (Anger, 2003) and diet (Dawirs 1984), if percentage larval survival is similar for both species, *C. maenas* would reproduce at a faster rate than *H. takanoi*. Furthermore, the eggs of *C. maenas* appeared to develop at a faster rate than those of *H. takanoi* at all temperatures (Wear, 1974, Figure 6.3). This suggests that while fecundity and the effect of temperature thereon are important factors, other factors also play a role in the success of *H. takanoi* in establishing and building a population in the Dutch delta. For example, competition in other life stages, perhaps in the plankton, or for shelter with juvenile *C. maenas*, as suggested in Chapter 7 and in Jensen et al.(2002). *Hemigrapsus species* are also known to be aggressive competitors and predators (Jacoby 1981, Jensen et al. 2002), and they were generally found aggregated under rocks in high densities, suggesting that their competitive success may be partly based on 'power in numbers'. Alternatively, *H. takanoi* may be filling a previously vacant, or mostly vacant niche in the environment of the delta waters.

Conclusions

- *Halicarcinus cookii* represents an example of one of various reproductive strategies in crabs, and illustrates the complexity and interconnectedness of crab reproduction.

  Each characteristic (sex ratio, separate growth and reproductive phases, continuous reproduction in females, structure of the seminal receptacles, male body size, mating behaviour etc.) influences, or is influenced by a combination of others.

- Changes in environmental temperature influence reproductive rates and thereby the Net Reproductive Rate, leading to changes in population growth and distribution.

  Despite their different reproductive strategies, environments and statuses as native and invasive, an increase in water temperature will increase the reproductive rate and potential distributional boundaries for both *Halicarcinus cookii* and *Hemigrapsus takanoi*. With a 2°C increase in temperature female *H. cookii* could produce one extra brood of eggs per lifetime (Table 5.3), resulting in 1000 extra larvae per female resulting in a 10–15% increase in fecundity (see Chapter 5). A similar increase is expected for *Hemigrapsus takanoi* in Europe (Chapter 6), and may lead to the spread of the species to new environments further north to locations now thought to be too cold for them to reproduce.

- Changes in distribution, whether by natural dispersal or by human driven vectors can lead to new interactions between previously allopatric species. In these cases reproductive output may contribute to the competitive success of one species over the other.

This thesis suggests that if the reproductive output of *H. cookii* increases as a result of temperature rise, it may help the species adapt to, or compete with newly arriving invasive species in the future, or aid in its dispersal and/or invasion of environments previously too cold. As a successful invader of habitats (Dauvin et al. 2009), *H. takanoi* has a reproductive output that allows the species to spread and establish quickly in a new environment. An increase in temperature may increase the fecundity of individual females, leading to rapid population growth and potential outnumbering of individuals of other species less affected by temperature, making it an even more successful invader.

- Interactions such as predation and competition for food or shelter are also important factors to the success of a species in a new environment.

While the fecundity of *C. maenas* appears higher than that of *H. takanoi*, evidence suggests that *H. takanoi* is taking over important habitats in the delta waters of the Netherlands for juvenile *C. maenas*, possibly contributing to the already declining *C. maenas* population. However, the wide range of habitats used by *C. maenas* (both soft and hard substrate and a wide range of temperatures and salinities) and its success as an invader elsewhere suggest that a situation will develop where the two species coexist and both occupy the niche originally occupied only by *C. maenas*.

- The differences observed between *Halicarcinus cookii* and *Hemigrapsus takanoi* shed some light on why the latter is such a successful invader and the former has no known invasion history.

The environments in which the crabs were found are likely to contribute to the chance that the crabs will be transported in the first place. *Halicarcinus cookii* was found in Kaikoura, New Zealand and is distributed along the intertidal shores of the South Island of New Zealand. Very little human activity that could result in the live transport of these crabs occurs on these coasts, although the case of *H. innominatus* being transported with oysters to Australia (Lucas, 1980) suggest that it is possible. In the Oosterschelde, the Netherlands, *Hemigrapsus takanoi* most likely arrived in Europe (originally in France) via ballast water (Dauvin et al., 2009) and from there to the Netherlands via oyster transport (Nijland, 2000, Nijland & Beekman, 2000, Faasse, 2002) and in the present study *H. takanoi* was found among oysters and mussels and in areas where human maritime activities are commonplace, suggesting that the risk of inadvertently transporting the crabs with shellfish, ballast water or other means is likely to be much higher.

Furthermore, although *H. cookii* reproduces continuously, its overall fecundity is lower than that of *H. takanoi* because the number of eggs produced is limited by female size after the terminal moult (which *H. takanoi* does not experience) and its adult life span of 2-8 years (Dauvin et al. 2009) is much shorter than that of *H. takanoi* at six months (Chapter 5). Temperature tolerance range is also likely to be a factor in invasive success as *H. takanoi* has a very wide tolerance range, allowing it to survive in a diversity of environments, while that of *H. cookii* is comparatively restricted, therefore restricting the environments in which it can survive.

Hemigrapsus takanoi has indeterminate growth which allow it to grow to larger sizes than *Halicarcinus cookii*. *Hemigrapsus* species are also known to be aggressive competitors and predators (Jacoby

1981, Jensen et al. 2002), which enable them to establish populations in the company of the much larger *Carcinus maenas*. *Halicarcinus cookii* lives cryptically in crevices and amongst seaweed, and no evidence available suggests it is a particularly strong competitor with other species. If *H. cookii* is not a strong competitor, the species may not be as successful in establishing a niche in a new habitat already containing resident crab species in or around New Zealand as *H. takanoi* has been shown to be in Europe.

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Summary

English - Dutch

English Summary

This thesis provides insights into the interconnectedness of crab reproductive biology, the selective forces leading to their development, the possible links to invasiveness and the influences of environmental factors thereon. The empirical data collected and presented in this thesis can be used to compare different crab species and make predictions about the effect of climate change on their population dynamics and invasiveness.

Two crab species in particular are examined in this thesis, *Halicarcinus cookii* and *Hemigrapsus takanoi ,* which share similar size and habitat preferences, but have different reproductive strategies and statuses as endemic and invasive. The potential effects of increased water temperature due to climate change on the reproduction and eventual population changes in the species are investigated as well as the possible links reproduction and temperature have with the invasiveness of a species.

Halicarcinus cookii is an endemic crab to the coasts of New Zealand and is not known anywhere else. The species shows determinate growth, hard shell mating, continuous brood production and ventral seminal receptacles. After the final moult the female produces numerous offspring limited only by sperm availability. With a hard shell the female also avoids mortality resulting from the regular vulnerable soft shell stage. Individuals mature over a range of sizes but do not continue growing after their pubertal moult. With such a terminal moult, brood size is limited by female size.

Hemigrapsus takanoi is native to the north west Pacific, but has been introduced and is very successful in Europe. The species shows indeterminate growth, hard-shell mating, a defined breeding season and ventral seminal receptacles. With indeterminate growth they continue moulting and growing throughout their adult life. After their pubertal moult, these species can mate throughout the year and produce 2-3 broods between each moult. They are not limited in growth or regeneration of limbs and can safely hide from predators during the vulnerable soft-shell inter-moult period rather than mating which exposes them to predators.

Despite their different reproductive strategies, broods of both species showed a similar reaction to increased water temperature in that the duration of development of the brood decreased as temperature increased. Extrapolating the results to a climate change scenario, it is suggested that with a temperature rise of 2°C *H. cookii* could produce one extra brood of over 1000 offspring per female life time, potentially leading to a 10-15% increase in fecundity and possible population growth. As *H. takanoi* does not show continuous brood production, predicting the effect of temperature rise is more difficult, but evidence suggests that fecundity is also likely to increase in this species with an increase in water temperature.

Temperature increase may also lead to a change in invasiveness of a species. If areas currently below the optimum temperature for a species become warmer, it is possible that a species may spread to the new locations. *Hemigrapsus takanoi* may spread further north in Europe than it's current distribution (assuming it is limited by temperature). Furthermore, if temperatures increase the rate of reproduction in a non-indigenous species, they may become more invasive in their present location.

The colonisation of a new habitat will involve new interactions, such as predation and competition, with species not previously encountered. The interactions of the two invasive crab species *H. takanoi* and *Hemigrapsus sanguineus* with the native *Carcinus maenas* in the delta waters of SW Netherlands was also investigated in this thesis. Whereas *C. maenas* was the most common shore crab in these waters, its

numbers have declined on the soft sediment substrates during the last 20 years. As the two invasive crab species were first recorded in the Dutch delta in 1999, they could not have initiated the decline of the native *C. maenas*. However, within a few years *H. takanoi* completely dominated the intertidal hard substrate environments; the same environments on which juvenile *C. maenas* depend. On soft sediment substrate the native and invasive crab species are presently more or less equally abundant. Nowadays *H. takanoi* appears to be a fierce interference competitor or predator for small *C. maenas* specimens by expelling them from their shelters.

Chapter 1 gives a general introduction to the topics covered in the body of the thesis and includes background information on reproduction in crabs, sexual selection in crabs, the role of temperature in reproduction rates, crabs as non-indigenous species and introduces the primary study species of the thesis.

In Chapter 2 (published in JMBAUK 2009) the relative growth and size at sexual maturity is presented for *H. cookii*. Allometric growth is also investigated and presented as a means to determine sexual maturity in the males of this species. While this crab undergoes a terminal moult, the size at which this occurs varies considerably to the extent that there is an overlap of 72% between mature and immature sizes. Relative growth of the abdomen width in immature females is positively allometric, but negatively allometric in mature females. Male abdomen growth is negatively allometric. There was no identifiable change in allometric growth in males to classify immature and mature males, but male cheliped growth was positively allometric, while in females cheliped growth was negatively allometric or isometric.

Chapter 3 (published in Zoologischer Anzeiger 2009) presents a comprehensive investigation into the mating strategy of *H. cookii*, and primarily focusses on how males maximise their fitness through mate choice. This chapter includes population dynamics of *H. cookii* in the Kaikoura peninsula, New Zealand, along with the operational sex ratio, gonosomatic index, mate guarding behaviour and mate attraction. No female choice was found in this species, yet females were able to build a sperm supply by mating with several males and storing the sperm in their seminal receptacle. Larger males were also found to transfer more sperm to the females, making them the more efficient mate for the female. Males mated preferentially with females carrying broods about to hatch. The determinate growth and continual brood production in this species indicate that the closer a female's brood is to hatching, the closer the female is to laying a new brood. Furthermore, post-copulatory mate guarding and male-male aggression also occurs in this species, particularly with females carrying late-stage broods. Male behaviour is therefore adapted to ensure he is the last to mate with a female before she lays a new brood.

Chapter 4 (published in Journal of Crustacean Biology 2009) presents the development and use of a technique to investigate sperm competition in internally fertilised crustaceans, using *H. cookii* as a study species. The technique involves the use of gamma rays to sterilise male crabs, followed by having females mate with known combinations of sterile and fertile males and analysis of the subsequent broods of eggs. In this way sperm mixing in the seminal receptacle (which would result in sperm competition), or sperm layering inside the seminal receptacle (resulting in no sperm competition) can be determined with live specimens. In *H. cookii* the majority of each brood was found to be sired by the last male to mate with the female, but consecutive broods showed that more eggs were fertilised by earlier mating partners. This information provides an explanation as to why males prefer to be the last male to mate with a female before she lays a new brood as described in Chapter 2.

Chapter 5 (published in JMBAUK 2012) investigates the effect of temperature on brood duration of three

Halicarcinus species. These three species (*Halicarcinus cookii*, *H. varius* and *H. innominatus*) all have determinate growth and continued brood production throughout their adult lives and occur in similar habitats in the Kaikoura peninsula in New Zealand where they experience the same temperatures. Total brood development at three different temperatures was investigated for each species, along with the interbrood period and the separate brood stages. For all species brood development and interbrood periods decreased with increasing temperature. These results are then compared with other species reported in the literature. While considering their brood production and mating strategy, if temperatures increase, reproductive rates will also increase so that more broods can be produced per female and potentially lead to population growth.

Chapter 6 (published in JMBAUK, 2012) is a similar study to that of Chapter 5, but with the invasive crab *Hemigrapsus takanoi* in the Oosterschelde tidal bay, the Netherlands. New information about brood development and the effect of temperature on the brood duration is presented and is expanded to discuss the effect of temperature on the Net Reproductive Rate (R_0) of the species and what that may mean for it as an invasive species in the Netherlands. As with the *Halicarcinus* species in Chapter 4, increased temperature led to a decrease in brood development time. Unlike *H. cookii* however, *H. takanoi* does not have constant brood production, and therefore reproduction is restricted to a specific season. The study indicates a potential trigger for the beginning ovary production to be when water temperatures reach about 15°C. Increased temperatures may lead to increased R_0 in *H. takanoi*, which is likely to influence its success as an invasive species in the Dutch delta waters.

Chapter 7 (published in Journal of Sea Research, 2012) delves further into *H. takanoi* as an invasive species in the Dutch delta waters and looks at the long term effects it has had on the native green crab *Carcinus maenas*. This study combines information gathered during a soft-substrate survey over a 20-year period, which encompasses the time before *H. takanoi* arrived, its initial arrival and establishment, and the years following its successful establishment and spread, as well as a 'snapshot' population survey of the hard substrate. This study provides a rare long-term perspective of the introduction of an invasive species and insights into its possible effects on a native competitor. Populations of *C. maenas* were declining before the arrival of *H. takanoi* , thus the arrival *of H. takanoi* could not be the original cause of decline in *C. maenas*. While adult *C. maenas* are often found on the soft substrate, juveniles tend to shelter around the rocks and other hard substrate areas while they are small and vulnerable to predators. It appears that *H. takanoi* took advantage of the decline in *C. maenas* and populated the hard substrate to the extent where they out-compete and/or preyed on the sheltering juvenile *C. maenas* of the same or smaller size. If juvenile *C. maenas* are being excluded from their previously safe shelters, this may have long term effects on the *C. maenas* population in the Dutch delta waters.

Chapter 8 discusses the links between Chapters 2-7 focussing on the objectives of the study: the reproductive biology of *Halicarcinus cookii*, the effect of temperature on the reproductive rate of both *H. cookii* and *Hemigrapsus takanoi* and predicting the possible effect of global temperature rise, and *Hemigrapsus takanoi* as a non-indigenous species in the Dutch delta waters.

The objective to provide new information about a rarely studied species (*Halicarcinus cookii*) was fulfilled in this thesis and the information can be used as bases for comparison for future research.

The hypothesis that temperature has no effect on the reproductive rate of crabs was rejected as both study species showed similar increases in brood development rate with increased temperatures. This suggests that global temperature rises may increase the reproductive rate of wider crab populations.

The hypothesis that the arrival, presence and effect of *Hemigrapsus takanoi* in the Dutch delta waters has had no effect on the native green crab *Carcinus maenas* was complicated by the fluctuations and the decrease in *C. maenas* numbers prior to the arrival of *H. takanoi*. It was concluded that while *H. takanoi* did not cause the initial decrease in the *C. maenas* population, it did take advantage of it and now dominates niches previously occupied by juvenile *C. maenas* where size dependent competition and/or predation on juvenile *C. maenas* occurs.

Nederlandse Samenvatting

Dit proefschrift geeft inzicht in de relaties tussen de voortplantingsstrategie van krabben, de selectiemechanismen in de ontwikkeling van krabben, het mogelijke verband met invasiviteit, en de invloed van omgevingsfactoren hierop. De empirische data die ten behoeve van dit proefschrift zijn verzameld en hier worden gepresenteerd kunnen gebruikt worden om verschillende krabbensoorten met elkaar te vergelijken en te voorspellen hoe klimaatsverandering de populatiedynamiek en invasiviteit van krabben kan beïnvloeden.

In dit proefschrift worden twee krabbensoorten onderzocht, *Halicarcinus cookii* en *Hemigrapsus takanoi,* die een vergelijkbare grootte en habitat voorkeur hebben, maar een verschillende voortplantingsstrategie hanteren. Ook zijn er verschillen in endemische en invasieve status. Een mogelijk effect van toename in water temperatuur als gevolg van klimaatsverandering op de voortplanting en mogelijke populatieveranderingen van de soorten is onderzocht, evenals de mogelijke verbanden tussen voortplanting en temperatuur enerzijds en invasiviteit anderzijds.

Halicarcinus cookii is een endemische krabbensoort langs de kust van Nieuw-Zeeland en komt niet elders voor. De soort groeit totdat het volwassen is en gaat paren, paart met een harde schaal, kan continu broedsel produceren, en heeft ventrale spermatheca. Na de laatste verschaling kan het vrouwtje continu nakomelingen produceren, waarbij de hoeveelheid enkel gelimiteerd wordt door de beschikbaarheid van sperma. Omdat het volwassen vrouwtje niet meer verschaalt, wordt sterfte als gevolg van verschaling voorkomen. Individuen kunnen volwassen worden bij verschillende groottes, maar groeien niet verder na hun laatste verschaling. Broedsel grootte wordt beperkt door de grootte van het vrouwtje na laatste verschaling.

Hemigrapsus takanoi komt oorspronkelijk uit het noordwesten van de Stille Oceaan, maar is geïntroduceerd in Europa waar de soort zich succesvol heeft gevestigd. De soort groeit ook tijdens het volwassen stadium en wisselt verschalen en groei af met productie van nakomelingen. De soort paart met een harde schaal, heeft een afgebakend broedseizoen en ventrale spermatheca. Na de verschaling in de puberteitsfase kan deze soort het gehele jaar paren en 2-3 broedsels produceren tussen elke verschaling. Ze zijn niet beperkt in groei of regeneratie van poten. Tijdens de kwetsbare periode na verschaling, waarin de schaal zacht is, verschuilen ze zich voor predatoren en paren ze niet.

Ondanks de verschillen in voortplantingsstrategie, zoals hierboven beschreven, laat broedsel van beide soorten een vergelijkbare respons zien op een toename in water temperatuur, namelijk een verkorte duur van de ontwikkeling van het broedsel bij een verhoogde omgevingstemperatuur. Als deze resultaten worden doorvertaald naar gevolgen van klimaatsveranderings dan kan *H. cookii* bij een toename van de temperatuur met 2°C een extra broedsel van meer dan 1000 nakomelingen produceren per vrouwtjesleven, wat leidt tot een toename van 10-15% in voortplantingssucces en een mogelijke populatiegroei. Omdat *H. takanoi* geen continue broedselproductie heeft is het effect van een toename in temperatuur moeilijker te voorspellen, maar de resultaten wijzen erop dat ook bij deze soort het voortplantingssucces lijkt toe te nemen bij een toename in temperatuur.

Een toename in temperatuur kan leiden tot een verandering in invasiviteit van soorten. Als gebieden opwarmen die momenteel een temperatuur hebben die onder de optimale temperatuur van een soort ligt, dan is het mogelijk dat een soort zich naar deze nieuwe locaties kan verspreiden. Zo kan *H. takanoi* zich verder naar het noorden verspreiden dan de huidige verspreiding (aangenomen dat alleen temperatuur limiterend is). Daarnaast kan een soort meer invasief worden op de huidige locatie als een temperatuurstijging de mate van voortplanting van niet inheemse soorten verhoogt.

De kolonisatie van een nieuw habitat leidt tot nieuwe interacties, zoals predatie en competitie, tussen soorten die nog niet eerder met elkaar in aanraking zijn gekomen. De interactie van twee invasieve krabbensoorten, *H. takanoi* en *Hemigrapsus sanguineus*, met de inheemse krabbensoort *Carcinus maenas* in de deltawateren van zuidwest Nederland staat in dit proefschrift beschreven. *Carcinus maenas* was oorspronkelijk de meest voorkomende krab in de kustzone van deze wateren, maar aantallen op het zachte substraat zijn afgenomen in de afgelopen 20 jaar. Aangezien de twee invasieve krabbensoorten voor het eerst in de Delta regio zijn aangetroffen in 1999, kunnen deze de afname in aantallen *Carcinus maenas* niet hebben veroorzaakt. Echter, *H. takanoi* is in een paar jaar tijd een dominante soort geworden op hard substraat in intergetijde gebieden; dezelfde omgeving waar juveniele *C. maenas* van afhankelijk is. Op het zachte substraat komen deze inheemse en invasieve krabbensoorten ongeveer evenveel voor. Tegenwoordig lijkt *H. takanoi* een pittige concurrent of predator van kleine *C. maenas* door hen uit hun schuilplaatsen te verjagen.

Hoofdstuk 1 bevat een algemene inleiding op de onderwerpen die in het proefschrift aan de orde komen. Hierbij komt de volgende achtergrondinformatie aan bod: voortplanting bij krabben, sexuele selectie bij krabben, de rol van temperatuur in de mate van voortplanting, krabben als niet-inheemse soorten, en een introductie van de krabbensoorten die in dit proefschrift zijn bestudeerd.

In hoofdstuk 2 (gepubliceerd in JMBAUK 2009) staat de relatieve groei en grootte bij seksuele volwassenheid van *H. cookii* beschreven. Allometrische groei is eveneens onderzocht en gepresenteerd als methode om seksuele volwassenheid bij mannetjes te bepalen. De grootte van mannetjes van deze krabbensoort verschilt zodanig bij de laatste verschaling, dat er een overlap van 72% is tussen adulte en juveniele individuen. De relatieve groei van de breedte van het abdomen van vrouwtjes is positief allometrisch bij juveniele vrouwtjes, maar negatief allometrisch bij adulte vrouwtjes. Het abdomen van mannetjes is negatief allometrisch. Mannetjes konden niet worden geclasssificeerd als juveniel of volwassen door het ontbreken van een waarneembare verandering in allometrische groei. Daarentegen was de groei van de scharen bij mannetjes positief allometrisch, terwijl dit bij vrouwtje negatief allometrisch of isometrisch was.

In hoofdstuk 3 (gepubliceerd in Zoologischer Anzeiger 2009) wordt een allesomvattende studie van de voortplantingsstrategie van *H. cookii* gepresenteerd, die voornamelijk gericht is op hoe mannetje hun fitness maximaliseren door partner keuze. Het hoofdstuk bevat de populatiedynamica van *H. cookii* op het Kaikoura schiereiland in Nieuw-Zeeland, de sex ratio, de gonosomatische index, de afscherming van de voortplantingspartner en de aantrekkingskracht van partners. Partnerkeuze door vrouwtjes werd in deze soort niet aangetroffen. Vrouwtjes waren wel in staat om een grote spermavoorraad op te bouwen door met verschillende mannetjes te paren en het sperma op te slaan in hun spermatheca. Grotere mannetjes konden meer sperma overdragen aan vrouwtjes, wat ze een efficiëntere partner maakte voor het vrouwtje. Mannetjes paarden bij voorkeur met vrouwtjes die broedsel droegen dat op het punt stond uit te komen. Het stoppen van groei in het volwassen stadium en de continue broedsel productie in deze soort wijzen erop dat hoe dichter het broedsel op uitkomen staat, hoe eerder een vrouwtje een nieuw broedsel zal aanmaken. Daarnaast komt afscherming van de voortplantingspartner na de paring en aggressie tussen mannetjes voor bij deze soort, met name in het bijzijn van vrouwtjes in het laatste stadium van het broedsel.

Het gedrag van de mannetjes is daarom aangepast, zodat hij de laatste is met wie een vrouwtje paart voor ze een nieuw broedsel legt.

Hoofdstuk 4 (gepubliceerd in Journal of Crustacean Biology 2009) behandelt de ontwikkeling en toepassing van een techniek om de competitie tussen spermacellen in bevruchte krabben te onderzoeken, met *H. cookii* als test soort. De techniek omvat het gebruik van gammastraling om mannetjes krabben steriel te maken, waarna vrouwtjes krabben in een bepaalde volgorde met steriele en vruchtbare mannetjes paren. Vervolgens wordt de broedsel productie geanalyseerd. Op deze wijze wordt in levende individuen sperma in de spermatheca gemengd (met competitie tussen spermacellen als gevolg) of gelaagd (zonder menging en competitie tussen spermacellen). Resultaten in *H. cookii* lieten zien dat de meerderheid van het broedsel bevrucht bleek te zijn door het mannetje dat als laatste met het vrouwtje gepaard had. Daaropvolgende broedsels lieten zien dat meerdere eitjes bevrucht werden door eerdere partners. Dit verklaart waarom mannetjes de voorkeur hebben om het laatste mannetje te zijn dat paart met het vrouwtje voordat ze een nieuw broedsel legt, zoals beschreven in hoofdstuk 2.

Hoofdstuk 5 (gepubliceerd in JMBAUK 2012) beschrijft het onderzoek naar het effect van temperatuur op de broedduur van drie *Halicarcinus* soorten. Deze drie soorten (*Halicarcinus cookii*, *H. varius* en *H. innominatus*) bezitten allemaal een groei die stopt tijdens het volwassen stadium en een continue productie van broed tijdens hun adulte leven. Ze komen in vergelijkbare habitats en omgevingstemperatuur voor op het Kaikoura schiereiland in Nieuw-Zeeland. De totale ontwikkeling van het broed bij drie verschillende temperaturen is voor elke soort onderzocht, evenals de periode tussen het leggen van broedsels en de afzonderlijke broed stadia. Voor alle drie de soorten nam de tijd voor ontwikkeling van het broed en de periode tussen het leggen van broedsels af bij een hogere temperatuur. De resultaten van deze soorten zijn vervolgens vergeleken met andere soorten die in de literatuur zijn beschreven. Broedselsproductie en voortplantingsstrategie in aanmerking genomen, blijkt de voortplanting toe te nemen bij een stijgende temperatuur. Hierdoor kunnen per vrouwtje meer broedsels worden geproduceerd en neemt de omvang van de populatie mogelijk toe.

Hoofdstuk 6 (gepubliceerd in JMBAUK, 2012) beschrijft een vergelijkbare studie als in hoofdstuk 4, maar dan met de invasieve krabbensoort, *Hemigrapsus takanoi*, uit de Oosterschelde, Nederland. Nieuwe informatie omtrent de ontwikkeling van het broed en effecten van temperatuur op de broedduur is beschreven. Daarnaast is het effect van temperatuur op de vervangingswaarde (R_0) van de soort bediscussieerd en is beschreven wat dit kan betekenen voor een invasieve soort in Nederland. Net als met de *Halicarcinus* soort in hoofdstuk 4 leidt een toename in temperatuur tot een verkorting van de ontwikkelingstijd van het broed. In tegenstelling tot *H. cookii* heeft *H. takanoi* echter geen continue broedsel productie, waardoor voortplanting beperkt wordt tot een specifiek seizoen. Het onderzoek laat zien dat er een potentiële trigger is voor de start van de eiproductie, als de water temperatuur 15°C bereikt. Een temperatuurstijging kan leiden tot een verhoogde R_0 in *H. takanoi*, wat het succes van de invasiviteit van de soort in de Nederlandse deltawateren mogelijk beïnvloedt.

Hoofdstuk 7 (gepubliceerd in het Journal of Sea Research, 2012) gaat dieper in op *H. takanoi* als invasieve soort in de deltawateren en onderzoekt de lange termijn effecten op de inheemse krabbensoort *Carcinus maenas*. Deze studie combineert data van een zacht-substraat monitoringsprogramma over een periode van 20 jaar met een momentopname van de populatie op hard substraat. De zacht substraat monitoring omvat de tijd voorafgaand aan de introductie van *H. takanoi*, de eerste aankomst en vestiging van deze soort, en de jaren na de succesvolle vestiging en uitbreiding van deze soort. Het onderzoek geeft een lange termijn vooruitblik op de introductie van een invasieve soort en inzicht in de mogelijke effecten op een inheemse concurrent. Populaties van *C. maenas* namen al af voordat *H. takanoi* aanwezig was. De introductie van *H. takanoi* kan dus niet de oorzaak zijn van de oorspronkelijke afname van *C. maenas*. Adulte *C. maenas* worden meestal aangetroffen op zacht substraat. Juvenielen zijn klein en kwetsbaar voor predatoren en schuilen onder rotsen en ander hard substraat. Het lijkt erop dat *H. takanoi* heeft kunnen profiteren van de afname van aantallen *C. maenas* en het hard substraat heeft kunnen domineren door succesvolle overheersing van en/of predatie op hier schuilende juveniele *C. maenas* van een vergelijkbare of kleinere grootte. Het verjagen van juveniele *C. maenas* uit hun schuilplaatsen kan lange termijn effecten hebben op de populatie *C. maenas* in Nederlandse deltawateren.

In Hoofdstuk 8 worden de relaties tussen hoofdstukken 2-7 beschreven waarbij de nadruk wordt gelegd op de doelstellingen van deze studie: de voortplantingsbiologie van *Halicarcinus cookii*, het effect van temperatuur op de voortplanting van zowel *H. cookii* als *Hemigrapsus takanoi*, een voorspelling van de mogelijke effecten van een wereldwijde temperatuurstijging, en *Hemigrapsus takanoi* als niet-inheemse soort in de deltawateren.

Aan de doelstelling om nieuwe informatie te beschrijven over een weinig onderzochte soort (*Halicarcinus cookii*) is voldaan in dit proefschrift en deze informatie kan worden gebruikt als basis voor toekomstig onderzoek.

De hypothese dat temperatuur geen effect heeft op de voortplantingscapaciteit van krabben is ontkracht, aangezien bij twee bestudeerde soorten een vergelijkbare toename in voortplantingscapaciteit bij een temperatuurstijging werd geconstateerd. Dit suggereert dat een wereldwijde temperatuurstijging de voortplantingscapaciteit van krabpopulaties kan laten toenemen.

De analyse van de hypothese dat de introductie, aanwezigheid en effecten van *Hemigrapsus takanoi* in de deltawateren geen effect heeft op de inheemse krabbensoort, *Carcinus maenas*, wordt bemoeilijkt door de fluctuaties en afname van aantallen *C. maenas* vóór de introductie van *H. takanoi*. Ondanks dat *H. takanoi* niet de oorzaak was van de initiële afname van *C. maenas* kon de soort profiteren van deze afname en de niche bezetten die eerst door juveniele *C. maenas* werd bezet door middel van grootte afhankelijke competitie met en/of predatie op juveniele *C. maenas*.

Acknowledgments

Several people have been pivotal in my journey to finish this thesis. In particular, I thank my supervisor Ass. Prof. Colin McLay at the University of Canterbury in New Zealand without whom I would not have even started, let alone managed to complete this study. Also thanks to IMARES, Wageningen in the Netherlands and in particular Prof. dr. Han Lindeboom and Prof. dr. Aad Smaal for supporting me and providing the means to finish the study. Thanks also to dr. Jeroen Jansen for igniting the drive to complete this work when I had set it aside.

Thanks to Kimberley Seaward who shared in my experiences at the beginning of this study (see page 164) and the University of Canterbury, New Zealand for the use of their facilities in Kaikoura, New Zealand. Mandy Godschalk was also a big help in the field and in the lab during the work in the Netherlands. Thanks also to my mother-in-law, Penny Hutchens for all her proofreading services.

I also thank my husband Jonathon Hutchens who has been there to encourage me to get motivatied and stop procrastinating, as well as helping out with field and lab work despite not sharing the same fondness for crabs as I do. Thanks to Martine van den Heuvel-Greve for always being available to chat to, provide advice and encouragement, patiently listen to my complaints, feed me and provide a shoulder to cry on when required. Thanks also to her husband Bart and artistically gifted children Mare and Nanne.

Thank you also to my paranymphs, Martine and Karin for their support in the final stages of putting this thesis together.

Finally thanks to my twin boys who are still in utero as I write this (but not for much longer!). They provided me with a very strict deadline, and therefore motivation to have this finished.

Curriculum Vitae

Anneke van den Brink was born on 13 August 1981 in Christchurch, New Zealand. From childhood on she has been fascinated with nature, animals and the environment. After completing her schooling education in 1999, she spent her gap year in 2000 as an au pair in Michigan, USA where she also volunteered as a camera operator at the Grand Rapids Community Media Center. She then returned to New Zealand in 2001 to begin university.

Anneke completed a bachelor of science degree at the University of Canterbury in 2003 majoring in Biological Sciences with a GPA of 8.62 (grade: A). During this degree she also completed courses in other disciplines including Antarctic studies, psychology, linguistics, American studies and Russian history. Anneke was awarded a university prize for her high academic achievement.

As a university student, Anneke also worked as Research assistant to a PhD student under Ass. Prof. Bruce Waldman in research to extract DNA and locate and identify the MHC gene in the South African clawed frog, *Xenopus laevis*. She also worked as a home assistant to the elderly at a local retirement home.

After graduating with a BSc, Anneke continued on at the University of Canterbury to complete a master's degree with first class honours in marine biology in 2006 with a grade of A+. During this time she was awarded two scholarships: the Free Mason annual scholarship and a university master's scholarship.

During her masters research Anneke worked as a course demonstrator for undergraduate marine biology field trips and laboratory classes; assistant teacher for middle and high school students during rocky shore field trips; Field assistant for various marine ecology and marine mammal research projects around New Zealand for the Marine Ecology Research Group (MERG) and for Dr Laura Boren; a junior editor of final draft of a bio-chemistry text book for Icarus Publishing Ltd.; maître d' in a local restaurant and a life guard at the local swimming pool. She was also called upon as expert witness by Maritime Investigations New Zealand to research barnacles found on a life raft after a fishing boat capsized and to produce a deposition for trial.

After graduating with an MSc Anneke worked at the university of Canterbury as a research assistant for an environmental consultancy contract with the National Institute of Water and Atmospheric Research New Zealand (NIWA) where she assisted in cumulating information for the production of a report on research of shellfish abundance and distribution around Banks Peninsula for MERG under Prof. David Schiel.

In 2006 Anneke moved to London, UK. In London she worked as a contract employee at Coby Philips in PA/secretary/document controller positions for various engineering, management and advertising companies including Gratte Brothers, Hoare Lea, McCann Erickson Advertising and Turner & Townsend. During this time she also spent two weeks on Mt Iglit on the island of Mindoro, The Philippines conducting Tamaraw (*Bubalus mindorensis*) population surveys.

In 2008 Anneke returned to New Zealand to work as a science technician in the biodiversity and biosecurity group at NIWA where she was involved in updating the Cranfield (1998) report of all invasive marine species in New Zealand. She was also involved in Port Survey fieldwork, database management, report writing and rapid response activities when the European fan worm, *Sabella spallanzanii*, was discovered in the Port of Lyttleton.

In 2009 Anneke moved to the Netherlands to work as a researcher at IMARES (Institute for Marine Resources and Ecosystem Studies) where she is currently involved with national and international projects including shellfish aquaculture and diseases, invasive species and effects of human activities on benthic communities, as well as some PR design activities. During this time she has also completed the research for this PhD.