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CHEMICAL COMMUNICATION
IN MATING SHORE CRABS
CARCINUS MAENAS

Mattias Ekerholm

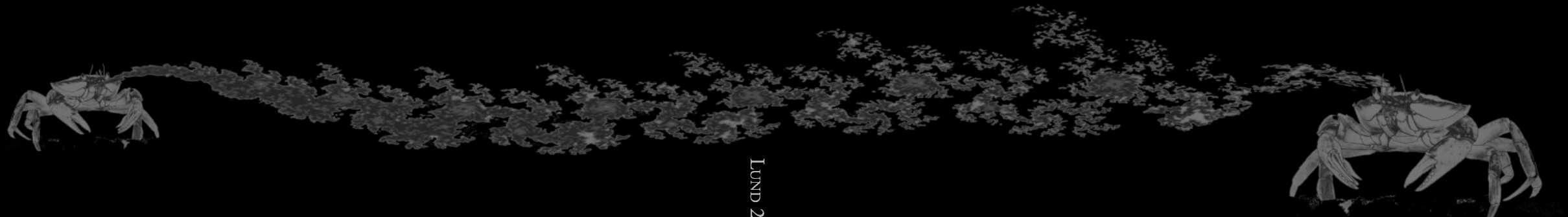
MATTIAS EKERHOLM

CHEMICAL COMMUNICATION IN MATING SHORE CRABS



LUND
UNIVERSITY

DEPARTMENT OF CELL AND ORGANISM BIOLOGY



LUND 2005

CHEMICAL COMMUNICATION IN MATING SHORE CRABS
Carcinus maenas

Mattias Ekerholm

Doctoral Thesis
Lund September 2005



LUND
UNIVERSITY

DEPARTMENT OF CELL AND ORGANISM BIOLOGY

Academic thesis in fulfillment of the degree of Doctor of Philosophy at the Faculty of Science at Lund University. The thesis defence will take place in the Zoology Building, Helgonavägen 3, Lund, at 10.00 am, September 29, 2005. Faculty opponent: Dr Thomas Breithaupt, University of Hull, Hull, UK.

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Title and subtitle Chemical Communication in Mating Shore Crabs <i>Carcinus maenas</i>			
Abstract <p>This thesis presents evidence that the shore crab <i>Carcinus maenas</i> utilize pheromones and other chemical signals for mating. Briefly, I have studied male and female responses to odors and urine from both sexes in long-range, near-range and at contact range. Males are attracted to female urine pheromones. When concentration and spatial scale are varied, the same chemicals (urine) evoke a chain of different behaviors. At close distance and high concentrations pairing-related behaviors are evoked, and search-related behaviors and display are abandoned. Conversely, low concentrations on longer distances show more search and display and no pairing behavior.</p> <p>Response variability plays a significant role in modifying behavior in this species. I have shown that much of this can be attributed to a novel primer pheromone, which increases male receptivity to the female pheromone. Female moult stage also affects male responses at contact-range, but not at near-range. Male status also affect his search, but not pairing behavior.</p> <p>The female pheromone has due to the lack of long-range properties not been able to explain how males locate females over a distance. Instead, present evidence suggested that the pattern may be reversed, with females locating males over a distance.</p> <p>I here present evidence that females of the right moult stage locate males over long distances. The females are able to discriminate between male and female odor and search preferentially for males. The pheromone in male urine evokes several pairing-specific behaviors in females, similar to the case of males and female pheromones.</p> <p>As a final step in linking pheromone communication to the lek mating system, we show that females are able to identify male status by odor, and preferentially search for the dominant male.</p> <p>This dissertation shows that chemical communication during the mating period is of utmost significance for a marine species, the shore crab. Apart from this I have shown that complex behavioral chains can be evoked by the same cue, presented at different spatial context and concentration. I have also shown how important it is to know the mating system of the species to be able to identify the steps where pheromones and other chemical signals affect behavior such as search and choice of a partner.</p>			
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MAIN REFERENCES

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Ekerholm, M. and Hallberg, E. 2005. Primer and short-range releaser pheromone properties of pre-moult female urine from the shore crab *Carcinus maenas*. *J. Chem. Ecol.* 31:1845-1864.
- II. Ekerholm, M. and Hallberg, E. 2005. Female moult state and male dominance modulate pheromone responses in the shore crab *Carcinus maenas*. (Submitted)
- III. Ekerholm, M. and Hallberg, E. 2005. Male odour evokes female courtship and attraction. -A male shore crab sex-pheromone? (Submitted)
- IV. Ekerholm, M. Wilke, L. and Hallberg, E. Odour-mediated female choice and dominance recognition in shore crabs *Carcinus maenas* (*Manuscript*)

CONTENTS

PREFACE	1
INTRODUCTION	2
BIOLOGY AND LIFE HISTORY OF THE SHORE CRAB	2
<i>Pairing and mating</i>	4
<i>A pest species, or not?</i>	4
CHEMICAL SIGNALS AND RECEPTION.....	6
<i>Chemical signals, infochemicals and pheromones</i>	6
<i>What is a pheromone?</i>	7
<i>What chemicals are pheromones?</i>	8
<i>The sense of smell and taste in crabs and crustaceans</i>	8
SEXUAL SELECTION AND MATING IN SHORE CRABS.....	10
MATING AND CHEMICAL SIGNALS.....	11
AIM.....	11
METHODOLOGY.....	12
<i>Assays at three different scales</i>	12
<i>Contact-range sponge assays</i>	12
<i>Near-range assays: olfactometer and Y-maze</i>	12
<i>Far-range trapping</i>	12
<i>Urine sampling</i>	14
<i>Determination of female moult stage</i>	15
<i>Conditioning of males</i>	15
PRIMER AND RELEASER PHEROMONES IN FEMALE URINE	15
STATUS AND MOULT STATE AFFECT MALE RESPONSES TO FEMALE URINE...	16
A MALE-EMITTED SEX-PHEROMONE	16
ODOUR MEDIATED FEMALE CHOICE	17
CONCLUSION AND FUTURE OBJECTIVES	17
REFERENCES	18
SWEDISH SUMMARY (HUR FÅNGAR HON DRÖMPRINSEN?)	23
ACKNOWLEDGEMENTS (TACK!)	26
PAPERS I-IV	

PREFACE

Chemical signals affect all organisms from bacteria to blue whales, fungi as well as plants and animals. In unicellular organisms, local interactions between the signal molecule (ligand) and receptors on the cell surface affect biochemical processes inside the cell, eventually resulting in a response such as altered metabolism, growth or perhaps movement. In multicellular organisms chemical communication occur both between cells within the organism and between the organism and its inner and outer environment.

Chemical signalling within our body allow control of growth, movement, and mental activity. Coordinated systems of neural activity mediated by neurotransmitters and modulators acting and reacting with pinpoint precision. Hormones released from organs in our bodies, carried by circulation, evoking a response wherever there is a receptor present, locally or globally.

Chemical signals between individuals, mediated by senses of smell and taste, allow communication, recognition of sex and physiological state, pairing, and eventually offspring.

*This thesis is focused on how chemical signals from males and females affect behaviour and physiology of the other sex, and how physiology of the sender affects the quality of these signals. The subject investigated was the shore crab *Carcinus maenas*.*

INTRODUCTION

Fishing crabs is one of the all time high summer occupations for kids anywhere along the west coast of Europe. Armed with mussels, shrimp, or fish entrails, and a simple string tied to the bait, the kids scuttle along sandy or rocky shores looking for the best crab site. Hours later the kids return to their parents exhausted, wet and in many cases with a skin tone too red to be healthy. The parents look at their son or daughter and the accompanying bucket, and are met with a “Mum, Dad, look!”. The bucket is filled with shore crabs (*Carcinus maenas*), an abundant resident under rocks, algae and sand all along the west coast of Europe. -Now, what to do with them? Cook them and eat them perhaps? Though they taste wonderful boiled with salt, half a sugar cube and crown dill, there is precious little to eat in them. Many kids (and students) arrange crab races, to see which crab first makes it to the water. But most often the answer is to let them back into the sea, to be taken up again the next day by other kids, fishermen, or an occasional scientist.

BIOLOGY AND LIFE HISTORY OF THE SHORE CRAB

The Shore crab *Carcinus maenas* occurs from Norway along the European west coast to West Africa. While ubiquitous in shallow waters it occurs down to a depth of 200 m. This crab species has been described as a voracious predator, but is actually omnivorous, eating live prey such as fish, gastropods, bivalves, annelids and crustaceans as well as carrion or algae (Johnston and Freeman, 2005). As many other crustacean species, it is cannibalistic. The adult colouration varies from green via yellow to red, almost always with a black pattern present on the upper surface of the carapace (head shield) and upper side and tips of the pereopods (walking legs) (Figure 1A).

The female crab grows to a size of up to 65 mm across the carapace, while males are considerably larger, reaching 80 mm, though occasional reports of larger crabs exist. Males also differ from females by having proportionately larger claws, used in competition for females. The most prominent difference is however, the seven segmented abdomen, which in females is broad, dark pigmented and serves as a brood chamber in which the eggs are attached to the four biramous pleopods (Figure 1B). The male abdomen is five segmented (3-5 are fused), more narrow, and lacks dark pigmentation (Figure 1C). In males, only the two first pleopods on each side are present. These are modified to form a copulation organ, transferring the spermatophores to the female gonopores. Fertilization occurs internally and the eggs are later extruded and attached to the pleopods. The egg hatch to a pelagic zoea larva, and after the fourth instar, the zoea (Figure 1D) goes through a metamorphic moult into the semi-pelagic settling instar called the megalopa (Figure 1E). When the megalopa finds a suitable environment, the larva moults into a 1.5 mm juvenile crab (Figure 1F). This occurs from 4 to 6 weeks after

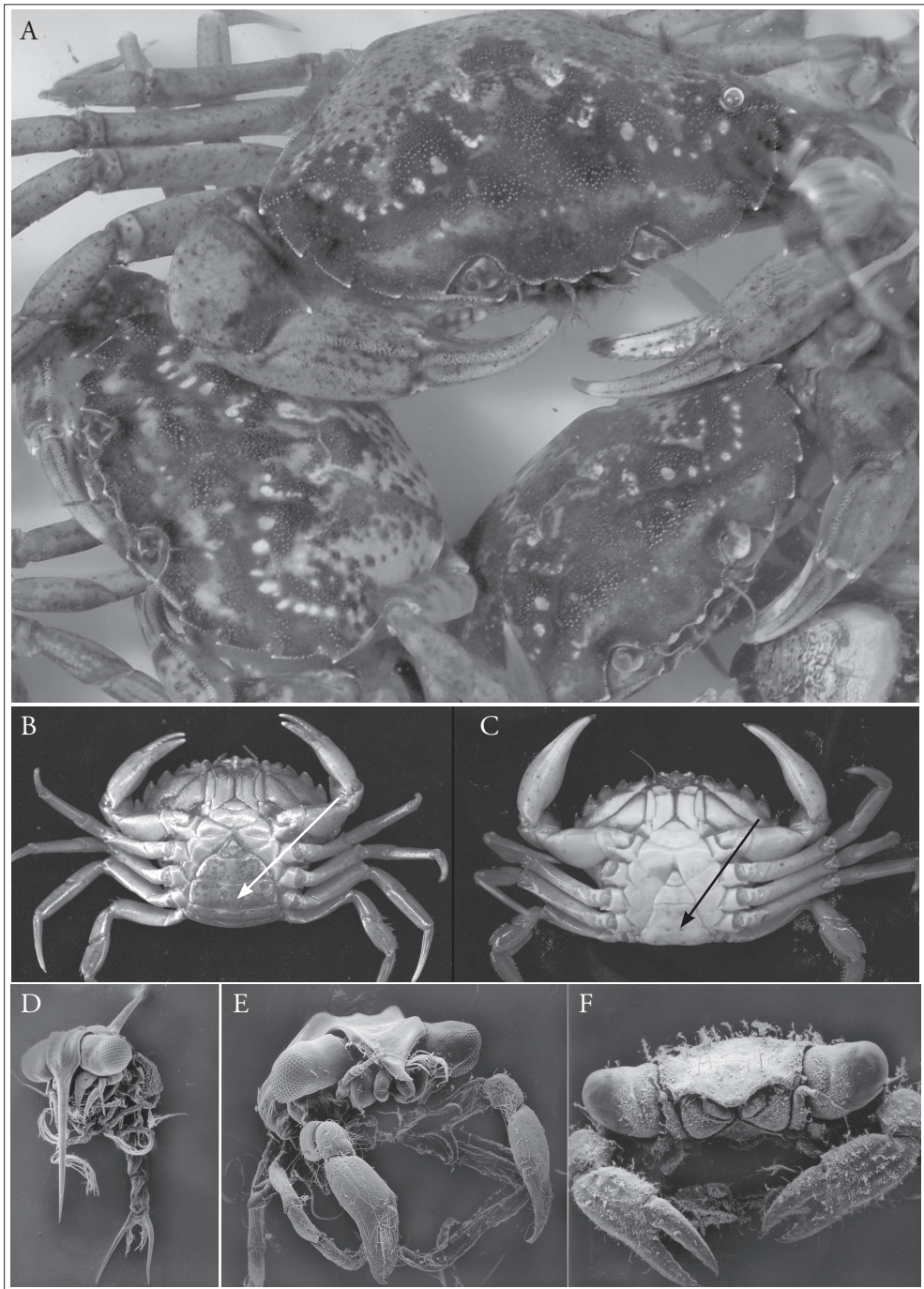


FIG. 1. Shore crabs of different sex and life stages. A) Photograph of adult males. B) Ventral view of a female crab, note the wider, dark pigmented abdomen (arrow). C) Ventral view of a male crab showing the narrow abdomen with fused segments (arrow). Scanning Electron micrographs of: D) Zoea IV larva, E) Megalopa larva, and F) First instar juvenile crab.

hatching, depending on food and water temperature. One to one and a half year later the crab becomes sexually mature. It has then moulted 10 to 11 times since it became a juvenile crab and has reached a carapace width between 30 and 45 mm (Rice and Ingle, 1975; Mohamedeen and Hartnoll, 1989).

Pairing and Mating

In spring, as the water temperatures start to rise the shore crabs start to move to shallower waters (Crothers, 1967; Crothers, 1968; van der Meeren, 1994). Mating season starts in June, with males aggregating at certain hot-spot areas. Here they compete for access to the females entering the area for mating (van der Meeren, 1994). When the male encounters the female, he displays a pose raising high up on walking legs 2 to 4, and holds the fifth pair of legs at or above the level of the carapace (Figure 2A) (Berrill and Arsenault, 1982). The male walks around posing and finally grabs the female with his pereopods but not the claws. The male holds the female in a pairing stance referred to as pre-copulatory embrace, or cradle-carrying (Figure 2B). Pre-copulatory cradle-carrying continues until the female moults, between a few days and a week later. When the female moults, the male stand guard over her and may even help her to remove the old exoskeleton. When the female first appears from the old shell, she is in a gelatinous state, but this quickly changes. After 30 min to an hour later the female shell is hard enough to allow mating. The male then picks up the female and carries her ventral side up, this is referred to as the copulatory embrace (Figure 2C). Both crabs then extend their abdomina (Figure 2D), and the male use his gonopods (modified pleopods) to transfer the spermatophores to the female. Copulation lasts between one and three days, followed by a period of post-copulatory cradle-carrying which lasts up to four days (Berrill and Arsenault, 1982). Male investment in post-copulatory cradle-carrying is thought to be advantageous for the male, increasing reproductive success by ensuring exclusive fertilization of the female by excluding other male copulations, and by increasing the females' probability to survive after moult through protection.

A pest species, or not?

The high numbers of active crabs during the summer months that makes kids so occupied with crab fishing also has its drawbacks. Fishermen get crabs in their nets, and since there are few uses for large amounts of shore crabs, there is no benefit from catching them. On the contrary, the crabs get entangled in the nets, and removing them may take hours. While in the nets, the claws gnaw through the nylon netting, leaving holes that have to be repaired. Finally, and most annoyingly, they eat the fish caught in the net and may reduce a sleepy fisherman's bounty to mere bones.

- Imagine the following scenario from somewhere along the coast of Europe:

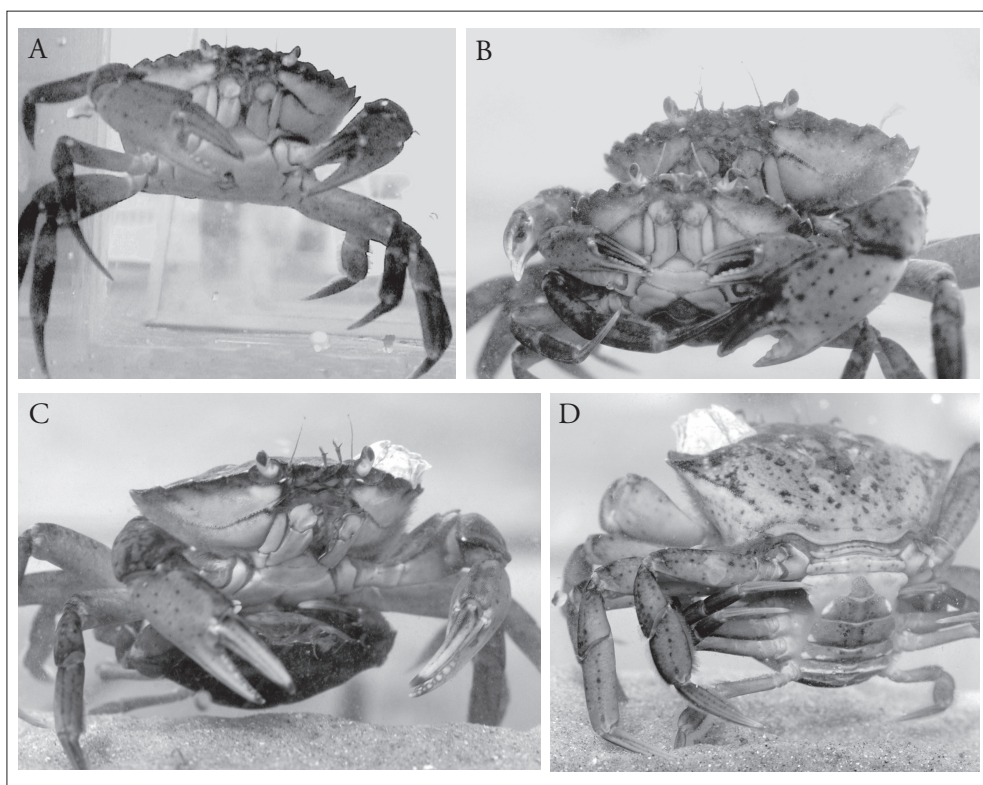


FIG. 2. The most obvious components of shore crab pairing and mating behaviour. A) The male pose. B) Pre-copulatory cradle-carrying, here the male carries the female under him using his walking legs. C) Copulation, this occurs shortly after the female moult. D) Rear view of copula, showing the unfolded male and female abdomens allowing spermatophore transfer.

Part-time fisherman Bob has just started up his small fibre-glass boat. Easing out of the harbour, he give his outboard engine more gas, and the steady hum turns into a roar, echoing out over the mirror blank morning sea outside the little village. A few hundred meters further ahead, he sees the red marker buoy indicating a string of gill nets. The boat eases down and glides over the calm water surface. Looking good, Bob states, seeing gaps in the gill net's floatline at a few places. There might be a few trout there, after all. The net feels heavy, there is definitely something there. Just hope that no crabs have gotten into the net, yet. After pulling in a few meters of net he spots the first crabs, not a good sign. Two hours later he pulls into the harbour to sum up today's catch. One small trout, thirty-three flounders (seven half eaten), three small cods, the carcasses of two trout and salmon between two and three kilos, and about five hundred shore crabs gnawing their way through the nets.

It is easy to understand that the shore crab generate mixed emotions. While not being a species of high direct economic impact in most European countries it nonetheless has a high general interest. There is also general consensus in that the numbers of shore crabs have increased along the Swedish west coast during the last decades. So, both children and fishermen will see a lot of these crabs in the foreseeable future.

In other parts of the world, another picture is emerging:

The shore crab, unintentionally introduced to North and South America, South Africa and Australia is a threat to native fauna and aquaculture. Due to its opportunistic life strategy and adaptability it has caused major concern to local governments, and several different strategies to monitor and control this species has been developed (Leroux et al., 1990; Griffiths et al., 1992; Cohen et al., 1995; Thresher et al., 2000; McDonald et al., 2001; Carlton and Cohen, 2003; Thresher et al., 2003).

None of these employ a pheromone based control or monitoring system, something that has been very successful in insect pest management. The two most common methods used for insect pest control are trapping methods, where males are lured to traps over long distances using synthetic female pheromones and mating disruption methods, where female pheromones are dispensed over an area, making males unable to track females due to the more potent synthetic sources of pheromones (Svensson, 2002).

CHEMICAL SIGNALS AND RECEPTION

Chemical signals, infochemicals and pheromones.

Chemical signals perceived by the sensory organs of olfaction and taste play an important role in everyday life for most organisms. The first thing that comes into mind when talking about odours and tastes, is often food, but chemical cues are used for so much more.

For example, oyster larvae use chemical signals for finding their preferred habitat (Zimmer-Faust and Tamburri, 1994).

Turtles (Lohmann et al., 1999), lampreys (Fine et al., 2004) and salmon (Doving and Stabell, 2003) migrate over long distances using odour trails as navigational cues.

Odours from predators such as pike evoke escape or hiding responses in prey organisms (Kats and Dill, 1998).

Subordinate lobsters and crayfish avoid dominant ones by using urine cues, which maintain dominance hierarchies (Breithaupt and Eger, 2002; Karavanich and Atema, 1998).

Finally, mate attraction and recognition in moth, polychaetes, crustaceans, fish, and several mammals are guided by odours from conspecifics (see Wyatt, 2003 for an overview).

Chemical cues may be divided into three groups, nutrients, toxins, and infochemicals. An infochemical is a chemical conveying information between two individuals, evoking a behavioural or physiological response in the receiver. Infochemicals are divided into two main groups, *allelochemicals* and *pheromones* (Dicke and Sabelis, 1988).

An allelochemical is a chemical that mediates an interaction between two individuals of different species. Depending on if it is beneficial to the sender, receiver or both it is classified as an *allomone*, *kairomone*, or *synomone*, respectively (Nordlund and Lewis, 1976).

An example of a species using an allomone is the spider orchid *Ophrys sphegodes*, which lures male bees with an odour resembling the female bee pheromone (Schiestl et al., 2000). When the male bee alights, the flower deposits pollinia on the bee. The bee then transfers the pollen to another flower, but gets nothing in return.

Kairomones are in many cases inadvertently emitted substances leaked from predators and used by prey for avoiding them (Kats and Dill, 1998). In extreme cases, exposure to a kairomone can lead to induced changes in body shape of the prey to avoid predation. This occurs in crucian carp *Carassius carassius* exposed to pike odour (Brönmark and Miner, 1992) and *Daphnia* exposed to odours from predatory fish and invertebrates (Laforsch and Tollrian, 2004).

The best examples of synomones are flower odours, which attract insects feeding on nectar in the flowers. The insect then transfers pollen to another flower, thus benefiting both parts.

What is a pheromone?

A pheromone is a chemical used for communication between members of the same species. While defined by Karlson and Lüscher (1959) as “substances that are secreted by an organism to the outside and cause a specific reaction in a receiving organism of the same species”, to differentiate these substances from hormones. This definition no longer holds true, mainly due to the expansion seen in the chemical communication research area. In this thesis I will instead use the definition proposed by Dicke and Sabelis (1988), which is based on cost-benefit analysis, a more suitable way of classifying an unknown infochemical (chemical utilized for communication purposes).

This states that a pheromone is:

An infochemical that mediates an interaction between organisms of the same species in which the benefit is to the origin-related organism ((+ , -) pheromone), to the receiver ((- , +) pheromone), or to both ((+ , +) pheromone).

Sexual pheromones (used for communication between the sexes) in general fall under the (+ , +) pheromone definition, while components mediating choice in the other sex may actually fall under the (- , +) pheromone category depending on quality of the emitting individual.

In addition to this, pheromones are also classified into two groups depending on the effects they have on the receiving organism. A pheromone evoking a direct behavioural response is classified as a *releaser pheromone*, while a pheromone altering physiology without directly affecting behaviour in the receiver is classified as a *primer pheromone*.

The silk moth, *Bombyx mori*, from which the first pheromone was isolated (Butenandt et al., 1959), illustrates the classic view on behaviour (what I later refer to as the “Simplified moth model”) in relation to insect pheromones. Here, the female emits a sex-pheromone into the air, evoking male upwind search over long distances. When the male has found the female, he lands next to her and emits his (short distance) pheromone. If he is accepted, mating follows. Today, knowledge on pheromones from a large variety of species has produced a much more diverse picture. The current view is instead that the actions and spatial effectiveness of a pheromone is correlated to the mating system, thus either the male or the female or both emit pheromones responsible for attraction and evaluation of the other sex.

What chemicals are pheromones?

A common misconception is that pheromones belong to a certain group of chemicals. While it is true that many Lepidoptera utilize structurally related compounds for sex-pheromone communication and use common biochemical pathways (see Rafaeli, 2002), a rich and diverse flora of mainly volatile chemicals are used by terrestrial organisms in general.

In aquatic environments, most signal molecules seem to be directly related to their physiological or energetical functions. Substances may be large or small, polar or volatile, and still be used for signal purposes due to the physical properties of water.

This suggests that no common chemical property of pheromones used in water need to be present (like volatility needed for dispersion in air). Instead, chemical methods to identify the nature of aquatic pheromones need to be developed on a case-by-case basis, making isolation a tedious business. Present evidence origin from fish (Dulka et al., 1987; Sorensen, 1992), worms (Zeeck et al., 1988; Zeeck et al., 1994; Hardege et al., 1996; Hardege et al., 2004), and brown algae (Maier and Müller, 1986; Boland, 1995). In fish, reproductive hormones such as steroids and prostaglandins function as reproductive pheromones (Stacey, 2003). Polychaete worms use several classes of compounds as sex-pheromones. Depending on species, volatile substances (Hardege et al., 1996), peptides (Hardege et al., 2004) and steroids (Zeeck et al., 1994) may be utilized. However, no crustacean sex-pheromone with verified activity is published to date.

The sense of smell and taste in crabs and crustaceans

Chemical cues are interpreted and coded by the sensory organs for smell and taste. In aquatic animals, these senses may best be separated according to their functional properties, such as spatial range and response threshold properties. Anatomical definitions do exist, but they differ between genera and are therefore not suitable for generalisation. Olfaction (the sense of smell) is defined as long range chemoreception, while taste is defined as close or contact-range chemoreception. Sensory cells in olfactory organs have low threshold sensitivities (sub-nanomolar range in crustaceans (Derby et al., 1991)), while response thresholds of taste receptors are generally higher (typically in the micromolar range (Corotto et al., 1992; Garm et al., 2005)).

Crustaceans, as all arthropods are covered in the exoskeletal cuticle, and this includes the sensory organs. In order to obtain contact with the environment, chemo- and mechanosensory organs are present in (most often) hair-like cuticular structures referred to as sensilla or seta. Specialisations in the cuticle such as basal articulations, pores or sponge-like structure mediate contact between sensors inside the sensillum and stimuli in the surrounding medium. The main olfactory organ of crustaceans are the aesthetasc sensilla (Figure 3A), located on the first antenna (antennule) (Hallberg et al., 1992; Hallberg et al., 1997). However, other sensilla present on the antennules also mediate chemosensory information (Horner et al., 2004). While both types of sensory organs mediate food-search (Horner et al., 2004), the aesthetasc pathway also controls more advanced processing and recognition tasks (Steullet et al., 2002), and is involved in pheromone detection (Gleeson, 1982).

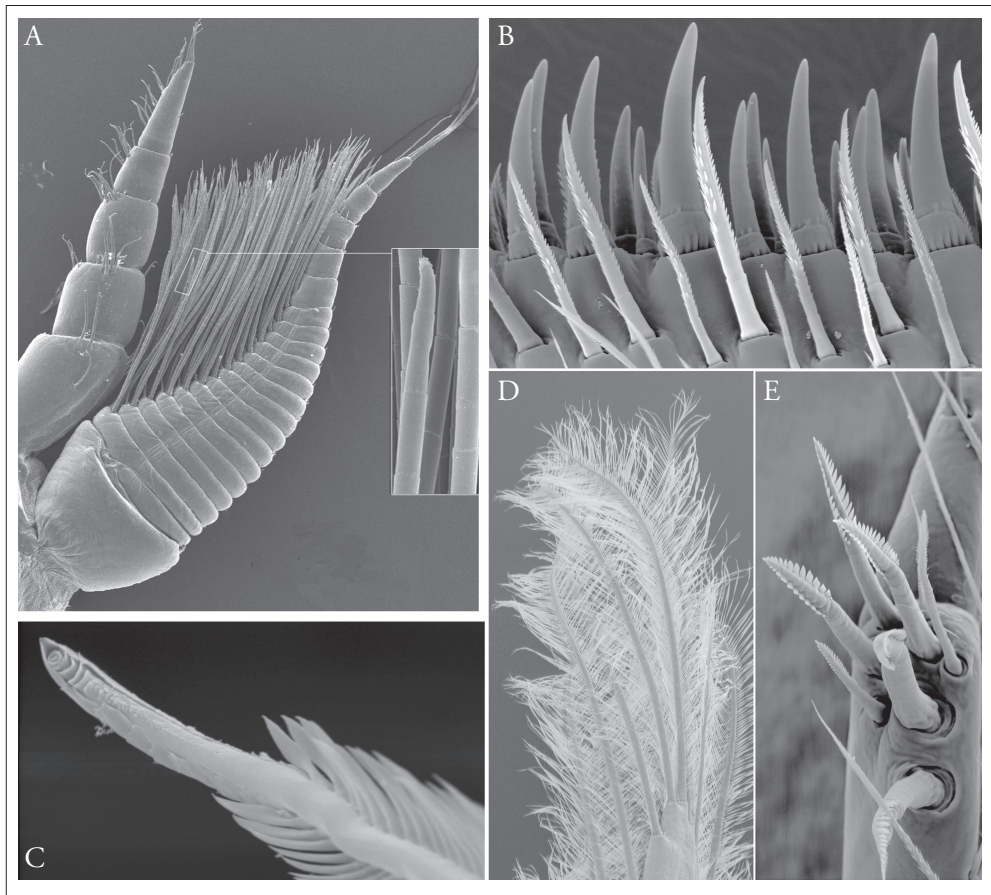


FIG. 3. Scanning electron micrographs of crustacean chemosensory organs. A) The medial and lateral flagellum of the antennula in *C. maenas*. The lateral flagellum contains a dense array of aesthetasc sensilla. Inset shows repeated annulations of the aesthetascs. B-E shows morphological variations in mouthpart setae of various decapod crustaceans. (A: courtesy R. Wallén. B-E: A. Garm)

The taste sensilla are located on claws, walking legs and mouthparts (Figure 3B-D). Most of these sensilla have mechano-, as well as chemosensory functions (Derby, 1982; Corotto et al., 1992; Voigt and Atema, 1992; Garm, 2004; Garm et al., 2004; Garm et al., 2005). Molecules used by the senses of olfaction and taste in the marine environment are often common tissue constituents such as amino acids, nucleotides, nucleotides and ammonium bases (Carr and Derby, 1986; Kasumyan and Doving, 2003).

Conversely, most molecules utilized by olfactory organs in terrestrial animals are volatiles. However, some taste receptors are sensitive to amino acids (Nelson et al., 2002) as well as commonly known: sugars, salts, acids and toxic substances (bitter). The evolutionary benefit of having receptors identifying amino acids is clear, since these substances are the building blocks of proteins and may also serve as metabolic fuel. Though terrestrial animals originate from aquatic ancestors, terrestrial animals are not able to use amino acids for olfactory based identification and food search. The reason for this is that they are non-volatile and thus not dispersed by the wind. Hence utilization of these chemicals in air became restricted to contact distances, and therefore taste.

SEXUAL SELECTION AND MATING IN SHORE CRABS

Sexual selection is one of the most important factors that influence the reproductive success in an individual (fitness). The two main forces shaping the outcome of this are intersexual selection and intrasexual competition (Darwin, 1879). Depending on the balance between these forces, and how they affect each sex, several different mating systems may develop, from strictly monogamous to highly polygamous. Lek mating systems have highly skewed operational sex ratios and the main selection pressures are male-male competition and female choice (Emlen and Oring, 1977). Shore crabs show lek behaviour in the field (van der Meer, 1994) and also show male-male competition for females and female choice of males in the lab (Sneddon et al., 2003). This is a normal scenario in lekking species, however males spend considerable resources fighting (Sneddon et al., 2000; Sneddon et al., 2003), and stopping takeover attempts during the period of pre-copulatory cradle-carrying (mate guarding) of the female. Because this is a costly behaviour, there is a possibility of male choice. Olfactory cues could be utilized for evaluation of choice factors, especially in females, but perhaps also in males. Given the high olfactory dependence in crustacean behaviour (Atema, 1995), it is likely that chemical cues play a role for several aspects of shore crab mating behaviour.

MATING AND THE SENSE OF SMELL (A SYNOPSIS OF MY THESIS WORK)

Crabs and lobsters have been known to utilize chemical cues in reproduction since the 1960s (Ryan, 1966; Atema and Engstrom, 1971; Christofferson, 1974; Eales, 1974; Gleeson, 1980). The general notion at that time was that a female pheromone was responsible for attracting males over a distance, much like the simplified moth model. This view prevailed and several attempts to isolate the pheromones from female crabs and lobsters using various behavioural assays failed, although the presence of a pheromone became more well-established. Since then, a few attempts at isolation has been made (Asai et al., 2000; Kamio et al., 2000; Asai et al., 2001; Hardege et al., 2002; Kamio et al., 2002) but no evidence of successful isolation is yet available. Studies on the mating system in *C. maenas* showed evidence for a pheromone, and subsequent results have shown that males exhibit higher activity and are attracted to premoult female cues over very short distances (< 0.5 m) (Seifert, 1982; Bamber and Naylor, 1996b; Bamber and Naylor, 1996a). While male attraction to a female pheromone is coherent with the simplified moth model (below), the crucial long distance search has not been shown in any crustacean.

AIM

The aim of my thesis work was to investigate if there is indeed a sex-pheromone present in the shore crab, and evaluate its actions eventually leading to the isolation and production of a sex-pheromone utilizable for monitoring of this species. Since many of the commercially utilized moth pheromones depend on the long-range properties of the pheromone, I started out with what I call the simplified moth model. Under this model the following three statements should be true:

1. There is a female pheromone selectively attracting males of the same species.
2. The pheromone should attract males over long distances to be effective.
3. A major part of the sexually mature males within the effective range of the pheromone should be attracted.

Published results present at the time of the beginning of my thesis work suggested that female shore-crab pheromones existed, but this needed to be verified (Eales, 1974; Seifert, 1982; Bamber and Naylor, 1996b; Bamber and Naylor, 1996a; Bamber and Naylor, 1997). There was also doubt about the generality of male attraction to females, which I aimed to investigate under the hypothesis of male choosiness. I ended up falsifying 2, and 3 (obviously), and was lead on another track during the way...

METHODOLOGY

Assays at three different scales

An animal tracking an odour to its source may go through three stages of search behaviours. First the long-distance search guides an animal to the vicinity of the odour source. At near distance the animal homes in on the odour source, and finally, when close to, or in contact with the odour source, it displays behaviours appropriate for the type of odour source it has found, such as eating, fighting, or in the present case, pairing. In order to evaluate the functions of a pheromone at all three spatial scales, I developed or modified methods to assess this.

Contact-range sponge assays.

Crab-sized polyurethane sponges are treated with pheromone solutions and function as dummies that are presented to crabs in small aquaria (see I and III in thesis for details) (Figure 4). This method has allowed me to verify that several mating-specific releaser behaviours are evoked by crabs presented with a sponge containing urine from the opposite sex (I, II, III). Behavioural analysis in the female pheromone assay utilizes seven individual behaviours and summed weighted behaviours. Most beneficial is that large numbers of crabs are easily tested using this quick, reliable and mobile assay.

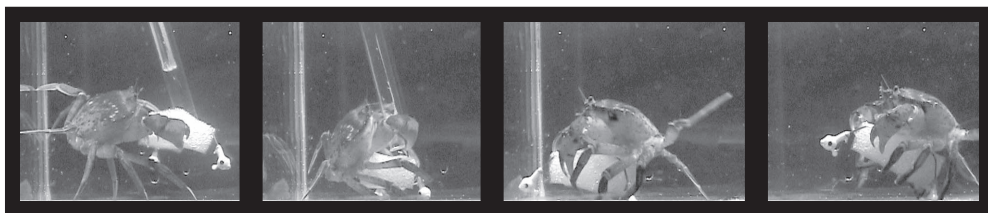


FIG. 4. Behavioural sequence shown by a *Carcinus maenas* male towards a female urine treated sponge served with a pair of forceps. From left to right: the posing reaction, the grabbing of the sponge, sponge-handling and cradle carrying, and finally cradle-carrying and stroking.

Near-range assays: olfactometer and Y-maze

Two different methods were used for studying near-range attraction, the olfactometer, measuring activity (Figure 5) (I, II) and later, the Y-maze (Figure 6) (III, IV). The Y-maze flume has proven to be a more versatile method allowing both choice and activity to be measured at the same time. Stimuli in both these tanks can be delivered as: 1) Whole animals in net cages. 2) Passive odour sources in sponges, plaster or glucose gel. 3) Odour solutions using a peristaltic pump.

Far-range, trapping experiments

Paired large traps (Figure 7) baited with live crabs or with crab odours are used for evaluation of long range properties of chemical signals through quantitative and qualitative analysis of animals caught (I, III). This also serves as validation that a specific odour source finally can be utilized for biological control purposes.

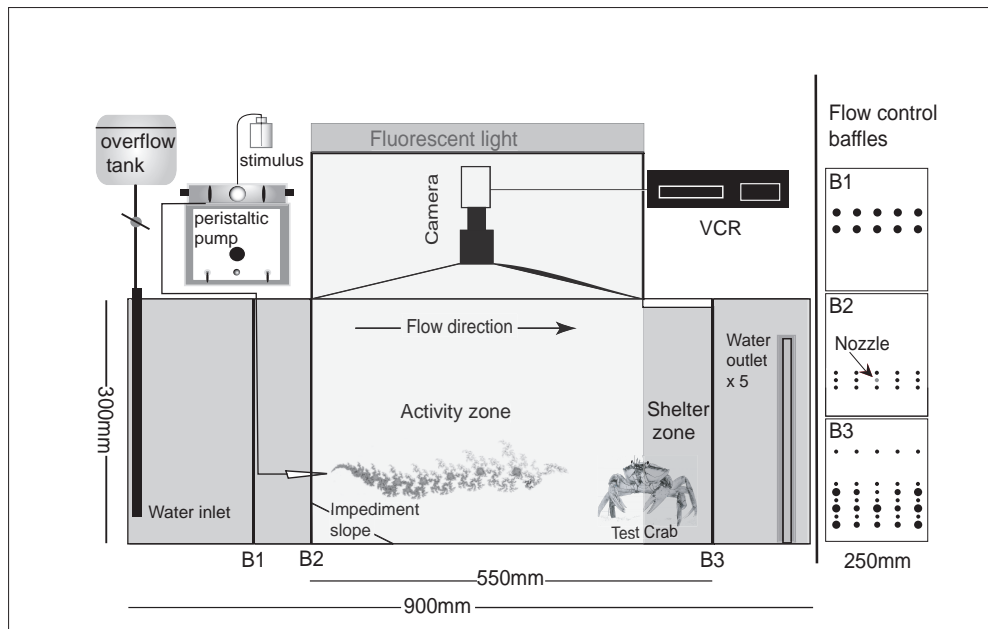


FIG. 5. The olfactometer tank setup, where I used a pump-generated plume as a stimulus source, and recorded male search activity patterns to female urine in a controlled flow environment.

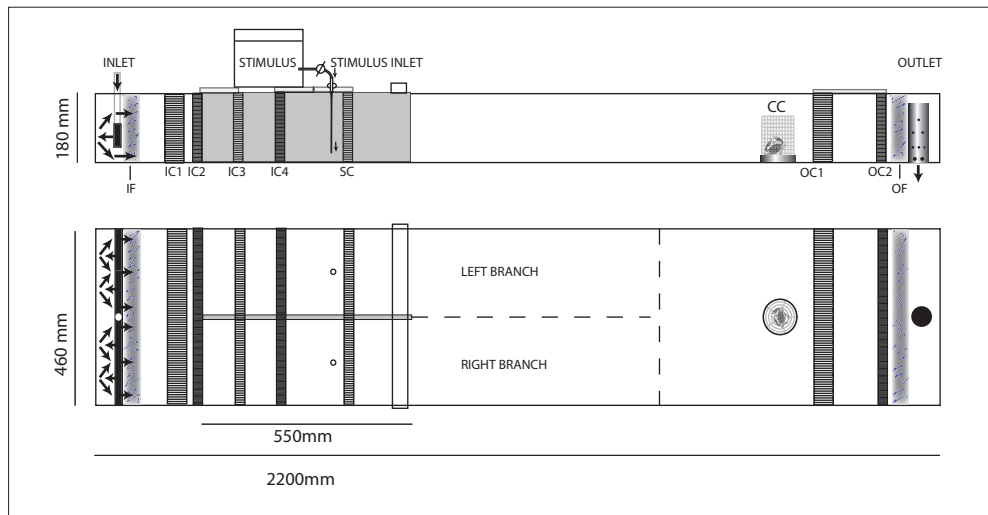


FIG. 6. The Y-maze flume used for studying female choice to conspecifics. Above: side view. Below: horizontal view. The upstream part of the y-maze is partitioned into two branches, left and right. Controlled flow maintains separation between the odour sources in the left and the right branch until they reach the downstream 1/3 of the arena (indicated by dashed vertical line). Odour water (stimulus) is gravity fed from a container above the flume.

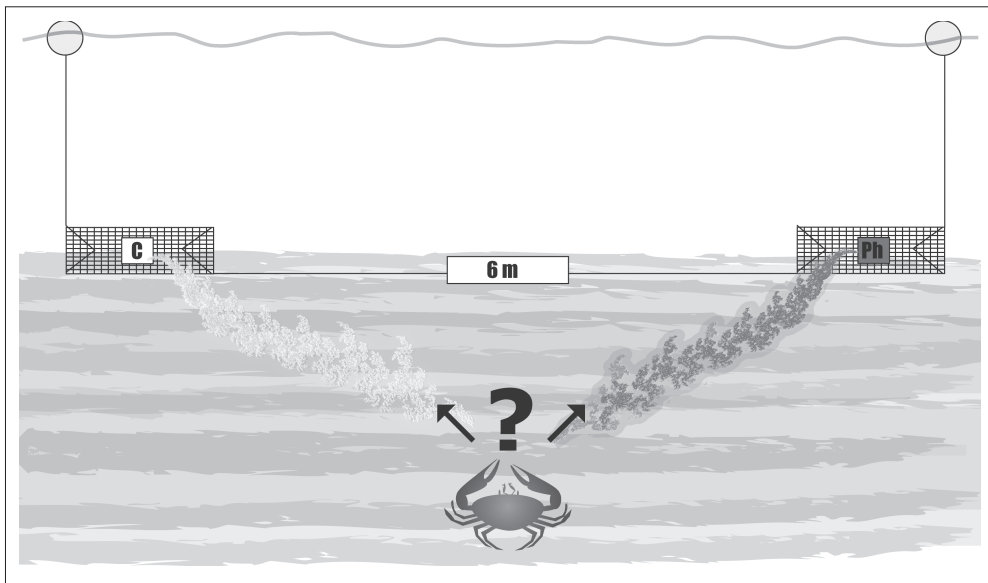


FIG. 7. The paired trap setup, used with several trap sizes to verify chemical attractiveness of the pheromones in the field. Ph=Pheromone, C=control

Urine sampling technique

A crab is strapped with rubber bands to a wooden board. The urine is then collected from the opening of the antennal opercula using a micropipette (Figure 8) (Bamber and Naylor, 1997). The urine from each individual crab is transferred to a microcentrifuge tube and immediately frozen at -20°C . Unimpaired activity of the urine pheromone is retained for more than four years if stored at this temperature. Collection from females is made at a daily basis until 7 days after moult. Time of urine sampling, and moulting of the female are noted for future reference.

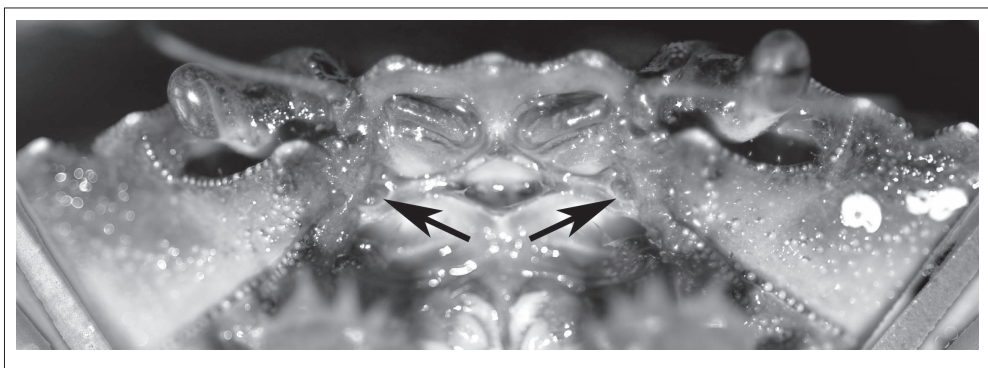


FIG. 8. Ventral view of a crab mounted for urine sampling. Arrows show the paired nephropore openings covered by an opercula. The operculum is lifted with a fine hooked needle to allow urine to be sampled using a micropipette tip and a syringe.

Determination of female moult stage

Female shore crabs are kept at high density (100/m²) in communal tanks. One male crab for every ten females is added to the tank to find females of the appropriate moult status, indicated by male pre-copulatory cradle-carrying. Tanks are searched for pre-copula pairs, which are removed from the communal tanks and separated. After separation, females are transferred to individual tanks for urine sampling.

Conditioning of males

Establishing subordinate or dominant status in male crabs is done by putting subject males in aquaria together with two other males not used for testing. Males conditioned to be subordinate are put in tanks containing two larger males, while males to be dominant are put in tanks containing two smaller males. An opened blue mussel *Mytilus edulis*, is lowered into the aquarium to promote conflict. Behaviour of the experimental animal during the fight for food is classified as dominant if the experiment male climbed on top of the other male(s) or elicited repeated retreats from the other males (Sneddon et al., 1997). The animal is considered subordinate if it was below the dominant and retreated repeated times. 98% of the crabs show successful conditioning using this method.

PRIMER AND RELEASER PHEROMONES IN FEMALE URINE (I)

In this paper, male responses to female urine pheromones at different concentrations in three spatial contexts were studied. We used the sponge assay, the olfactometer and trapping, to see which behaviours was evoked by pheromones delivered at different scales, and to see which concentrations was needed to evoke a certain behaviour. Our results state that there is a chain of behaviours evoked by increasing pheromone concentrations. Male response to female pheromones can vary considerably. To avoid this, some earlier investigations used only pre-copula males for studies of female pheromones (Bamber and Naylor, 1996a; b; 1997). Differential responses may be caused by several different factors. In goldfish, pheromones act both as primer and releasers, with the primer reinforcing releaser responses (Stacey, 2003). In analogy to this, I speculated that better pheromone responses from paired males may be caused by previous exposure to the pheromone, reinforcing the releaser response through priming. This turned out to be the case, but even so, priming does not explain all variation.

STATUS AND MOULT STATE AFFECT MALE RESPONSES TO FEMALE URINE (II)

Another thing worth considering when it comes to the differential expression of male response is male status. Though several papers have described shore crab distributions in the field, these only provide secondary evidence about mating system in this species (Edwards, 1958; Naylor, 1962; Crothers, 1968; Reid et al., 1994). To my knowledge, only one thorough field study has addressed the mating system of the shore crab (van der Meer, 1994). However, this study provides valuable insight, and states that the males gather at leks visited by females. A lekking mating system is also what would be suggested on pure theoretical grounds, (Emlen and Oring, 1977). Since different status-dependent strategies at a lek may affect male choosiness, this may be another cause for differences in male pheromone response. To evaluate if male status indeed affect male responsiveness, I performed a series of experiments in which I manipulated male status irrespective of male size, and tested responses at near and close/contact distance. Female moult state was also taken into account. We found out that males are choosy only at short distances, and that male search patterns vary depending on status.

A MALE-EMITTED SEX-PHEROMONE (III)

Since all my evidence suggested a short-range pheromone and conformed to a lek mating system, I realized that the simplified moth model did not fit crab pheromone communication, and had to search for another model. I realized that the lekking may be the key to the pheromone communication system. One of the main issues regarding lek formation discussed in undergraduate textbooks is the hot-spot theory. Here, the sensory stimuli from a group of males at a hot-spot attract females. A mating system where groups of males attract females from a distance (according to the hot-spot theory), and then females attract males nearby would make more sense than the moth model for shore crab communication. The first thing I had to prove was female attraction to males. Luckily, an ongoing project studying effects of copper poisoning on pheromone communication (Ekerholm and Krång, in prep), showed female search for males, since the males acted sluggish due to effects of copper. Later, another study verified female attraction to males under laboratory conditions (Sneddon et al., 2003). Since females seemed to be attracted to males under laboratory conditions, I now switched to the hot-spot model and, began investigating if females were attracted to males in the field and also, more specifically attracted to male odours. In a series of experiments collected under paper III, I investigated the effects of males, male odour and male urine on female search, and stereotyped releaser behaviours. Again we used three different spatial contexts to validate the findings. After this, it was clear that females are indeed attracted to groups of males. Male odour induced increased activity and search for males at a meter scale in the Y-maze. Finally, female courtship behaviours could be released using only a male-urine treated sponge, stating that there is indeed a pheromone present in male urine.

ODUR-MEDIATED FEMALE CHOICE (IV)

In most lekking species, female choice is pronounced, and since males fight readily for access to females (Sneddon et al., 2003), a female who chooses a large dominant male is more well protected towards takeover attempts. Lobster females choose dominant males for mating (Cowan and Atema, 1990). Likewise, female shore crabs choose larger, more dominant males (Sneddon et al., 2003). In both European (Skog et al, in prep) and American lobsters (Karavanich and Atema, 1998) and crayfish (Breithaupt and Eger, 2002), males are able to discriminate between dominant and subordinate state individuals on olfactory basis. If I wanted to further validate the hot-spot model, I needed to find out if females could choose between dominant and subordinate males. More specifically, could they discriminate between the odours of dominant and subordinate males, and choose one in particular? This was actually the case, which leads to several important conclusions. We now have evidence that support a pheromone communication system tuned to hot-spot mating. Under this model, females about to moult locate male groups from long distance based on pooled male odour cues. At the hot-spot, females search for and choose between males based on male status, reflected by odour. At the same time, females emit pheromones with the urine, attracting males from the neighbourhood. The male and female, perceiving each others odour, pose and initiate contact behaviour. The male then grabs and cradle-carries the female, influenced by the female pheromone. A primer pheromone reinforces male cradle-carrying during this time.

CONCLUSION AND FUTURE OBJECTIVES

During the progress of my thesis I have been able to verify the presence of pheromones in female urine, and during the course of time revised the chemical communication model from a single pheromone system to a mating system-related, three pheromone-dependent system relying on both primer and releaser components, male as well as female pheromones. Moreover, urine in both males and females contain cues mediating choice in this species. I have been able to show that stereotyped mating behaviours in males as well as females are evoked by chemical cues from the opposite sex, and that dominance and physiological state affect the quality of this signal and how it is perceived.

This new view of chemical communication in crabs has left a field wide open for subsequent investigations. Male cues and interactions between male and female cues should be investigated further. From a pheromone isolation, and applied biological control point of view, I am more or less back where I started, with one big difference: We have found a putative pheromone with long-range properties. Therefore, chemical identification of the male pheromone, which could be used for biological control, would make even more sense than for the female pheromone. Especially since the target is now the limiting sex, making biological control more effective. During the development of this pheromone based biological control system, a series of questions of high scientific interest will also be answered.

In my opinion this should begin with an attempt to isolate the male pheromone, starting with localisation of the source. Apart from being given off in the urine, other sources may exist. Odours from males with putative pheromone-release sites blocked will be tested on females in Y-mazes. Potent sources of pheromones will be identified by using male tissue extracts. Pre-preparation purified extracts, followed by HPLC fractionations of the most potent stimulatory extracts will be tested using the sponge assay.

Another important question that should be addressed at the same time is how pheromone odours are spread in nature, and how crabs locate their source. Hence, a closer look on how odour release around a hot spot works is required. Artificial hot-spots can be created in the field, using large traps "baited" with different numbers of crabs, catching attracted individuals. A simultaneous release of marked crabs and crabs with transponders which will be traced using telemetric methods would shed quantitative as well as qualitative detail of this. Attraction of transponder-marked animals to naturally occurring hot-spots should also be studied. Of vital importance is a simultaneous monitoring of currents in the adjacent water to enlighten mechanisms of odour spread, such as effective attraction distances in relation to currents.

GC-EAG has proven valuable for identification of odour molecules in many insect species. To get an electrophysiological backup to the behavioural assays, we first need to find out which sensory organs are associated with pheromone reception. Selectively ablating sensilla on the crabs, and subjecting the crabs to sponge assays and y-maze tests should shed some light on this. The dual tests will allow investigation if both long-range and contact-range pathways are involved.

To optimize the chemical mixture of the synthetic pheromone, an investigation on how carapace colour, size and status affect odour-based female choice is necessary. How these factors influence female search and mating behaviours should be tested using the Y-maze and the sponge assay. If female choice of male odour is affected by the above factors, their chemical identity should be investigated. When all this is done, we may be close to an effective synthetic pheromone, utilizable for control purposes in the field. Some scientific curiosity should also have been satiated during this process.

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HUR FÅNGAR HON DRÖMPRINSEN?

Hur gör man för att finna en partner om man är liten, lever i vatten och inte ser särskilt bra? När varenda sten är ett berg, och varje tångruska en skog. Det undrade såväl jag som den lilla honkrabban som snart ska ömsa. Strandkrabbor kan bara para sig precis efter att honan har ömsat. Därför har honan ett mycket starkt intresse av att finna en perfekt partner inom några få dagar. Hur gör hon då detta, när hon varken syns eller ser särskilt bra? Tja, det finns ju andra sinnen än syn...

Luktsinne viktigt i havet

Hos krabborna är lukt- och smaksinnet viktigare än synen. Faktiskt är det så att de flesta ryggradslösa djur som lever i havet är mer beroende av luktsinnet än av synen. Näsan används exempelvis för att känna igen och leta upp mat, undvika rovdjur, hitta en lämplig boplatz eller för att läsa kartan när djuret flyttar. Dessutom kan den användas till att känna igen artfränder, eller som i våra krabbors fall, till att finna en partner.

Näsan på skaft

När vi människor vill känna igen lukten av något sniffar vi på det. Det innebär att vi förflyttar luft som innehåller molekyler som vi känner igen som en specifik doft, till de sinnesceller i näsan som känner igen molekyler. Krabban gör precis tvärtom. Istället för att flytta dofterna till näsan, flyttar krabban näsan (med sina sinnesceller) till doftmolekyler. Detta kan den göra eftersom första antennen, där näsan sitter, är ledad och kan röras i flera riktningar. Genom små ryckningar i antennskaftet fångar krabban in doften i en liten pensel av hår som sitter längst ut på antennen. Håren i penseln kallas för aesthetascer och innehåller krabbans luktsinnesceller. Förutom luktsinnet använder krabborna även smaksinnet när de letar efter något intressant. Över hela kroppen finns små smakorgan som kallas för smaksensiller utplacerade. Längst ut på gångben och klor finns fler smaksensiller än på övriga kroppen. De används för att identifiera kemiska signaler och för att följa doftspår som finns på botten.

Parningsbeteenden styrs av feromoner

Vår lilla honkrabba använder även hon sitt luktsinne för att finna sin drömprins. Hon spårar med hjälp av luktsinnet upp lekar, det vill säga platser där en stor mängd hanar samlats för att kämpa och etablera dominanshierarkier, må bäste hane vinna! Vinnaren och ett antal andra dominanta hanar kan sedan para sig med flera honor, medan förlorarna antingen inte får para sig alls eller tar till tjuvknep för att få tillgång till honor. Förlorarnas strategi går ut på att hålla sig i utkanterna av parningsområdena, för att försöka fånga honor som rör sig in mot centrum av leken där de dominanta hanarna finns.

Hon stannar en bit utanför leken och flickar med antennerna och känner genast var de dominanta hanarna finns och rör sig mot deras doft. De andra hanarna försöker hon nogsamman att undvika. När hon kommit nära den dominanta hanen så gör hanens doft att hon ställer sig upp på tåpetsarna och släpper ut ett kemiskt lockmedel med sin urin

och pumpar iväg det med mundelarna mot hanen. Kemiska signaler som används för att kommunicera mellan könen inom en art kallas för sexualferomoner. Och det är precis ett sådant som honan släpper ut.

Den stora dominanta hanen som vår hona valt ut reagerar på doften. Han börjar gå omkring på tåspetsarna med klorna utbredda och försöker se ståtlig ut. Han har fått vittring på vår hona. Han sniffar och smakar sig ivrigt fram längs hennes doftspår och lyckas efter en stund finna henne. De cirklar runt varandra en stund, när plötsligt hanen fattar galoppen. Istället för att långsamt och metodiskt följa doftplymen, rusar han rakt mot henne. Han försöker greppa tag i henne för att bära henne under sig. Hon gör vilt motstånd för att bedöma hanens kvalitet. Om hanen lyckas hålla fast henne, slutar hon efter en stund att göra motstånd. Han har klarat testet. För att försäkra sig om att hanen inte släpper henne när hon är sårbar, så släpper honan ut ett annat feromon, ett så kallat primerferomon. Detta gör att hanen svarar ännu bättre på det feromon som gör att han bär henne. Han bär sedan henne under sig tills hon ska ömsa (allt från en dag till en vecka). Medan hon skiftar skal står han hela tiden ovanför och beskyddar henne. En timme efter det att honan har ömsat sker parningen, medan honan fortfarande är mjuk.

Strandkrabban och vi

Krabbfiske är många barns favoritsysselsättning varma sommandagar, längs hela den svenska västkusten. Utrustade med flytväst, musslor, räkor, eller fiskrens och ett snöre att binda fast det med, springer barnen runt på sand- och klippstränder medan de letar efter det bästa krabbstället. Åtskilliga timmar senare återvänder de blöta och utmattade till sina föräldrar, i många fall med en alltför röd nyans på solbrännan. Föräldrarna ser på sina söner och döttrar och den medföljande hinken, och möts med ett glatt "Mamma, pappa, kolla vad många jag har fått idag!". Hinken är full med strandkrabbor (*Carcinus maenas*), en mycket vanlig art längs Sveriges, samt Europas västkust. Efter krabbfisket uppstår det vanliga dilemma, vad ska man göra med alla dessa krabbor? Fastän välsmakande, kokade med salt och krondill, så är det inte mycket att äta på dem.

Lösningen brukar bestå i att återbörda krabborna till havet, för att återigen nästa dag fiskas upp av andra barn, fiskare, eller någon nyfiken vetenskapsman.

Ett skadedjur?

Det råder samstämmighet om att strandkrabban ökat längs kusten de senaste decennierna, vad detta beror på vet man inte idag. Olika teorier förekommer, från minskade torskbestånd (torsken äter strandkrabbor), till övergödning (fintrådiga grönalger är viktiga för överlevnaden hos småkrabbor), men ingen vet säkert. Även om barnen blir glada åt rikliga mängder krabbor, så är fiskare inte lika överförtjusta i ökningen, då krabborna skadar nät och äter upp fisk som fångas i nät och ryssjor.

Ännu större skada ställer krabborna till i andra delar av världen, där de oavsiktligt introducerats. I både Nordamerika och Australien så har skadegörelsen varit av sådan omfattning att man tvingats till nationella åtgärder. Konferenser har anordnats där man gemensamt försökt diskutera fram lämpliga bekämpningsmetoder som inte skadar omgivande miljö, men än så länge utan lyckat resultat.

Avhandlingsarbetets syfte och resultat

Syftet med mitt avhandlingsarbete var att studera om, och hur doftkommunikation sker under parningen hos denna art. Detta framförallt av två skäl, dels för att vetenskapligt kunna fastställa hur kemiska signaler kan användas för kommunikation mellan könen i marina miljöer, och dels för att om möjligt ge grunden till bekämpningsmetoder baserade på sexualferomoner (kemiska ämnen som används för kommunikation mellan könen inom en art, oftast i anslutning till parning).

Feromonbaserad bekämpning där man lockar till sig, eller stör hanars spårande av feromondofter från honor, har hos flera olika insektsarter visat sig vara effektiv. Dessa metoder har inte använts för marina djur, men skulle teoretiskt kunna ge en selektiv bekämpning av krabbor. För att utveckla en feromonbaserad bekämpningsmetod så krävs goda kunskaper om hur djuren använder sig av feromoner för att hitta och välja en partner. Förutom detta behövs även kännedom om feromonernas kemiska sammansättning.

Jag har med min avhandling visat att parningsbeteendet hos strandkrabban är nästan helt styrt av doftsignaler, och att hierarkier av beteenden styrs av avstånd och koncentration. Dessutom påverkar faktorer som hanens status både hans respons till hondoft och kvaliteten i hans doftsignaler. Honans ömsningsstatus påverkar på liknande sätt både hennes sökbeteende och kvaliteten i hennes doftsignal.

Detta leder till att jag tror att, för att kunna bekämpa strandkrabbor, så bör man rikta in sig på att isolera och framställa det hanliga feromonet. Dels beroende på att detta har visat sig ha långdistansaktivitet, och dels beroende på att honan är det begränsande könet i naturliga populationer, vilket gör det möjligt att effektivare reglera populationstillväxten genom att fånga honor.

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