THE UNIVERSITY OF HULL

Sex, Pheromone and Aggression in Norway lobster (*Nephrops norvegicus*):

For a better future of Scampi

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by

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Abstract

With a steadily increasing world population the demand for seafood has been growing rapidly over the past century. This has led to overfishing and decreasing catch rates in many seafood species. High fishing activity has endangered several aquatic species and pushed others to extinction. Signs of high fishing activity were also found in the *Nephrops*. In order to secure sustainability of important seafood species such as the Norway lobster (*Nephrops norvegicus*) it is important to intensify the research efforts on these species. Aggressive behaviour and injury are major constraints of communal holding of aquatic animals. A good knowledge of reproductive behaviour and larvae development is important for any hatching programs. Therefore, the aim of this thesis was to provide a research base that can improve sustainability of *Nephrops* and their well being in captivity, in order to culture them.

Both male and female *Nephrops* show fighting behaviour. However, only in fights with males a clear dominance relationship was maintained. Males and females recognise the higher status of their male opponent. Blocking of urine release showed that chemical communication by urinary signals is important in maintaining dominance relationships between males.

When comparing communal holding conditions to individual holding conditions over one months, no difference in death rate was found, indicating that a stable dominance hierarchy reduced aggression between animals that were kept in communal tanks. Although females lack the ability of recognising dominance in other females, they do recognise dominance in males. Male *Nephrops* have larger claws compared to the females showing additional sexual dimorphisms in the species. Moreover, *Nephrops* with larger claws tend to win the fights showing that claw size affects the outcome of fights.

In Lobsters, mating usually occurs after the female has moulted, and is in the soft shell condition. In *Nephrops* the highest number of matings occurred when the females were in the soft shell (postmoult) stage, but many males also tried to mate

with a hard shelled (intermoult) female when the odour of a soft shell female was present. This indicates that soft shelled female odour has an important effect on male behaviour. Similar to European lobsters (*Homarus gammarus*) and American lobsters (*Homarus americanus*), some *Nephrops* males also mate with hard shelled females even if no chemical cues from soft females are present. Thus, intermoult mating indicates the presence of female sex pheromone beyond the post moult stage.

This thesis provides applicable information to improve the *Nephrops* fishing industry and gives further details to enable *Nephrops* culturing in the future.

Introduction

Aquaculture is a fast growing business whose global production needs to reach 80 million tonnes by 2050, in order to maintain the consumption demand (FAO, 2011). In 2005, 52 million tonnes of shellfish were produced worldwide from aquaculture (Utting, 2006). The main species of shellfish farmed currently are oysters, mussels and scallops (Defra, 2004). In 2004, the farmed shellfish production in the UK was over 27,800 tonnes, and 128,000 tonnes were from wild caught (Cefas, 2005). The United Kingdom has the world's largest share of Norway lobsters, *Nephrops norvegicus* (Scottish Executive, 2006) and landed a total of 30,516 tonnes, which had a value of £70.5 million in 2004 (Defra, 2004). The Norway lobster is the most valuable species and therefore commercially the most important shellfish species in the UK (Howard, 1989). They are consumed within the UK (mainly as breaded scampi) as well as exported dead or alive, to countries such as Italy, France and Spain.

The *Nephrops* industry is dependent on weather and seasonal factors, which can decrease the volume of catch and drive the prices up (Aguzzi et al., 2004b). Biological rhythms affect commercial catchability at a daily and seasonal rate (Chapman, et al., 1975; Aguzzi and Sardà, 2008). Emergence and peak catch cycles are nocturnal on the shallow continental shelf (20-50 m), crepuscular on the lower shelf (50-200 m) (Moller and Naylor, 1980) and diurnal on the slope (400-430 m) (Chapman and Rice, 1971; Aguzzi et al. 2003). Although fishermen have adapted very well to the biological rhythm of *Nephrops* to catch them, there are factors, such as weather, that they are powerless to control. Therefore the product supply is unreliable. Yet, new rules for increasing mesh size or cutting days at sea have more of an influence on the total catch. Briggs et al. (1999) showed that an increase in mesh size of 10 mm decreased the catch by approximately 30%. In 2004 the catch decreased by approximately 10 000 tonnes relative to 2003 (Defra, 2004).

The Sea Fish Industry Authority (Seafish), who are interested in improving the sustainability of British seafood have looked at improving the quality, and therefore maximising the value, of *Nephrops*. They are helping to improve the handling methods on board and they are recommending that fishermen tube the healthier

Nephrops from the trawled catch to increase the profit and subsequently landing less (Linkie, 2007). Tubing is a method mainly used by the creel boats where *Nephrops* are kept individually in plastic tubes to avoid injuries during transportation. The value of live *Nephrops* is higher compared to tails therefore the trawlers can improve their profit by tubing healthy *Nephrops*. Improving holding facilities for *Nephrops* is becoming more important because during times when abundance and prices are low suppliers could use them to keep excess *Nephrops* to sell when landings drop *Nephrops*. Thus, suppliers have more control over the volume of the product and can be more reliable. Despite 50 years of Norway lobster fishing (Howard, 1989) there have been few attempts to find an alternative to harvesting *Nephrops* from the natural environment. Yet, the importance of aquaculture in securing the demand for seafood in the future was mentioned in the 1995 Kyoto Conference (Muired and Nugent, 1995).

History of *Nephrops* Fishing

Prior to the 1950s, *Nephrops* were mainly landed as bycatch and also discarded as unwanted bycatch (Phillips, 2006). In 1985, France and Spain each had landings exceeding 8.000 tonnes, while Scotland alone landed 17.000 tonnes (Howard, 1989). *Nephrops* fisheries have been developing in Scottish waters since the early 1950s (Phillips, 2006). In the UK, most *Nephrops* were landed in the ports of Eyemouth, Anstruther and Lossiemouth in Scotland (Howard, 1989). By 1954 landings amounted to 575 tonnes, valuing at £52,000 (Howard, 1989).

Nephrops became a very valuable species and around this time the Scottish vessels started to fish specifically for them (Howard, 1989). From 1959, the *Nephrops* fisheries became increasingly important so that laws were introduced in 1962 to permit greater control over fishing activity (Howard, 1989). However, Irish fishing law differed from the Scottish and English law, by allowing trawling with nets of less than 70 mm mesh size (Howard, 1989). The most popular commercial fishing method is trawling with mesh size between 70 mm and 100 mm (Seafish, 2010). Traditionally; single net otter trawls (Figure 1) have been used. However, since the early 1990s the use of twin rigged trawls (Figure 2) has been increasing (Seafish, 2010). The length of time spent at sea depends on boat size. Larger boats can go out

fishing for weeks while smaller boats mainly going out for daily trips. Another method of fishing *Nephrops* is called creeling, where the fishermen use baited pots (Figure 3). The fishermen, particularly in inshore coast waters use creels to catch *Nephrops*. Although the catch rate is lower using this method, it results in higher value compared to the trawl catch *Nephrops* (Seafish 2010). Currently, the total catch for this species in the North East Atlantic is approximately 80,000 tonnes per annum, valuing at more than £50 million annually (Seafish, 2007).

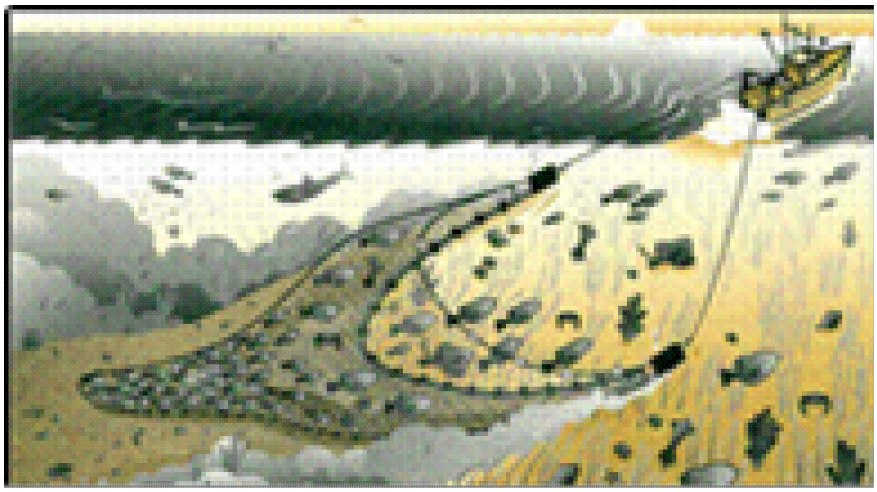

Figure 1. A picture of a bottom/otter trawl gear (Alaska Marine Conservation, 2008)

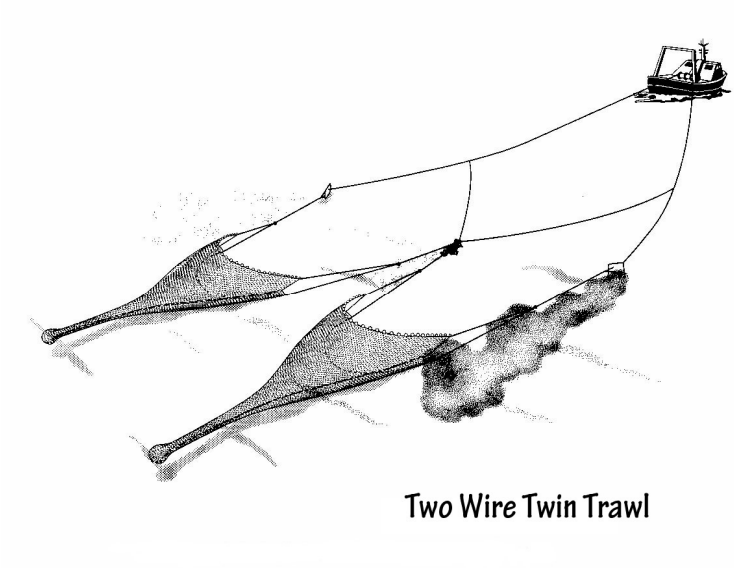

Figure 2. A picture of a twin rigged trawl (Crimond Enterprise Ltd., 2008).

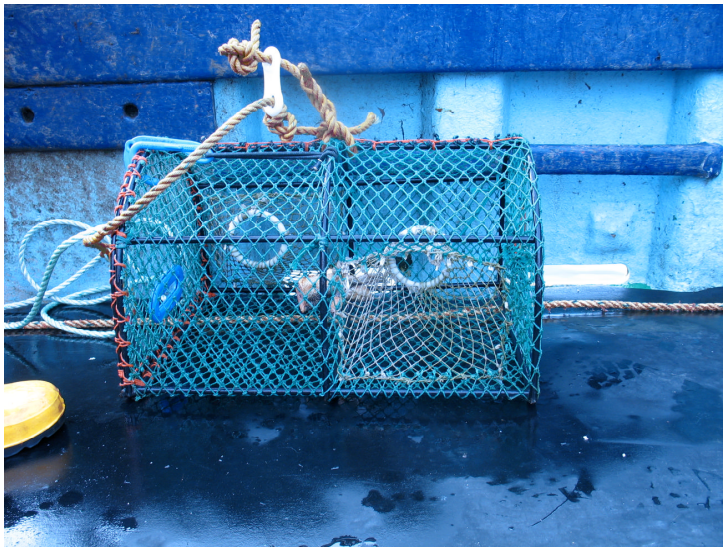

Figure 3. A *Nephrops* pot. Photography by E. Katoh.

Fishery management

The assessment of *Nephrops* stock has improved substantially with the use of independent estimates from underwater television surveys (Chapman, 1979) and estimation of fecundity to determine the biomass of spawning stocks (Briggs et al., 2002). However, illegal fishing activities make obtaining accurate catch rates still difficult (Barclay, 2011). It has also proved difficult to define adequate precautionary reference points for *Nephrops* fishing (ICES, 2006). An additional issue is the bycatch in the *Nephrops* trawl fisheries. Due to the small mesh size the discard of fish species such as cod, hake, or haddock can be significant in *Nephrops* trawl fisheries (Seafish, 2010). In order to reduce the bycatch rate and to allow recovery of these fish species, the number of days trawlers could spend at sea has to be better controlled. Management should also take into account creel fisheries, as they tend to catch a higher proportion of larger *Nephrops* and also berried females (Seafish, 2007). *Nephrops* fisheries are mainly managed by: raising mesh size (e.g. 80 – 95 mm in Scottish water); increasing minimum landing size (e.g. 25 mm CL in the Scottish waters and 20 mm CL around Portuguese coast, (Castro et al., 2003)); and, defining TAC (total allowance catch) (Cefas, 2008). Current UK legislation on twin rigs prevents the use of twin or multi rigs when fishing for *Nephrops* unless the mesh size is the white fish size of 80 to 100 mm (The Scottish Fishermen, 2000). The ICES (2008) are targeting an increase in mesh size (110 mm) to reduce bycatch.

In general, further research is necessary to improve the management of the fishing industry, and any decisions must take into account aspects of growing fish farms and hatcheries.

Aquaculture

The number of animal species suitable for aquaculture is steadily increasing worldwide. Atlantic salmon (*Salmo salar*) are cultured in the Bay of Fundy, Canada (Burridge et al., 2007); Pacific white shrimp (*Penaeus vannamei*) are farmed in northwest Mexico (Castillo-Juarez et al., 2007); and, there are commercial rock lobster farms in New Zealand and southern Australia (*Jasus edwardsii*) (Johnston et al., 2008; Radford et al., 2007). While other species are at the beginning of commercial aquaculture, a species such as the giant freshwater prawn is already a high valued cultured species. This is because of its wide acceptance from consumers, due to its dainty taste, ease of culture and export potential. Notably, the commercial production of giant fresh water prawns, *Macrobrachium rosenbergii*, has expanded in Asia with overall production increasing from 18,451 tons in 1990 to 203, 903 tons in 2005 (Whangchai et al., 2007).

Aquaculture is a new technique of producing seafood rather than obtaining it directly from aquatic environments. Aquaculture is considered as part of the food production sector, by supplying the population with important proteins (Sheriff et al., 2008). Therefore, artificial breeding, rearing and feeding must be studied, developed and improved. Previously, many species were only available through wild caught fisheries, but now a lot are cultured or in the experimentation process (Garibaldi, 1996). There are three different techniques of aquaculture: extensive, semi-intensive and intensive systems. In extensive systems, humans have only little or no impact on the procedure and the organisms rely on natural food, for example in Philippines carps are cultivated in extensive ponds. In intensive systems humans have a huge impact on the production by controlling the product with different diets and antibiotics (FAO, 2008). For example, trout are produced in intensive ponds in the UK. Semi-intensive systems are combination of the previous mentioned systems. In

New Caledonia, the blue shrimp, *Litopenaeus stylirostris,* is commercially produced under semi-intensive rearing conditions (Chim et al., 2008). There are two other practised culture systems, monoculture and polyculture. In monoculture only one organism is used, which is more time consuming and larger financial investments are necessary. On the other hand, in polyculture systems organisms are cultivated in combination with plant or animal husbandry and built into a miniature ecosystem. Therefore, not much human intervention is needed (Jennings et al., 2001). Jennings et al. (2001) approximates that 300 aquatic species are cultivated worldwide and more species are studied. For example, work on culturing the east coast rock lobster, *Panulirus homarus rubellus,* in 2008 by Kemp and Britz was carried out in South Africa, or along the southeast coast of India spiny lobsters *Panulirus homarus* were grown in sea cages (Vijayakumaran, et al., 2009).

Hatcheries are similar to the aquaculture farms mentioned above, only with the difference that the species are released after they have reached a certain size. This is usually when they are strong enough to survive in their natural environment. They are not kept until they reach plate size and are ready to sell. Mainly species, such as European and American lobsters are reared in hatcheries and take approximately 5 to 9 years until they reach maturity (Whale et al. 2006). The legal landing size is 87 mm CL (Seafood Scotland, 2010). Although, there was a study done where they farmed European lobster to plate size (250 – 300 grams) in 24 – 30 months (Drengstig et al., year not given). However, commercial lobster farms do not exist. At the moment, lobster farming is not economically feasible for aquaculture however; research into this field is helping move lobster farming to a point of economical viability. A hatchery is more of a stocking enhancement program to support the natural population from declining. Moreover, they are used as a public education and research source. Within the United Kingdom there is one in Cornwall, the National lobster hatchery in Padstow and another one in Orkney (Orkney Lobster Hatchery). There is also a semi-commercial lobster hatchery in Anglesey Zoo North Wales, which is mainly focused on research. Currently, there is a debate over whether to build lobster hatcheries in Eyemouth, Scotland and Scarborough, England.

Outside the UK there are lobster hatcheries located on the Kvitsøy Island in Norway and the Main Lobster hatchery in Canada. There are also projects around the world

working on rearing different species such as the spiny lobster in Florida (Palm Beach Post, 2005) or the gastropod *Concholepas concholepas*, also known as "loco" in Chile, as they are of high economic and ecological importance (Manriquez et al., 2008).

Consequences and Issues of the Fishing industry and Aquaculture

Industries have a history of depleting natural resources and damaging the environment. In the fishing industry, populations have been overfished or are in danger of being so. Fishermen and the government are usually too late to realise the issue, which brings the fishermen's living in danger. It is understandable that people are sceptical, but it seems likely that many are more worried about competition rather than sustainability or preventing the collapse of populations e.g. some fishermen do not see the purpose of investing and having a lobster hatchery (Scarborugh Evening News, 2008). However, some fisherman starting to observe declining size of individual animals and catch rate, which is starting to concern them (Cameron, 2010). Many think that the lobster is by no means an endangered species, so have no contingency plan if the lobster population crashes, as it did in Long Island Sound in 2000 (IntraFish, 2005).

Large commercial fisheries, such as benthic and beam trawlers have been overfishing in the North Sea (Ducrotoy and Elliot, 2008). There is concern that the loss of mature and breeding fish populations as well other ecosystem changes within the North Sea are being caused by the high fishing pressure (Kaiser and Spencer, 1996). Large seabed trawling has resulted in a change to the community structure in certain areas, not just through the removal of the stocks but also the damage that fishing is doing to the sea floor. Beam trawling has a history of changing the ecosystem in a large way (Ducrotoy and Elliot, 2008). Heavy towed gears disturb the uppermost layer of the seabed and cause the death of benthos (Kaiser and Spencer, 1996). Moreover, in this type of fishery, discarded *Nephrops* tend to have a high mortality rate due to the stress of being exposed to low salinity surface water (Harris and Ulmestrand, 2004).

The United Nation's Food and Agriculture Organisation commission published a review of the impacts of trawling and scallop dredging on benthic habitats and communities (Løkkeberg, 2005). This paper wrongly concluded that fishing has no disturbing affects on the seabed (Løkkeberg, 2005). Yet, the finding of the benthos showed obvious effects of trawling. This was demonstrated in the Gullmarsfjord area using a sediment profiling image camera, which showed clear changes in sediment structure (Rosenberg and Nilsson, 2005). A multibeam survey of the Tromsøflaket in Norway, conducted in June 2006 by the Institute of marine Research, Bergen, showed that rich sponge communities have been destroyed by trawling (Gray et al., 2006). The collapse of the Atlantic cod in Canada came as a stunning shock, which made it clear that even seemingly stable fishery resources are subject to a far greater degree of uncertainty than once thought (Lauck et al., 1998). As other fisheries have collapsed one by one in the past 15 years, fishermen turned to lobstering to make a living, but even lobstermen agree that lobstering has grown too popular (Daley, 2000). Moreover, as fishery resources decline, the excessive economic waste has become an increasing global concern (Eggert, 2001).

On the other hand, while considerable time and finance is invested in aquaculture to develop techniques to rear aquatic organisms, the environmental damage that might occur is not considered (Jennings et al., 2001). It was found that the use of pesticides by salmon farmers has caused concern among environmentalists and those involved in traditional fisheries. In the Bay of Fundy, Canada, cultured Atlantic salmons (*Salmo salar*) are treated against Sea lice (ectoparasites) with azamethiphos formulation Salmosan. In this area the American lobster (*Homarus americanus*) is the most commercially important indigenous species and Burridge et al. (2007) showed that the Salmosan pesticide can negatively affect spawning and can be lethal to adult female American lobsters. In Southern bay scallops, *Argopecten irradians concentricus* and American bay scallops, *Argopecten irradians irradians*, respectively observed that inbreeding could cause lower hatching, less viability and slow growth (Liu et al., 2011; Zheng et al., 2008). In order to have a better control on aquaculture, those problems need to be resolved before expanding aquaculture further (Jennings et al., 2001).

The Biology of Decapods Crustacean

Morphology and Growth

Crustaceans have a hard, jointed, external shell that encases the body and limbs. They use their gills to exchange gas by diffusing water through the branchial (gill) surface (Brusca and Brsuca, 1990). *Nephrops* are closely related to clawed lobsters such as the European lobster (*Homarus gammarus*) and American Lobster (*Homarus americanus*) (Bell et al., 2006), but they can be easily distinguished by their smaller size, orange-red colour, long slender chelae and prominent kidney-shaped (reniform) superposition compound eyes, in which light is reflected back through the rhabdoms (Johnson et al., 2002). *Nephrops* eyes are large in proportion to their bodies compared to lobster eyes. The bodies of clawed lobsters are divided into two parts: the fore part of the body (head and thorax) is covered by continuous shell, the cephalothorax; while the hind part (abdomen) is divided into six flexible segments (Brusca and Brsuca, 1990). They also have two pairs of antennae, one pair of antennules and one pair of antennae. They flick the antennules, which are also used for "sniffing". It is a mechanism to collect odour information from their environment (Koehl et al., 2001). The most often used size measurement in denoting the size of a lobster or *Nephrops* is to measure the carapace length (CL). The carapace length is the distance from the orbit behind the eye to the end of the carapace before the abdomen (Figure 4). *Nephrops* have 20 pairs of jointed appendages that are specialised for performing different functions. The two largest claws (Cheliped) are usually used for catching prey, and in encounters with other animals. When they catch the prey, it will then be passed to the different parts of their mouth (Farmer, 1974a). The external part of the mouth includes three maxillipeds, three maxillae and one pair of mandibles which all have their own specialised function (Farmer, 1974a). The so-called four pairs of walking legs (pereiopods) are used, as the name says, for walking, cleaning and also for digging burrows. Males use, in addition to the third maxilipeds, the second and third pereiopods to grasp the female during copulation (Farmer, 1974b). The swimmerets (pleopods) on the abdomen of a female are used for carrying and oxygenating the eggs (Farmer, 1974b). In stressful or dangerous situations, *Nephrops* may discard one of their large claws as a defence mechanism. The appendage becomes detached at a breaking point near its base; there is little bleeding and the

limb regenerates and grows with successive moults (Howard, 1989). Sexes are separate and can be only distinguished by turning the *Nephrops* on their back. The first pair of pleopods on the abdomen is thicker in the male, and forms a forward-pointing tube that is used to pass a sperm package to the female during mating (Farmer, 1972b). The sperm then fertilises the eggs when they are laid. In the female the first pair of pleopods are significantly thinner (Howard, 1989).

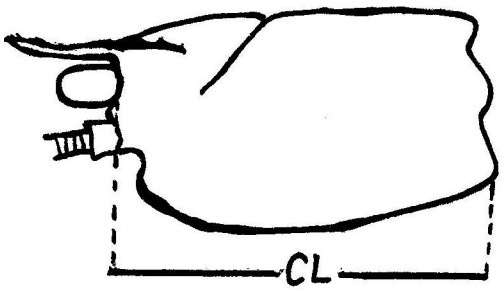

Figure 4. An Example of *Nephrops norvegicus,* taken with standard measurements, Carapace length (CL) (adapted from Calderón-Pérez, 1986).

Chemosensory and mechanosensory sensillae are located on all the cephalothoracic appendages, including the antennules, antennae, six pairs of mouthparts and five pairs of pereiopods (Derby, 1982; Hallberg and Skog, 2010). There are three main chemoreceptor organs located in the antennules for smell, and the walking legs for taste (Devine and Atema, 1982). The major chemoreceptor organ is located in the antennules (Voigt and Atema, 1992). They smell by flicking (or so called sniffing), this mechanism will lead the odour to the enhancing odour receptor areas (Koehl et al., 2001). The chemoreceptors on different appendages of lobsters (*Homarus americanus*) fulfil different functional roles (Derby, 1982; Derby and Atema, 1982). The presence of chemosensory sensilla are also described in *Nephrops* (Laverack, 1968) and showed the use of antennae and antennules to locate females (Farmer, 1964). In many species such as American lobsters (Karavanich and Atema, 1998a, Breithaupt et al., 1999), Crayfish (*Orconectes virilise* and *Orconectes propinquus, Astacus leptodactylus*) (Tierney and Dunham, 1982; Breithaupt and Eger, 2002) and big-clawed snapping shrimps (*Alpheus heterochaelis*) (Obermeier and Schmitz, 2003) it was found that they use urine to communicate; whether that be intraspecific and interspecific.

As with other crustaceans, the growth of the Norway lobster is a discontinuous process consisting, known as moulting or ecdyses (González-Gurriarán et al., 1998). Moulting consists of a five-stage process, where they absorb water to increase the hydrostatic pressure within the body in order to break the old shell (Wahle and Fogarty, 2006). Then the old exoskeleton parts from the layer underneath the new cuticle and splits between the carapace and the abdomen (Wahle and Fogarty, 2006). After the animal withdraws itself out of the old shell, the animal has a soft exoskeleton, this being when it is most vulnerable and endangered (Wahle and Fogarty, 2006). This process takes approximately 30 minutes and is followed by an increase in size (Wahle and Fogarty, 2006). The new soft exoskeleton will then thicken and harden gradually through the deposition of calcium salts (Wahle and Fogarty, 2006). When the mouthparts are hardened they also consume the cast-off shell as an additional resource of calcium (Wahle and Fogarty, 2006). After approximately two weeks in the moult stage, the new shell is fully hardened and no further growth can occur until the next moult (Horwards, 1989). In decapods it is generally found that as the animal grows the moult frequency falls, the absolute size increment increases and the percentage increment decreases (Hopkins, 1967; Farmer a, 1973). Moreover, Farmer (1973a) found that moulting in the laboratory occurred throughout the year in males, but there was a peak of moulting activity in females from June to August. Similar moulting activity was observed in the monthly samples from the natural population. Farmer (1975) determined that in the Irish Sea, juveniles moult approximately 15 times before reaching sexual maturity at about 21 mm CL. In most areas females moult once per year in spring and spawn annually (Bell et al., 2006). There is a difficulty in assessing the age of *Nephrops*. Since the growth rate varies seasonally, intervals being shorter and increments greater in summer than in winter, such extrapolation may give unreliable results (Hillis, 1972). The moulting rate reduces with increasing carapace length (e.g. freshwater crayfish *Paranephrops planifrons*, Hopkins, 1967), this may well be a growth rate change at sexual maturity such as is known to occur in many crustacean species (Hillis, 1971). In one of Hillis‘ (1971) studies in the Irish Sea, he defined age groups by carapace length; carapace-length of 7 – 10 mm (age group 0), 15 – 17 mm (age group 1) and around 24 mm (age group 2). Moreover, the number of moult were approximately 5 – 6 times in year 0 – 1, 3 – 5 times in year 1 – 2 and about 1 or 2 times for the larger, older specimens (Hillis, 1972). The growth of a juvenile in its first year of life (age-group 0) at a

carapace length of 7-10 mm is rarely seen formerly (Hillis, 1971). In one year the juveniles can grow to about 18 – 19 mm (CL), at which stage they are mainly recruited to the Irish trawl fishery though they are still sexually immature (Hillis, 1973).

Reproduction and Mating

Many lobster species support valuable fisheries; therefore their reproduction has been investigated since the onset of commercial exploitation (MacDiarmid and Sainte-Marie, 2006). Male lobsters have paired testes that lie dorsally in the body cavity and lead via vas deferentia to gonopores at the base of the fifth pair of walking legs (Meglitsch, 1967). Female lobsters have paired ovaries, which also lie in the body cavity but leads via paired oviduct to the reproductive aperture at the base of the third walking legs (Meglitsch, 1967). In females the abdominal pleopods and the inner branch (endopods) have long developed setae, specialised to carry eggs during the incubation period (Farmer, 1972b). The first walking leg in females terminates in a small pincer and is used to groom the setae and manipulate the egg mass (Farmer, 1972b). The detailed morphology of reproductive structures in lobsters have been reasonably well described for those species that support large commercial fisheries.

Mate searching in crustaceans depends on different communicational cues, of which chemical and visual cues are most important (Atema and Engstrom, 1971). An example of an early step in the evolution of chemical communication systems were found in goldfish. They are able to control the release of urinary prostaglandin pheromones to communicate their condition and location. Furthermore, receptive female goldfish show a strong tendency to urinate when placed with males and when rising into spawning substrate (Appelt and Sorensen, 2007). In rock shrimp, *Rhynchocinetes typus*, receptive females use chemical cues emitted by males to select robustus males (hard shell males) while males on the other hand use visual cues to find receptive females (Diaz and Thiel, 2004). Female American lobsters use smell to choose the dominant male to mate with. Moreover, it was found that female lobster urine reduces the incidence of male aggressive behaviour and induces male mating behaviour (Bushmann and Atema, 1996).

In *Nephropidae*, such as *Nephrops, Homarus americanus*, and *H. gammarus*, mating takes place usually while the female is still soft, directly after the female has moulted and before hardening of the new exoskeleton (Farmer, 1975; Howard, 1989).
When the males detected the females, they stroke females with their antenna for 2 – 20 minutes (Farmer, 1974). After the stroking period, the male will turn the female onto her back, so they are in a ventral to ventral position (Farmer, 1974). In this position the male is able to transfer his spermatophores into the females thelycum, performing thrusting movement (Farmer, 1974). In achelate lobsters (e.g. *Panulirus argus*) mating behaviour is similar by being in ventral to ventral position, however, the difference is that the female is on the top instead of the male (Childress and Jury, 2006). Although, most mating occurs when the female is in the soft shelled stage, it was observed that male lobsters also mate with hard shelled females (Skog, 2009; Waddy and Aiken, 1990; 1991; **Paper IV**). Intermoult mating is an alternative mating strategy to postmoult mating, and gives the female the opportunity to mate in cases such as, when they failed to mate in postmoult stage or when they received small amount of spermatophores (Waddy and Aiken, 1990; 1991).

Agonistic behaviour and Dominance hierarchy of decapods

Research into the aggressive behaviour of cultured species is important, because aggression between individuals is a problem for the aquaculture industry. Fights between animals can cause damage, by puncturing the carapace or even leading to a loss of claws or other appendages, which decreases their value. In the worst case they die after the fight as a result of the combination of damage and stress. In wild-caught specimens puncture wounds suggest intraspecific aggression (Karnofsky et al., 1989). Moreover, space availability in their natural environment makes significant difference to captive holding conditions as they can avoid encounters (Baird et al., 2006).

Shelters are of prime importance in the life of the lobsters such as American lobsters (*Homarus americanus*) and Norway lobsters (*Nephrops norvegicus*) (Chapman and Rice, 1971; Chapman, et al., 1975; Chapman and Horward, 1979; Karnofsky, et al., 1989). They spend most of their time in shelters, but emerge at dusk to dawn under

optimum environmental illumination to feed, however there are other factors which affects the emergence of *Nephrops*, such as looking for mating partner or changing burrows (Aguzzi and Sarda, 2007; Chapman and Rice, 1971; Chapman et al., 1975; Chapman and Horward, 1979; Karnofsky, et al., 1989). They dig shelters under eelgrass, rocks, or built burrows into the sand and their shelter locations appear clustered. Some animals change shelters frequently (Chapman and Rice, 1971), whereas other occupies the same shelter for up to 10 weeks. Cohabitation in shelter or multiple shelter use (when two or more animals are in one burrow) has been observed (Chapman and Rice, 1971). It often occurs during periods of pair formation or when post-larvae settle on the sea floor and use the burrows of the adults (Karnofsky et al., 1989). In the mating season a mature female inhabits a male's shelter prior to and following her moult. Moreover, 34% of *Nephrops* borrows were found with other species such as echiuran worm or goby (Tuck et al., 1994). In captivity it was found that habitat complexity reduces the number of agonistic interactions and the total time spent interacting (Baird et al., 2006).

In captivity lobsters like *H. americanus* and *H. gammarus* are highly aggressive and need to be kept separately or with taped claws so that they are unable to use them in encounters (Cenni, et al., 2010). The chemical composition in the body may influence the fighting duration, such as reduced levels of serotonin increased the amount of time animals engaged in fighting behaviour (Edwards and Kravitz, 1997; Huber et al., 1997). The findings include the demonstration that serotonin injections will cause renewed willingness of subordinate animals to engage dominants in further agonistic encounters (Edwards and Kravitz, 1997; Huber et al., 1997). No significant effects were seen on who initiated encounters, who retreated first, or who the eventual winner would be. Thus, in this model elevation or reduction of serotonergic function affects the tendency of animals to engage in agonistic encounters (Doernberg and Cromarty, 2001).

In many species size plays an important role in the outcome of encounters, as larger animals usually win the fights (Thorpe et al., 1994; Huntingford, 1995,). Smaller crabs were able to win a fight, when the size difference was small, in which case encounters tend to be long and more aggressive (Huntingford, 1995). In *N. puber* both larger and smaller crabs were more likely to win fights that they initiated, suggesting

that motivation plays a role (Huntingford, 1995). In breeding season small crabs were as likely as larger ones to win fights, by persisting much longer than they did in the absence of such stimuli (Huntingford, 1995). The juvenile crayfish (*Procambarus clarkia*) the largest animal is always the superdominant animal and this does change when another juvenile is becoming larger. In juvenile signal crayfish (*Pacifastacus leniusculus*) a feeding hierarchy has been observed and it has also been noted that juveniles can raise their rank by consuming high amounts of food or by being very efficient (Ahvenharju and Ruohonen, 2006). The fighting declines over the first few hours and to low levels by 24 h (Issa et al., 1999).

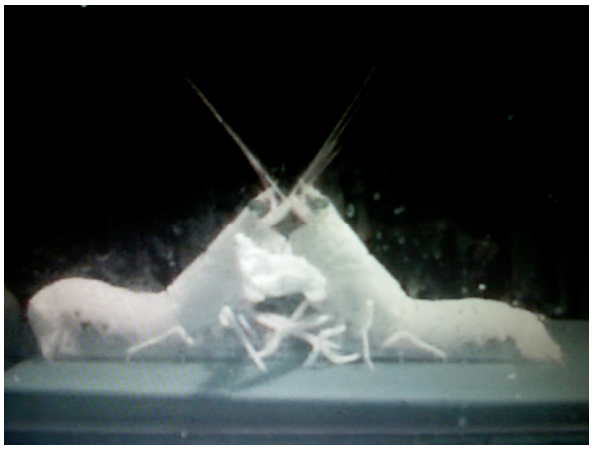

Figure 5. Aggressive interaction between two *Nephrops*, showing meral spread. Photograph by E. Katoh

In many animal species conflict will arise between individuals over limited resources such as food, space and mating opportunities. These situations are often regulated by the formation of dominance hierarchy (Drews, 1993). **Paper I** demonstrates that male *Nephrops* do fight (Figure 5) and are able to build dominance hierarchies, where the aggression levels decrease. Development of a social hierarchy generally leads to a reduction in the frequency and intensity of fights (Wilson, 1975). It was found that chemicals play a role in aggressive behaviour (Breithaupt and Atema, 2000; Breithaupt and Eger, 2002, **Paper I**). Crayfish (*Orconectes propinquus and Orconectes virilise*) show the ability to discriminate species by chemicals. Individuals of both species and both sexes show a significantly higher attraction to conspecific conditioned water than to heterospecific conditioned water (Tierney and Dunham, 1982). *Nephrops* are unable to recognise the opponent in the absence of urine (**Paper I**).

There are three possible mechanisms to maintain dominance formation: individual recognition, status recognition, or winner/loser effect (Mesterton-Gibbons and Dugatkin, 1995; Goessman et al., 2000; Hsu and Wolf, 2001; Gherardi and Daniels, 2003; Dugatkin and Earley, 2004). In some fish species a winner from a previous fight tend to win again and loser are more likely to lose in future fights, which is the winner and loser effect (Hsu and Wolf, 2001). The 'winner-loser effects' does not depend on sensory assessment of the opponent (Chase et al., 1994; Hsu and Wolf, 2001) but can produce divergence of hierarchical ranks: when winner effect is dominant, when an excess of loser effect is determined or when there is a balance of winner loser effects (Hock and Huber, 2005). However, depending on the species the winner-loser effect has different lasting affects (Bergman et al., 2003). In some species of crayfish (Seebacher and Wilson, 2007), hermit crabs (Gherardi and Atema, 2005; Gherardi et al., 2005; Gherardi and Tiedemann, 2004; Hazlett, 1969), mantis shrimps (Caldwell, 1979; Caldwell, 1985) and lobsters (Karavanich and Atema, 1998; Johnson and Atema, 2005) use individual recognition for maintaining dominance. These species have the ability to recognise conspecifics individually from previous encounter. The subordinate animal usually avoids the dominant animal, which leads to less fighting and maintaining dominance formation. Status recognition is used by other species of crayfish (Copp, 1986; Zulandt-Schneider et al., 2001), hermit crabs (Winston and Jacobson, 1978) and *Nephrops* (**Paper II**). In this mechanism the animals are able to recognise the status of an opponent from a previous encounter. They do not recognise whom, as an individual they are meeting, however, they will know whether the opponent is dominant or subordinate compared to their own status. Most of the time the subordinate animals tend to avoid dominant individuals and therefore maintain a dominance hierarchy. It is often assumed that the formation of these hierarchies depends on learned recognition of dominants by subordinates (Bovbjerg, 1956) or learned recognition of individuals (Hazlett, 1969) as occurs in some vertebrate species (e.g. Bernstein and Gordon, 1980). Although it was demonstrated that *Nephrops* are able to recognise the status of an opponent, female *Nephrops* lack the ability of the recognition of same sex opponent (**Paper III**).

The Biology of *Nephrops*

The Classification of *Nephrops norvegicus*:
Phylum: Arthropoda,
Subphylum: Crustacean
Class: Malacostraca
Order: Decapoda
Family, Nephropidae,
Genus: *Nephrops*
Species: *Nephrops norvegicus*

Figure 6. Norway lobster, *Nephrops norvegicus*. Photography by E. Katoh.

Nephrops (Figure 6) are also known as Norway lobster, Dublin Bay prawn, scampi or langoustine, which makes it sometimes difficult when communicating. Therefore, it is preferred to call them *Nephrops*, as their scientific name is *Nephrops norvegicus* and this avoids confusions.

The geographical range of *Nephrops* extends from Iceland southwards to Morocco and the Mediterranean as far as Egypt. Although there is a mitochondrial DNA variation between the Northeast Atlantic and the Mediterranean, interestingly the most difference was found in the Irish Sea population of *Nephrops* by having a reduced level of diversity in the genetic structure (Stamatis et al., 2004). *Nephrops* are found in depths ranging from 15 m to more than 800 m. In Scotland the main

populations are found between 40 m and 200 m. As the Norway lobster is a demersal species it is sensitive to light of longer wavelengths (510 to 525 nm), like the *Crangon allmani* and *Pandalus montagui* (Johnson et al., 2002). *Nephrops* distribution depends on the sea floor sediment, since they prefer fine cohesive mud in which they can construct burrows (Aguzzi, et al. 2004a). The burrows can extend 20 – 30 cm below the mud surface and range from simple tunnels with a single opening, through to more typical forms with a wide sloping front entrance and a small rear entrance, to complex tunnels with more than two openings (Chapman, 1979). As mentioned before the population density on the commercial ground is determined by using television and photographic survey. With this method it is provided that the *Nephrops* burrows can be distinguished from those of other species (Chapman, 1979). At one extreme in Loch Torridon at 30 m depth, the density averaged one *Nephrops* in every 5 m^2 whereas in the Firth of Forth, the density was as high as 4 *Nephrops* per 1 m^2 (Chapma, 1979; Howard, 1989). The density and spacing of the burrows in *Nephrops* inhabited areas varies considerably dependent on the availability of suitable mud substrate (Howard, 1989). Large *Nephrops* at low densities tend to be characteristic for areas with fine sediments, whereas in areas with coarser sediments the *Nephrops* size is smaller and the population densities are much higher (Howard, 1989). Coarse mud is a sediment mix with a high proportion of sand, silt and clay, while fine mud has low sand content (Tuck et al., 1997a).

The age at which maturity was reached appeared to be between 4 – 4.5 years for males and between 3 – 3.5 years for females (Tuck et al., 2000). There is a difference in carapace length at onset of maturity between areas ranging from 18 mm CL to 26 mm CL (Farmer, 1975; Figueiredo and Thomas, 1967; Howard, 1989). Around Scotland and northeast England the lowest recorded size of berried female is between 20 and 23 mm CL.

The ripe ovary in the mature female can be seen through the carapace as a dark, green-black area, during the late summer and early autumn. Generally, mature females reproduce every year and fertilise their eggs (Symonds, 1972) however, the hatching period varies depending on geographical differences, e.g. in Iceland from April to July (Eriksson, 1970), in Portugal from February to April (Figueiredo and Barraca, 1963) and in the Adriatic from January to March (Frogila and Gramitto,

1981). The varying hatching period can be caused by different temperature regimes and or food sources (Bailey, 1984; Redant, 1994, Relini, 1998). The dark green fertilised eggs are carried on the pleopods of the female's abdomen and are incubated between 6 months and 13 months depending on the latitude (Chapman, 1980). As the embryo develops within the egg there is a gradual change in colour from dark green through pale green and finally to a pinkish brown colour just prior to hatching. A female of 25 mm CL carries about 500 eggs and one of 35 mm CL about 1,500 eggs (Howard, 1989). The larvae are very different in appearance from the adult (Howard, 1989). The *Nephrops* larval period, in the laboratory, from the beginning of stage I to the end of stage III was estimated to range from 43 – 90 days (Dickey-Collas et al., 2000b), while Howard (1989) estimated a larval period ranging from 20 – 40 days. The larval duration can be between 4 and 8 weeks depending on factors such as temperature and food (Wahle and Fogarty, 2006). When juvenile Norway lobsters settle on the bottom sea floor they are about 16 mm in total length (Howard, 1989). They enter existing, previously inhabited burrows and often they cohabit with adults *Nephrops* and remain within their burrows without emerging for about one year (a strategy that protects this vulnerable stage from predation) (Tuck et al., 1994). *Nephrops* are opportunistic predators and are very active foragers (Howard, 1989). The larvae are carnivorous, actively preying on a wide range of planktonic organisms (Jones et al., 1997). Juveniles and adults feed on a wide variety of material including mollusc, annelids, crustaceans, echinoderms and small fish (Howard, 1989). They can also feed on very small organisms such as the microscopic foraminifera found in the mud, catch active prey by snapping with their claws, or search for food material lying on or within the surface of the mud (Howard, 1989). The estimates of an adult daily food consumption obtained varied from 1.098 to 1.170 g dry food per 100 g body wet weight in males and 1.642 to 1.755 g dry food per 100 g body wet weight in females (Cristo and Castro, 2005). The constantly changing life cycle of *Nephrops* larvae makes rearing them in captivity a complex affair.

Project descriptions

Aims

The aim of this thesis is to study the behaviour of males, females, berried females and larvae of *Nephrops* to contribute to the development of a method for culturing Norway lobster *Nephrops norvegicus*, in the future. The aim is to investigate factors affecting the well-being of adult *Nephrops* in captivity, by understanding the agonistic behaviour, investigating different holding conditions and the effect of conspecific odour and elucidating mating behaviour and the role of female odour.

Paper I: Fighting behaviour and the role of urinary signals in dominance assessment of Norway lobsters, *Nephrops norvegicus*

This paper investigated the fighting behaviour of size-matched male *Nephrops,* with a particular emphasis on the role of urinary chemical signals in the assessment of dominance. In order to provide the best holding conditions, it is important to understand the agonistic behaviour of *Nephrops* kept in captivity.

General procedure for all contests

Norway lobsters were paired in dyadic encounters on two consecutive days. Observation of male pairs was made using a 70-l glass tank (38 x 61 x 30 cm) which had three sides darkened with a black sheet. All observations were carried out in the dark, with only the tank illuminated by using a 25-W bulb suspended 36 cm above the surface of the water. The water temperature in the experimental tank was between 10 and 12 ˚C. One of the two animals was marked with duct tape around the propodus of the chelipeds in order to allow easy identification. The pair was introduced into the experimental tank on opposite sides with an opaque plastic sheet separating the two sides. The animals were given thirty minutes to acclimatize to the new environment. After this time, the divider was lifted, the video recording started and the animals were allowed to interact for 30 min. Based on stereotypical agonistic behaviours

(Table 1) the animals were identified as winners or as losers. The fight ended when one of the animals (the loser) showed avoidance (level −1) or escaped (−2) from the winner at the end of a bout and did not show any aggression exceeding level 3 for the remaining time of the interaction.

Table 1. Definition of agonistic levels for fighting *N. norvegicus* (adapted from Atema and Voigt, 1995)

Level	Behaviour	Definition
-2	Fleeing	Walking backwards, walking away or turning away, tailflipping
−1	Avoidance	Walking around but avoiding opponent, body pressed to the ground
0	Separate	No activity
L	Separate	Locomotion, cleaning
1	Approach	Animals within reach of claws, facing approaching, turning towards, following
2	Touching	Some body parts (e.g., abdomen, pereopods) touch for extended time without any higher levels of aggression
3	Threat display	High on legs, meral spread (horizontally spread chelipeds without display physical contact)
4	Cheliped pushing	Combatants push each other face to face in meral spread position pushing
5	Wrestling	Smacking, pushing, antennal touching claw grabbing, punching

Experimental treatment: preventing urine release

Animals were allowed to use chemical communication to establish dominance in the first encounter. During second encounters urine release was blocked by diverting the urine into catheter tubes, so that the opponent could not smell it. Catheterisation (Figure 7) is a method used to prevent urine release. A modified version by Breithaupt et al. 1999 was used. *Nephrops* were fixed in a sponge to avoid injuries and autotomy. Silicon tubing of 20 cm length and 1.5 mm diameter was attached to the carapace surrounding the nephropores. Cyanoacrylate glue was used to attach the tubing and an additional layer of cyanoacrylate was applied to the tube to prevent leakage. To accelerate the drying process Zip Kicker, Pacer fluid was used. Both tubes were crossed in front of the *Nephrops* and attached to the carapace and connected to floating 1.5 ml collective vials. In order to prevent a pressure build up, small holes were pierced in the top of the vials. Carbon filtered water was used to wash the equipment between use.

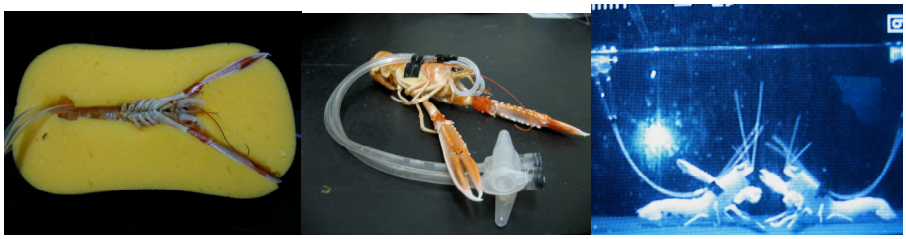

Figure 7. Catheterisation technique. The *Nephrops* on the left is fixed in the sponge. Silicon tubing has been attached to both nephroperes using cyanoacrylate glue. The *Nephrops* in the middle has the vials attached to the tubes. On the right is an encounter between two male *Nephrops* with catheters.

Analysis of behaviour and fight duration

Fights were analyzed at 5-s intervals. For each interval a behavioral category was assigned for both winner and loser (Table 1). Behaviours were analyzed for 30 min after lifting the divider. If animals displayed more than one behaviour in one interval an overall level was assigned for that interval on the basis of the following ranks: levels 5, 4 and 3 outranked (>) level 2, 1, 0, −1 and −2; level 5 > 4 > 3 > 2 > 1; level −2 outranked level −1, and both level −2 and −1 outranked levels 2, 1 and 0. Olfactory sampling behaviour (antennule flicking) of both combatants during first and second encounters was recorded separately using a stopwatch. Fight duration was measured as a sum of the duration of individual bouts that occurred within each encounter. Bouts included aggressive behaviours of both combatants higher then level 3. A bout started when the combatants were within reach of the claws (level 1 or higher) and ended at the start of a separation of at least 15 s. The fight ended when one of the animals (the loser) showed avoidance (level −1) or escaped (−2) from the winner at the end of a bout and did not show any aggression exceeding level 3 for the remaining time of the interaction.

Results

Agonistic encounters of male *Nephrops* follow a common pattern starting with approach, then followed by threat displays and physical interactions (see definition of behaviours in Table 1). A decrease in fight duration from first to second encounter indicates that *Nephrops* are able to maintain dominance in sequential contests. The main difference between the two encounters is in the behaviour of the loser. Losers strongly reduce their aggression level from first to second encounters. Olfactory

sampling behaviour (antennule flicking) of eventual losers is higher than that of the winner indicating that the loser assesses chemical signals of the dominant male. When urine release is blocked for the second encounter, there is no difference in fight duration between first and second encounter. The results suggest that Norway lobsters develop lasting dominance relationships. The study also provides preliminary evidence that urine-borne chemical signals play an important role in mediating dominance.

Paper II: Communal holding conditions and the effects of social hierarchy in Norway lobster, *Nephrops norvegicus*: dominance pheromones reduce aggression in groups of *Nephrops*

In this paper two holding conditions for Norway lobsters (*Nephrops norvegicus*) were tested. Moreover, this paper also investigated how *Nephrops* recognise conspecifics. Forming dominance relations within a group leads to a reduction in aggressive encounters and therefore less injuries and deaths. The ability to understand the formation and maintenance of dominance relations could be used for aquaculture purposes to maintain commercial species in communal tanks.

Communal holding conditions are less time consuming and more economically efficient compared to individual holding conditions. Since, Paper I showed that pairs of male *Nephrops* are able to form dominance relationships, the next step was to test whether they are able to form a dominance hierarchy in a group and if so what mechanisms they use to maintain the dominance hierarchy.

Holding conditions

One population of 25 *Nephrops* were separated in individual compartments, whilst the other twenty-five animals were not separated and could therefore interact with each other (Figure 8).

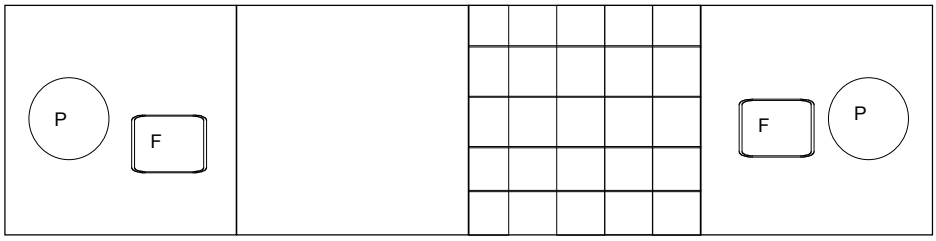

Figure 8. A schematic drawing of the holding tank. The two outer compartments ($1m^2$) where provided with a proteinskimmer (P) and biofilter (F). The two middle compartments ($1m^2$) are different by the right part having 25 smaller compartments (each $40cm^2$).

Individual or Status recognition

The Norway lobsters participated in two rounds of encounters. First fights enabled *Nephrops* to assess the opponent. The second fights were designed as either familiar or unfamiliar treatment. In familiar treatments *Nephrops* encountered the same opponent that they fought in the first fight. In this case both the identity of the opponent and the status of the opponent have been encountered previously by the combatants. In the unfamiliar fights, both *Nephrops* had the same fight history as in the familiar treatment but had not previously encountered the opponent. In this case the individual identity of the opponent is unfamiliar but the opponent's status was not (Figure 9).

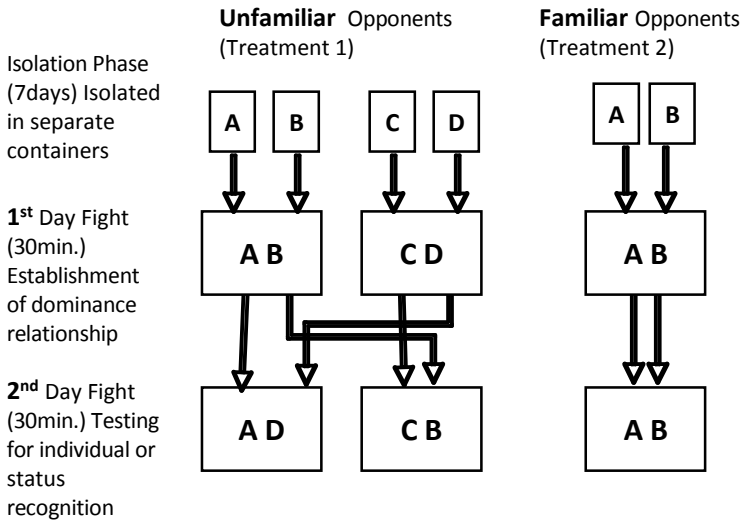

Figure 9. Schematic diagram showing the experimental fight set up. The letter A, indicates individual with dominant status and, B signifies individuals with subordinate status. Animals were isolated for at least one week to eliminate any prior experience. A, B, C, and D represent *Nephrops* isolated in separate tanks during the isolation phase. In the familiar experiment animals fought their opponents from the first fight, while in the unfamiliar treatment animals' status had been encountered before but is unknown to the opponent. Arrows indicate the movement of *Nephrops* from treatment to treatment.

The effect of conspecific odour in agonistic interactions

The role of conspecific odours was tested in Norway lobsters agonistic encounters. Dominant male odour, female odour and sea water (control) were added to male fights to investigate the effect of conspecifics odour on fight durations.

Results

In the two different holding conditions (Figure 6) where 25 animals were kept together and 25 animals kept individually in the same water condition, no significant difference was found in the survival rate.

In both treatments familiar and unfamiliar experiments were conducted to investigate which recognition mechanisms Norway lobster use. However, no significant difference was found in the fight durations between days and treatments.
When adding conspecific odour to a male encounter depending on the odour (e.g. female or dominant male odour), the fight durations varied significantly.
The results showed that *Nephrops* can be kept in communal holding conditions and that they develop dominance relations using status recognition. The study also provides evidence that dominance odour plays an important role in regulating aggressive behaviour.

Paper III: Do female Norway lobsters (*Nephrops norvegius*) build dominance hierarchy? Or is it a male thing?

This paper investigates the agonistic behaviour of female *Nephrops* and was compared to the behaviour of their male counterparts. When planning to culture a species it is also important to know the behaviour of the females, and whether they need to be treated differently than males in captivity.

Experimental treatment

The general fight procedure described in Paper I was followed. Three experimental treatments were used. In the first treatment a female encountered another female (female fights). In the second treatment a male fought a female (mixed sex fights) and in the last treatment a male encountered another male (male fights).

Results

In female fights no difference was found between the first and the second day. However, when comparing the mean fight durations between all three treatments on the second day a significant difference was found ($p = 0.04$, Kruskal-Wallis test, $N = 45$ pairs; Figure 1). While in the female fights the mean fight duration from the 1st Day to the 2nd Day increased by 73%, in the mixed sex fights the fight duration decrease by 90% and in male fights by 55%. The fighting behaviour between males

and females did not differ. The behaviour of the females was not affected whether they were a loser or winner. The results also show that males have significantly larger claws compared to females and that claw size affects the outcome of a fight. The results suggest that females do fight, however the mechanisms of maintaining dominance hierarchies are more variable than in males.

Paper IV: Mating behaviour and evidence for female sex Pheromones in Norway lobster (*Nephrops norvegicus*)

In this study the mating behaviour of *Nephrops* was studied. Moreover, the role of female odour during the mating season was investigated. The study of mating behaviour can lead to alternative methods of cultivation to avoid overfishing. This understanding can help improve fishing management and thus the sustainability of wild populations.

Mating interaction

Similar procedure as in Paper I for fight procedure were used apart from that male encounter female. Three different treatments were tested. In the first treatment a postmoult females were encountered with a males adding female postmoult odour. In the second treatment, intermoult females were encountered with males adding female postmoult odour. At last, intermoult females were encountered with males and adding female intermoult odour.

Analysis of mating behaviour

All behaviours were noted and categorized in order to compare the behaviours between all experiments. Mating behaviour in Norway lobsters (*Nephrops norvegicus*) were divided into 6 stages (Table 2). All 6 behaviours were described and used in the analysis for both male and female behaviour. Mating success was determined by whether or not the male performed thrusting with his abdomen. Mating attempt was described as grabbing the female from behind and trying to turn her

around, yet further copulation behaviour, e.g. thrusting, will not follow. The latency to onset of mating was calculated from when the animals were introduced to the start of mating behaviour 2 (mounting; Table 2). The mating duration started from the mounting behaviour to when the animals were separated.

Table 2. Definition of courtship levels for mating *N. norvegicus* (adapted from Skog, 2009).

Level	Behaviour	Definition
1	Male Approach	The male approaches the female by walking towards her from the front (N = 6), the side (N = 2) or from the back (N = 8)
2	Mount	The male climbs onto the females' carapace, from behind or from the side when they are parallel to each other, using his pereiopods. The female is usually passive during mounting and does not move unless she refuses to mate. In the latter case, the female tries to escape, which occurred 8 times out of 24 mounting attempts
3	Turn	The male turns the female using his walking legs (pereiopods) onto her back (N = 6) or onto the side (N = 6). During this procedure the male often holds a claw or antenna of the female with one cheliped (N = 11). In some cases there was no reason for the male to turn the female, since in 4 out of 16 matings the female turned onto her back herself while the male tried to climb on her
4	Positioning	The male positions himself on top of the female (N = 6 out of 16 matings) so the ventral-to-ventral and face-to-face position can be maintained and the male gonopods are closest to the female seminal receptacle. This stage is skipped if the male has turned the female to the side directly instead of turning her onto her back
5	Rolling	The males who turned females on their backs and positioned themselves on top, will now turn with the females to the side while the hold on to the female with their pereiopods. Usually at this point the males' claw lets go of the females' claw or antenna. The female are in a torpedo shape with outstretched chelipeds
6	Thrusting	The male moves his abdomen rapidly, while the uropods at the telson open and close (Indication of spermatophore transfer and thus mating success (Skog, 2009))

Results

The mating behaviours of *Nephrops* were defined into 6 stages. The highest number of "matings + mating attempts" (N = 13) were achieved in the first treatment when the male encountered a postmoult female and potsmoult odour was added. In the second treatment were the female was in intermoult stage and female postmoult odour was added 10 "matings + mating attempts" were achieved. In the last treatment were the female is in an intermoult stage and female intermoult odour was added, 3

"matings + mating attempts" were achieved. The results show that postmoult mating and intermoult mating occur in *Nephrops*. The results suggest that female postmoult odour plays an important role in inducing mating behaviour in males. However, the results also suggest that intermoult female are able to produce sex specific pheromones to reduce male aggression and to induce mating behaviour in males.

Discussion

Over the four years of this project the total catch production of *Nephrops* declined by 3909 tonnes (FAO, 2011) and started to show signs of high fishing activity. For example, the *Nephrops* fisheries on the west coast of Scotland (Loch Torridon) have lost their Marine Stewardship Council (MSC) eco-label (Smith, 2010), which certifies the sustainability of a fishery. Moreover, fishermen have observed a decline in size of individual animals and a decrease in catching rates indicated by W. Cameron, (personal communication, 7 February 2010). Also, the finding of plastics in the stomachs of *Nephrops* by Murray and Cowie (2011) raises concerns for *Nephrops* fisheries, since this issue may be effecting the general *Nephrops* population it could have implications on both the health and the catch rate of the *Nephrops*. There is also a possibility that the *Nephrops* contaminated with plastic are a risk to the health of humans. However, *Nephrops* fisheries remain one of the most important shellfish resources in the UK (Scottish Executive, 2006). Although the high economic importance, nutritional benefit and decreasing catch rate makes them highly attractive to culture, only a few studies have conducted research on culturing *Nephrops* (Anger and Püschel, 1986; Dickey-Collas et al., 2000; Farmer, 1972b; Figueiredo, 1979; Figueiredo and Vilela, 1972; Hillis, 1975; McQuaid 2000; Thompson and Ayers, 1989; Rotlland et al., 2001; Smith, 1987), all with little success.

The aim of this study was to first gain a better understanding of adult *Nephrops*' social and reproductive behaviour before starting to rear larvae. The reason for this is that gaining a better understanding of adult *Nephrops* behaviour will help improve transportation methods and holding conditions in captivity since transportation methods and holding conditions affect animals' health and survival rates. This is particularly significant in the *Nephrops* industry where quality is an important factor,

especially when exporting them to countries, such as France, Spain or Italy. Moreover, the condition of the berried females affects the quality of eggs and thus the development of the larvae. After adult behaviour was further understood, the hatched larvae were kept using a new holding methods (circular tray).

Firstly, this thesis tested the agonistic behaviour of male *Nephrops* by conducting consecutive fight experiments. Then, in further experiments, the urine release was blocked on the second day to investigate the importance of chemical cues in the maintenance of dominance in repeated encounters. The results suggest three main conclusions: Male Norway lobsters fight in a ritualized manner and form dominance relationships; urinary signals play a role in maintaining dominance; and, losers are more active in olfactory assessment during fights than winners. In total 9 different fighting levels were described ranging from defensive behaviour, level -2, to aggressive behaviour, level 5 (see table 1; in paper 1). Although *Nephrops* have well developed claws (Parslow-William et al., 2002) that they could use to injure their opponents, no unrestrained physical aggression that could inflict injury was found. However, they do use their claws to push, smack, punch the opponent or to grab appendages of the opponent. Similar behaviour was found in other decapod crustaceans, such as: American lobsters (Atema and Voigt, 1996); crayfish (Moore, 2007); brachyuran crabs (Sneddon et al., 2003). Yet, cheliped pushing has not been described in other decapods. This behaviour in which opponents press the ventral sides of their chelipeds firmly against the chelipeds of the opponent may be used to assess the relative size of the claws using tactile information. Over the two consecutive fights, males built a dominance relationship where the losers from the previous fights behave very passively. Therefore, on the second day less bouts occurred during the experiments and fight duration was shortened. However, on the second day when urine release was blocked, the losers behaved more active and the fight durations between the first day and second day did not differ. Therefore, it can be concluded that in male *Nephrops*, urine plays an important role in establishing and maintaining dominance relationships. However, it was still unknown how male Norway lobsters establish dominance.

The second part of this thesis tested two different holding conditions and investigated which mechanisms male Norway lobsters use to recognise conspecifics. The results indicate several important key points about the aggressive behaviour and abilities of

the male *Nephrops*. In the holding experiments it was shown that there is no difference in the survival rate between communal and individual holding conditions. Contrary to expectations, competitions between individuals in communal holding conditions did not result in high death rates. This suggests that male *Nephrops* were either not fighting or established dominance hierarchies thereby considerably reducing aggression. Similar behaviour was also found in other crustaceans, such as crayfish and crabs (Bovbjerg, 1956; Gherardi and Daniels, 2003; Hazlett, 1968). In communal holding conditions, encounters between individuals occurred immediately after *Nephrops* were introduced into the tank. After a few hours, the *Nephrops* seemed to acclimatise to the new environment and established a dominance hierarchy and thus the fighting behaviour decreased. However, the mechanism behind the establishment and maintenance of dominance was still unknown.

In other crustaceans, there have been shown to be three possible mechanisms for the establishment and maintenance of dominance: winner-loser effect, individual recognition and status recognition (Mesterton-Gibbons and Dugatkin, 1995; Goessman et al., 2000; Hsu and Wolf, 2001; Gherardi and Daniels, 2003; Dugatkin and Earley, 2004). The results of this study indicate that male *Nephrops* use status recognition to maintain dominance hierarchies. Since, the winner-loser effect can be ruled out from the first paper, where second day fights were as long as first day fights when urine release was blocked on the second day. Being, if the winner-loser effect were occurring, it would be expected that on the second day there would be a reduction in the loser's aggressive behaviour, whether or not its opponents' urine was present. Individual recognition can also be ruled out, because no difference was found between the fight durations of familiar and unfamiliar fights. Moreover, the results show that in both treatments the fight durations from the first day to the second day decreased, indicating that male *Nephrops* have the ability to recognise conspecifics by status. On the other hand, there was found to be no significant difference in fight durations between the first and second day when urine release was blocked on the second fight (Katoh et al., 2008). This indicates that odour/chemical communication plays an important role in Norway lobsters. After it was found that dominance is based on status recognition and not on individual recognition, adding a chemical such as dominance odour would be an efficient procedure that would further reduce aggression in communal holding tanks. The results showed that although the

dominant male was not present the odour alone was enough to subdue the agonistic behaviour of the two other males; by decreasing fight durations. Although it is now known that male *Nephrops* use status recognition to form and maintain dominance hierarchies and that chemical signals play an important role in communication. Very little is known about female agonistic behaviour.

In this thesis, female agonistic behaviour was investigated by conducting female fights, mixed sex fights and male fights, then comparing the results. Moreover, it was looked at whether claw size plays a role in the outcome of the fights and if sexual dimorphism exists. In competitions between size-matched animals, fights tend to last longer and involve more potentially injurious behaviour than those between disparate animals (Smith et al., 1994). On second day encounters, social conditioning, as well as olfactory assessment of the opponents' identity and/or social status, has been found to be important (Caldwell, 1979; Karavanich and Atema, 1998a, b; Goessman et al., 2000; Gherardi and Daniels, 2003; Obermeier and Schmitz, 2003a; Moore and Bergman, 2005). Internal hormone levels can alter as a result of a fight experience and influence the outcome of a second fight (Huber and Delago, 1998; Goessman et al., 2000; Daws et al., 2002; Bergman and Moore, 2003, 2005).

This study found no difference in fighting method between male and female *Nephrops*. However, the fight durations in female fights were longer on the second day compared to the first day; while male and mixed sex fights were shorter on the second day compared to the first. Some animals such as female crabs show less intensity in terms of content or length compared to the males (Thorpe et al., 1994), this was not the case in female *Nephrops*. Although there is no significant difference in the female fight durations between the first and second day, Paper III, Figure 1 shows that there is a tendency that second day fights are longer than first day fights. This shows that no dominance hierarchy was established in female fights. On the other hand, there was an establishment of dominance between the individuals in the male and mixed-sex fights, which was demonstrated by shorter second day fight durations (Paper III, Figure 1.). The results indicate that female *Nephrops* do not recognise other females encountered previously in the same way as males do. Males that won the fight on the first day would often go on to win again on the second day. Moreover, males with bigger claws seemed to have a higher probability of wining

their fights. Yet, in the female fights, winners from the first day fights did not necessarily go on to win the second day fights, and claw size did seem to affect the outcome of the fights on both days.

This was unexpected, as females in other lobster species, e.g. European lobsters, have the ability to recognise the status of conspecifics (Skog, 2009). Therefore, other factors must induce fights in female *Nephrops*. In other species, agonistic encounters are affected by the environment (Bergman and Moore, 2003) and changing social circumstances (Ahvenharju and Ruohonen, 2007; Patullo et al., 2009). However, neither the environment nor the social circumstances changed for the *Nephrops* used in these experiments therefore, this cannot be the cause of female agonistic behaviour. Although, even though all the *Nephrops* were kept in the same conditions, motivational differences (perhaps limited resources, such as food or space) may have influenced the behaviour of female *Nephrops* during the fights.

It is now known, that male and female *Nephrops* use the same method (Paper III) to fight and that they are sexually dimorphic, since females have smaller claws than males. Yet, knowledge of the ability of female *Nephrops* to recognise individuals and/or status is lacking. The agonistic behaviour of both, males and females have been studied, however the detailed mating behaviour and the role of female odour during mating season is unknown.

The final area to be investigated in this thesis is the mating behaviour and the role of freshly moulted female odour has been investigated by testing three different treatments (Paper IV). In this study, six distinct mating stages were observed: approaching, mounting, turning, positioning, rolling and thrusting. In the first treatment where the female was freshly moulted, only two females managed to escape the mating attempt of their male *Nephrops*. The vulnerable and fragile condition of the moulted females may mean that they do not have a choice whether they want to mate or not. On the other hand, it could also mean that a female's willingness to mate may be higher when she is freshly moulted. Female blue crabs, demonstrated a willingness to mate by backing under the males body to initiate precopulatory mate guarding (Jivoff and Hines, 1998). Yet, moulted female *Nephrops* showed no such willingness. However, been that only three 'matings + mating attempts' occurred

when the female was in intermoult stage and when intermoult odour was added. This result may be an indication that for successful mating the consents of a female is necessary when in intermoult stage. Similar finding where observed in the stream-dwelling isopod that pair formation could only occur when the female did not resist (Sparkes, 2000). These findings indicate either that, females in the moult stage cannot choose their mating partners, but females in the intermoult stage can. Or, females in the moult stage are more receptive to mating advances than females in the intermoult stage.

Unlike in other species, such as the American lobster (*Homarus americanus*) (Atema *et al*., 1979), male *Nephrops* did not show any guarding behaviour, in particular carrying females or burying them in the substrate. The odour of a freshly moulted female induces mating behaviour in male *Nephrops* even when the female is in the intermoult stage. This outcome shows that the odour of moulted females contains sex-specific substances, which induces mating behaviour in males. Similar outcomes have been observed in lobster courtship behaviour (Atema and Cowan, 1986; Hughes and Matthiessen, 1962). Moreover, during the mating process the males showed no aggression to females. A possible reason for this is that female odours may suppress male aggression towards females (Cowan, 1991).

Although most of the matings occurred when the females were freshly moulted or when the odour of a freshly moulted female was added, this study presents the first intermoult mating in Norway lobsters. The occurrence of both intermoult and postmoult mating indicate the presence of female sex pheromones during the whole moult cycle as was also shown in the European lobster, *Homarus gammarus* (Skog, 2009). It is believed that intermoult mating is caused by a lack of sperm to fertilize eggs (Gosselin et al., 2005; Waddy and Aiken 1990) due to unsuccessful copulation during the moult stage.

Conclusion

This study widened our knowledge of *Nephrops* in several aspects: agonistic behaviour of both males and females, reproductive behaviour; berried female holding conditions and larvae holding. These are important results, which can be applied

immediately in the *Nephrops* industry, such as improving transportation methods and holding conditions whether they are males, females or berried females. Furthermore, it is recommended to use the chemical cues, such as dominant male odour to reduce aggressive behaviour in communal tanks when introducing new arrivals or freshly moulted female odour to induce mating behaviour in the male or in lobster pots to attract males and not females.

Overall, this study gained important information for the *Nephrops* fishing industry, and results on which further research in culturing Norway lobsters, *Nephrops norvegicus*, can be built.

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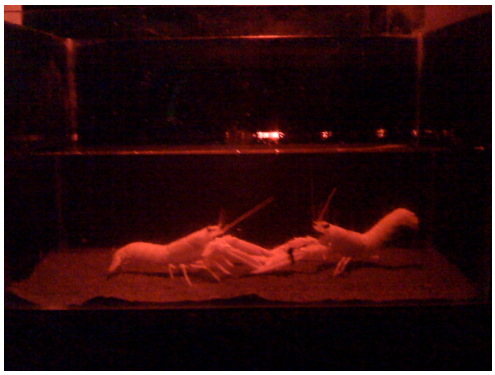
Photo gallery of *Nephrops*

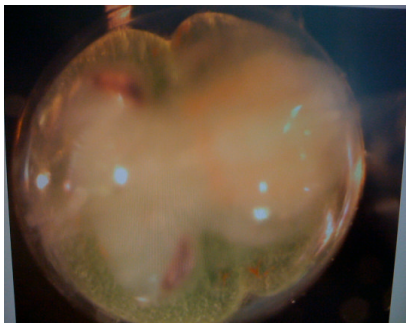
Holding claws!?



You are too smelly!

Its nice and warm sharing a shelter!

Is that an alien?



The eggs are too heavy!

The baby *Nephrops* is lost!

***Nephrops* Jacuzzi!**

Paper I

Fighting behaviour and the role of urinary signals in dominance assessment of Norway lobsters, *Nephrops norvegicus*

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Summary

Norway lobsters, *Nephrops norvegicus*, live on the bottom of the continental shelf where they construct and defend burrows. Little is known about their agonistic behaviour and potential mechanisms of dominance. This paper investigates fighting behaviour of size-matched male Norway lobsters with a particular emphasis on the role of urinary chemical signals in the assessment of dominance. Norway lobsters were paired in dyadic encounters on two consecutive days. A decrease in fight duration from first to second encounters indicates that *N. norvegicus* are able to maintain dominance in sequential contests. The main difference between the two encounters is in the behaviour of the loser. Losers strongly reduce their aggression level from first to second encounters. Olfactory sampling behaviour (antennule flicking) of eventual losers is higher than that of the winner indicating that the loser assesses chemical signals of the dominant male. When urine release is blocked for the second encounter, there is no difference in fight duration between first and second encounter. The results suggest that Norway lobsters develop lasting dominance relationships. The study also provides preliminary evidence that urine-borne chemical signals play an important role in mediating dominance.

Keywords: Norway lobster, *Nephrops norvegicus*, dominance fights, chemical signals, urine, decapod crustacean.

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Introduction

Norway lobsters, *Nephrops norvegicus* appear to be solitary living decapod crustaceans that inhabit individual burrows on the ocean floor (Marrs et al., 1996). Although they are of economic importance there is little knowledge of their social behaviour.

Norway lobsters have been observed to fight over burrows (Chapman &Rice, 1971; Farmer, 1974) but their agonistic behaviour has not been analysed in any detail. This study focuses on the fighting behaviour of Norway lobsters with emphasis on the formation of dominance and the particular role of chemical signals in the assessment of dominance.

In Norway lobsters as in many other animal species, conflict will arise between individuals over limited resources such as food, space and mating opportunities. These situations are often regulated by the formation of a dominance hierarchy (Drews, 1993). Development of a social hierarchy generally leads to a reduction in the frequency and intensity of fights (Wilson, 1975). Dominance hierarchies typically reflect differences in resource holding potential (RHP) between individuals (Parker, 1974). Correlates of RHP could be body size, weapon size, prior ownership, energetic state, or the winning/losing history of individuals (Dugatkin & Earley, 2004; Gherardi, 2006; Briffa & Sneddon, 2007).

Dominance is generally established in dyadic fights and maintained in subsequent contests (Drews, 1993). Dominance can be maintained by three possible mechanisms: individual recognition, assessment of social status, or winner/loser effect (Mesterton-Gibbons & Dugatkin, 1995; Goessman et al., 2000; Hsu & Wolf, 2001; Gherardi & Daniels, 2003; Dugatkin & Earley, 2004). For example, some insect species form linear hierarchies based on differences in confidence obtained through the previous winning/losing history of individuals (Alexander, 1961).

In crustaceans, difference in body size is an important determinant biasing the outcome of fights (e.g., in American lobsters: Scrivener, 1971; freshwater prawns: Barki et al., 1992; swimming crabs: Huntingford et al., 1995; snapping shrimp: Hughes, 1996; crayfish: Schroeder & Huber, 2001, Bywater et al., 2008). If opponents differ in body size or size of weapons fighting ability can be assessed visually or through tactile interactions (Bruski & Dunham, 1987; Hughes, 1996).

In competitions between size-matched crustaceans, social conditioning, as well as

olfactory assessment of the opponents' identity and/or social status, were found to be important (Caldwell, 1979; Karavanich & Atema, 1998a,b; Goessman et al., 2000; Gherardi & Daniels, 2003; Obermeier & Schmitz, 2003a; Moore & Bergman, 2005). Several studies of crayfish showed that previous social experience (winner/loser effect) and internal hormone level can alter the outcome of a fight (Huber & Delago, 1998; Goessman et al., 2000; Daws et al., 2002; Bergman & Moore, 2003, 2005). In addition, chemical assessment of the opponent appears to be crucial for the dynamics and outcome of fights. Urine release has been shown to play an important role in fighting behaviour of American lobsters and crayfish. American lobsters communicate via urine signals during fights (Breithaupt et al., 1999; Breithaupt & Atema, 2000). In American lobsters, these signals are used for olfactory recognition of the individual identity of their previous opponent (Karavanich & Atema, 1998a,b). Similar to lobsters, crayfish release urine signals during fights correlated with aggressive behaviours (Breithaupt & Eger, 2002; Bergman & Moore, 2005; Simon & Moore, 2007). Blocking of urine release in crayfish leads to an increase in fight duration (Zulandt Schneider et al., 2001). In contrast to lobsters, crayfish appear not to be able to recognize opponents they have fought previously (Zulandt Schneider et al., 2001; Breithaupt & Eger, 2002; Gherardi & Daniels, 2003), suggesting that winner/loser effects or olfactory assessment of social status is important (Bergman & Moore, 2003). Since in the decapod crustacean species tested so far the timing of urine release is adaptive for the sender and since urine release evokes an adaptive response in the receiver, it can be classified as a signal according to the recently refined definition of biological communication (Maynard-Smith & Harper, 2004; Scott-Phillips, 2008).

Urinary signals are released through the anterior located nephropores (Breithaupt et al., 1999). The urinary bladder is connected to the nephropores via a ureter and in American lobsters the urine is transported by the strong anterior projecting gill current away from the animal (Atema & Voigt, 1995). In this way urine signals can be sent over distances up to seven body lengths (Atema & Voigt, 1995). In marine crustaceans, urinary signals are received by the first antennae (antennules) (American lobster: Johnson & Atema, 2005; snapping shrimp: Obermeier & Schmitz, 2003b). The animals use the lateral flagella of the antennules to 'sniff' by flicking it. This mechanism enhances odour penetration into the receptor area (Koehl et al., 2001).

Norway lobster fight when they compete over burrows (Chapman & Rice, 1971). In

the field they have been observed to change burrows frequently (Chapman & Rice, 1971). The animals live at depths ranging from 20 to 800 m (Holthuis, 2006) and are well adapted to the low light environment by having large reniform reflecting superposition eyes (Shelton et al., 1985; Johnson et al., 2002). Chapman & Rice (1971) reported that Norway lobsters have a crepuscular habit and are rarely active during the day. Norway lobsters build burrows in sandy or muddy sediments, which can extend 20 – 30 cm below the surface and vary from simple burrows with one opening to more complex tunnel systems with more than one entrance (Howard, 1989).

Norway lobsters are solitary animals and live at densities from one animal in five square metres to as many as 4 animals/m2 (Howard, 1989). Population density depends on the substrate and is generally higher in course sand than in fine mud (Chapman & Bailey, 1987). Growth is negatively correlated with population density and it has been suggested that at high population densities, competition for food could be a limiting factor (Tuck et al., 1997).

These studies suggest that competition for food and shelter may have a strong effect on productivity of Norway lobster populations in the field.

The Norway lobster is one of the most commercially important European crustacean species (Scottish Government, 2006). However, knowledge of

Norway lobster behaviour is very limited and aggressive behaviour of this commercially important species has not been studied in any detail. A better understanding of social behaviour may allow improved holding conditions in captivity.

This study examines the mechanisms of dominance in *Nephrops norvegicus*.

Based on knowledge of other decapod crustaceans, we hypothesise that Norway lobsters form lasting dominance relationships. Dominance relationships may be mediated by visual, tactile and/or chemical signals. We investigate whether Norway lobsters, despite having eyes well adapted to the low lights environment, employ urine-borne olfactory signals to mediate dominance.

Methods

Animals and housing conditions

Norway lobsters, *Nephrops norvegicus* (Linnaeus, 1758) were caught with creel pots by fishermen from Eyemouth (Scotland) and from Amble (England). After an transportation period of approximately 8 h the animals were kept in 800-l communal holding tanks (15–25 animals per tank) provided with half cut clay pots that served as shelter. The water temperature was maintained constant at 8◦C. All tanks were equipped with a proteinskimmer, a biofilter and two to three air stones. The animals were kept in a light/dark (12 h : 12 h) cycle, and the light intensity was reduced using neutral density Wratten Gel filters. They were fed with frozen, cooked prawns (*Pandalus borealis*) once a week; the leftovers were taken out the next day.
Only males with hard shells were used, carapace length sizes ranging from
43 to 53 mm (46.7±2.7 mm, mean ± SE). The males selected were healthy with all appendages intact and without any signs of disease.

General procedure for all contests

Animals selected for observation were isolated by keeping them individually in plastic boxes for seven to ten days to ensure no physical, visual or chemical contact with other animals. To avoid any influence of size on the agonistic behaviour the carapace length of each pair did not differ by more than 5% (Karavanich & Atema, 1998a). Observation of male pairs was made using a 70-l glass tank (38 °— 61 °— 30 cm) which had three sides darkened with a black sheet. All observations were carried out with only the tank illuminated with dim red light using a 25-W bulb suspended 36 cm above the surface of the water. The water temperature in the experimental tank was between 10 and 12◦C. One of the two animals was marked with duct tape around the propodus of the chelipeds in order to allow easy identification. The pair was introduced into the experimental tank on opposite sides with an opaque plastic sheet separating the two sides. The animals were given thirty minutes to acclimatize to the new environment. After this time, the divider was lifted and the animals were allowed to interact for 30 min.

Animals were fought on two consecutive days with a 24 ± 2 h isolation period between encounters. We refer to encounter as the 30-min time period animals were allowed to interact. Fight refers to the time periods when the animals are interacting. A separation time of 24 h has been used in previous studies of dominance in decapod crustaceans and has proved to be long enough for complete recovery between encounters (Karavanich & Atema, 1998a,b; Zulandt Schneider et al., 2001; Breithaupt & Eger, 2002; Gherardi & Daniels, 2003; Johnson & Atema, 2005). The encounters were recorded using a Sony digital video camcorder (DCR-TRV480E) with camera and observer in the dark. All encounters were coded by date for the identification of the participants to aid in analysis of the fights at a later date. Based on stereotypical agonistic behaviours (see Table 1) the animals were identified as winners or as losers. When the first fight did not include any aggression above level 3 it was excluded from analysis ($N = 2$). Overall 52 animals (26 pairs, 13 pairs per treatment) were used.

Experimental treatment: preventing urine release

Animals were allowed to use chemical communication to establish dominance in the first encounter. During second encounters urine release was blocked by diverting the urine into catheter tubes, so that the opponent could not smell it. This method of urine blocking was previously used in order to investigate the role of urine signals in the maintenance of dominance (Karavanich & Atema, 1998a). Urine release was blocked only in second encounters as the focus of the study was on the role of urinary signals in the maintenance of dominance. Urine release was not blocked in the first fight to allow dominance to be established normally. Diverting the urine into catheters rather than completely sealing the nephropores with adhesive prevents the build-up of internal back pressure due to accumulation of urine in the body, a source of potential pain and eventual death of lobsters (personal observation on American lobsters by Breithaupt). Fight duration was recorded on the two consecutive days in 13 size-matched pairs of Norway lobsters. Animals were catheterized under a dissection microscope using a technique modified after Breithaupt et al. (1999). The pairs were prepared with catheters three hours before the fight. One end of the silicone catheter tubes (1 mm inner diameter, 30 cm long) was attached to the cuticle surrounding the nephropores (urinary pores) of the animal using cyanoacrylate glue (Zap-A-Gap).
The bond was quickly fixed with a drop of accelerator fluid (Zip Kicker). Tubings

looped up around each side of the carapace under the eyestalks and were fixed with cyanoacrylate glue and duct tape to the dorsal carapace. The other ends of the tubings were connected to two 5-ml syringes floating vertically on the water surface supported by air filled containers. Syringes were sealed by a perforated rubber plunger.

On the first day, Norway lobsters were sham-catheterized by attaching tubings to the carapace but not to the nephropores. The resulting handicap for the movement of Norway lobsters was similar between sham-catheterized and catheterized animals. Despite this handicap, sham-catheterized first fights did not differ in duration from unrestrained fights ($p = 0.91$; Mann–Whitney test) indicating that fighting behaviour was not restricted by catheterization. After the first fight the animals were returned to their plastic container and held in isolation for 24 ± 2 h. On the next day, urine release of the lobsters was blocked by fully catheterizing the animals, i.e., attaching the catheter tubes to the cuticle surrounding the nephropores. After acclimatization, the pair was allowed to interact for another thirty minute fight.

Analysis of behaviour of unblocked animals

The videotapes were copied to DVDs and analyzed on a PC. Fights were analyzed at 5-s intervals. For each interval a behavioural category was assigned for both winner and loser (Table 1). Behaviours were analyzed for 30 min after lifting the divider. If animals displayed more than one behaviour in one interval an overall level was assigned for that interval on the basis of the following ranking: levels 5, 4 and 3 outranked (>) level 2, 1, 0, −1 and −2; level 5 > 4 > 3 > 2 > 1; level −2 outranked level −1, and both level −2 and −1 outranked levels 2, 1 and 0. Olfactory sampling behaviour (antennule flicking) of both combatants during first and second encounters was recorded separately using a stopwatch.

Fight duration were measured as a sum of the duration of individual bouts that occurred within each encounter. Bouts included aggressive behaviours of both combatants higher then level 3. A bout started when the combatants were within reach of the claws (level 1 or higher) and ended at the start of a separation of at least 15 s. The fight ended when one of the animals (the loser) showed avoidance (level −1) or escaped (−2) from the winner at the end of a bout and did not show any aggression exceeding level 3 for the remaining time of the interaction. In order to test for

potential observer bias, six long control fights were re-analysed by an independent observer without knowledge of fight treatment. Differences in fight durations between experimenter and independent observer were between 4 and 11%. These changes did not effect the outcome of the statistical test. Differences in fight duration of control animals between first and second day were highly significant in both cases ($p < 0.01$; Wilcoxon signed-ranks test).

Statistics

Durations of offensive behaviours, defensive behaviours and total fight durations were analysed using non-parametric statistics since the data were not normally distributed (Shapiro–Wilk test, $p < 0.05$). The fight duration of first and second encounters were independently evaluated for both experiments.

Difference in duration between first and second fights was determined using two-tailed Wilcoxon signed-ranks tests. We regarded a significant decrease in fight duration from the first to the second encounter as evidence that dominance was maintained. No difference in fight duration indicates that dominance was not maintained (see also Karavanich & Atema, 1998). Details of the statistical results can be found in the figure legends.

Duration of flicking behaviour was normally distributed (Shapiro–Wilk test, $p > 0.05$) and analyzed using parametric statistics (mixed model ANOVA with fight number as random effect and outcome (winner/loser) and fight day (day 1/day 2) as fixed effects).

Results

Behaviour during control fights

Agonistic encounters of male *N. norvegicus* follow a common pattern starting with approach, followed by threat displays and physical interactions (see definition of behaviours in Table 1). The fight ends when one of the two combatants retreats or escapes (agonistic levels −1, −2, Table 1). Physical interactions (levels 4, 5) consist of ritualized aggressive elements including cheliped pushing and wrestling behaviour

(Table 1). Cheliped pushing (level 4) is a behaviour in which the combatants align the anterior body face to face while displaying meral spread and pushing forward (Figure 1). This behaviour (level 4) was regularly observed in fights that lasted longer than 60 s and in such fights occurred with a probability of 86%. Fights never escalated to behaviours that inflicted injury on the opponent. In general, winners showed

Table 1. Definition of agonistic levels for fighting *N. norvegicus* (adapted from Atema & Voigt, 1995)

Level	Behaviour	Definition
-2	Fleeing	Walking backwards, walking away or turning away, tailflipping
−1	Avoidance	Walking around but avoiding opponent, body pressed to the ground
0	Separate	No activity
L	Separate	Locomotion, cleaning
1	Approach	Animals within reach of claws, facing approaching, turning towards, following
2	Touching	Some body parts (e.g., abdomen, pereopods) touch for extended time without any higher levels of aggression
3	Threat display	High on legs, meral spread (horizontally spread chelipeds without display physical contact)
4	Cheliped pushing	Combatants push each other face to face in meral spread position pushing
5	Wrestling	Smacking, pushing, antennal touching claw grabbing, punching

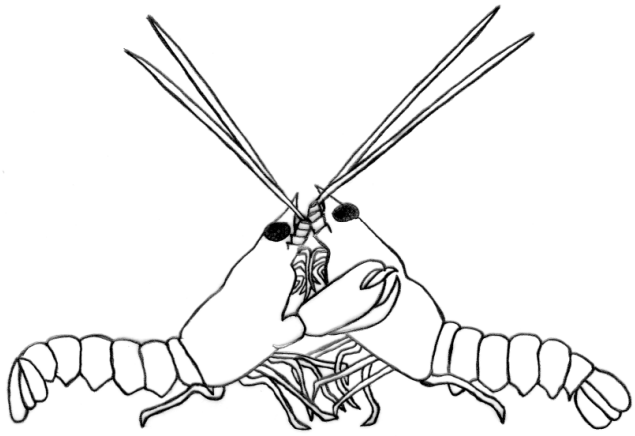

Figure 1. Sketch illustrating the behaviour 'cheliped pushing' in fighting Norway lobsters. Combatants align the anterior body face to face while displaying meral spread and pushing forward.

more aggressive behaviour than losers (p = 0.0001; Wilcoxon signed-ranks test). Losers, but not winners, reduced their offensive behaviour from the first to the second

encounter (losers: $p < 0.0001$, winners: $p = 0.54$; Wilcoxon signed-ranks test; Figure 2). In both fights, losers showed significantly more defensive behaviour than winners ($p < 0.0001$, Wilcoxon signed-ranks test; Figure 3). However, within winners and losers there was no difference in defensive behaviour between first and second encounter (loser: $p = 0.41$, winner: $p = 0.64$; Wilcoxon signed-ranks test; Figure 3).
Both winner and loser displayed antennule flicking (olfactory sampling behaviour; Schmitt&Ache, 1979) during fights. Losers spentmore time with flicking than winners ($F1,36 = 64.4$, $p = 0.0001$, mixed model ANOVA; Figure 4) No difference in flicking duration was found between first and second encounters in winners or losers.

Duration of control and urine-blocked fights

There was no reversal of outcome from first to second encounter in control fights or in urine-blocked fights. In control experiments, fight duration was significantly reduced from first to second day ($p = 0.0005$; Wilcoxon signed-ranks test, Figure 5). When urine signalling was prevented on the second day, there was no difference in duration between first and second day fights ($p = 0.64$; Wilcoxon signed-ranks test, Figure 5).

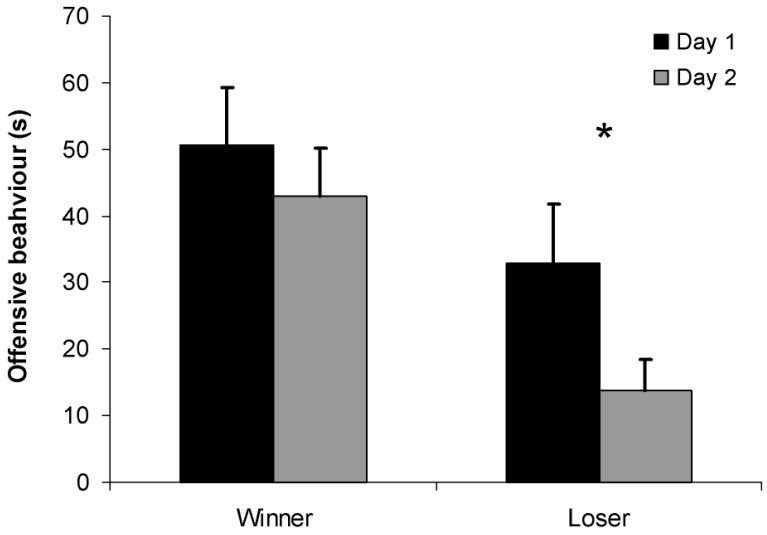

Figure 2. Offensive behaviours (levels 4, 5; see Table 1). Comparison of mean duration spent with offensive behaviour (seconds) between winner and loser in *N. norvegicus* on two consecutive days (mean + SE, $N = 13$). Asterisk indicates a significant difference in duration of loser offensive behaviour between day 1 and day 2 ($p < 0.05$;Wilcoxon signed-ranks test).

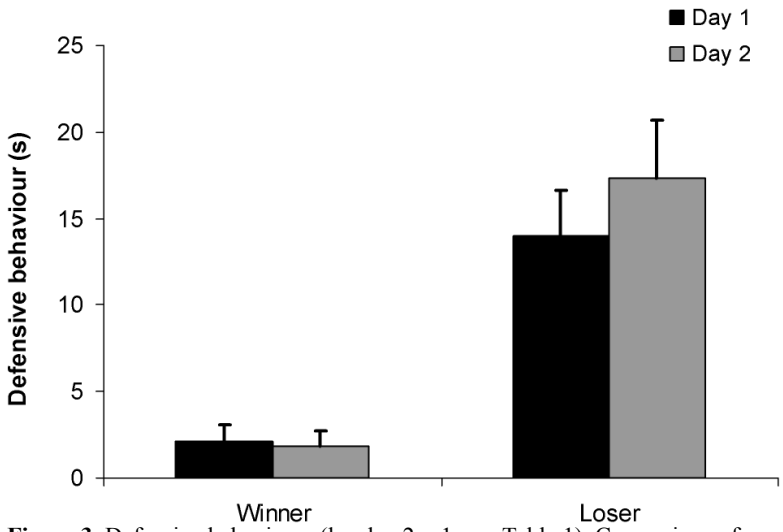

Figure 3. Defensive behaviours (levels −2, −1; see Table 1). Comparison of mean duration spent with defensive behaviour (seconds) between winner and loser in *N. norvegicus* on two consecutive days (mean + SE, $N = 13$). Losers show significantly more defensive behaviour than winners on day 1 and on day 2 ($p < 0.0001$ for both days,Wilcoxon signed-ranks tests).

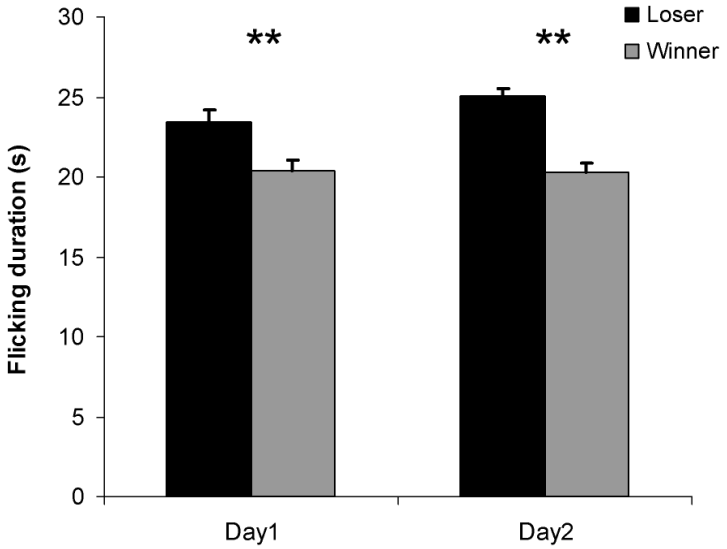

Figure 4. Antennule flicking. The duration of antennule flicking (s) in paired fights of sizedmatched *N. norvegicus* on two consecutive days (means + SE, $N = 13$). Asterisks indicate significant differences in flicking duration between winner and loser both on day 1 and on day 2 ($p < 0.01$; mixed-model ANOVA).

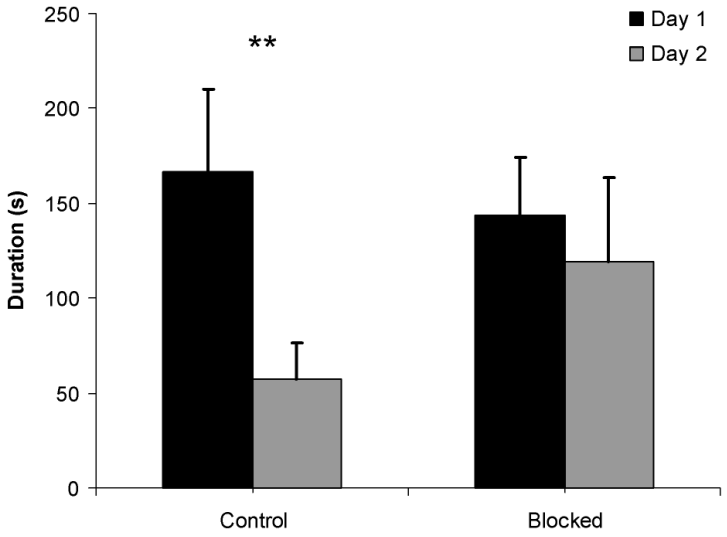

Figure 5. Fight durations. Fight duration (s) of unrestrained (control) and urine-blocked size-matched *N. norvegicus* on two consecutive days (means + SE, $N = 13$). Asterisks indicates significant differences between day 1 and day 2 in control animals ($p < 0.01$; Wilcoxon signed-ranks test).

Discussion

The results of our study suggest three main conclusions: (i) Norway lobsters form lasting dominance relationships in ritualized fights; (ii) dominance appears to be mediated by urinary signals; (iii) losers are more active in olfactory assessment during fights than winners. In the following we will first evaluate alternative explanation for our findings. We will then discuss the elements of fighting behaviour in Norway lobsters and the mechanisms they may use to maintain dominance.

The criterion for maintenance of dominance was a reduction in fight duration and intensity from the first to the second fight. Shorter, less intense fights in the second encounter could also have resulted from fatigue. However, this is unlikely since second encounters were only shortened in unrestrained combats

but not in fights between catheterised opponents. Combatants fitted with catheters did not reduce fighting time in the second encounter in spite of their additional physical burden. Furthermore, the detailed behavioural analysis revealed that only losers but not winners reduced their aggression from first to second encounter. If fatigue was a factor we would expect that both winners and losers would reduce their aggression. The results, therefore, suggest that the decreased fight duration is due to the maintenance of dominance, not due to fatigue.

Blocking urine release in second encounters prevents the reduction in fight duration

associated with the maintenance of dominance. This could be caused by three alternative effects. Firstly, the physical constraint of carrying catheters could have caused the fight to settle later than in unconstraint animals. Although we cannot completely exclude this possibility it is rather unlikely because fight duration of sham-catheterized first fights did not differ in duration from unrestrained fight (see Methods). Secondly, urinary signals in Norway lobsters may be general signals of fighting ability in first as well as in repeated aggressive encounters between individuals. Hence, preventing urine release may lead to a general increase in fight duration no matter if it is the first or second fight. Zulandt Schneider et al. (2001) found in crayfish *Orconectes rusticus* that the duration of first encounters was significantly increased when urine signals were blocked by catheters in first encounters.

Urine visualisation during first encounters of crayfish (*Astacus leptodactylus*) showed that aggressive behaviours are only effective in changing the behaviour of the opponent if they are accompanied by urine signals (Breithaupt & Eger, 2002). Thirdly, urine signals may be involved in the recognition of dominance in only the second encounters between individuals. Karavanich & Atema (1998a) demonstrated in American lobsters *Homarus americanus* that blocking urine release in the second encounter had the same effect as inactivating their olfactory receptors; urine blockage prevented a significant abbreviation of fights. Further experiments on Norway lobsters are necessary to discriminate between these three possibilities. The current study provides preliminary evidence, that urine signals play a similarly important role in dominance interactions of Norway lobsters as they do in agonistic interactions of crayfish and of American lobsters.

Fighting behaviour of N. norvegicus

Norway lobsters have well developed claws that they use for feeding on crustaceans and molluscs (Parslow-Williams et al., 2002). In a fight they could use these claws to injure their opponents. However, in 52 fights we did not find any examples of unrestrained physical aggression that could inflict injury. Other decapod crustaceans have been shown in previous studies to use their claws in order to inflict injury on the opponent, e.g., by pulling or tearing appendages off individuals (e.g., American lobsters: Atema & Voigt, 1996; crayfish: Moore, 2007; brachyuran crabs: Sneddon et

al., 2003). Similar to other decapod crustaceans, Norway lobsters use their claws to push, smack, punch the opponent or to grab appendages of the opponent. Some fight elements such as threat display (meral spread) and cheliped pushing appeared to be highly ritualized.Meral spread has been described in many other crustacean species (Sinclair, 1977; Atema & Voigt, 1996; Sneddon et al., 1997; Moore, 2007). Cheliped pushing, in contrast, has not been described in other macruran decapods. This behaviour in which opponents press the ventral sides of their chelipeds firmly against the chelipeds of the opponent may be used to assess the relative size of the claws using tactile information (Figure 1). These aggressive behaviours, perhaps together with other non-visible displays such as chemical signalling, were used to establish dominance in *N. norvegicus*. On the second day, the loser significantly reduced aggression level, leading to a reduction in the duration of the fight. The subordinate males often avoided encountering the dominant male by remaining motionless.
The dominant male, in contrast, adopted a 'dominance posture' with extended legs and meral spread. This posture resembled the dominance posture described in American lobsters *H. americanus* (Livingstone et al., 1980; Kravitz, 1988) and in squat lobsters *Munida quadrispina* (Antonsen & Paul, 1997) that can be elicited by injection of serotonin into the haemolymph (Livingstone et al., 1980; Antonsen & Paul, 1997).

Potential mechanisms of dominance maintenance in N. norvegicus

Dominance can be maintained by social conditioning (winner/loser effects), recognition of social status, by individual recognition or by a combination of these mechanisms. Our data suggest that in Norway lobsters olfactory assessment of urinary signals is important for the maintenance of dominance. Antennule flicking is higher in losers than in winners suggesting that the loser assesses chemical signals of the winner. Blocking urine release in second encounters prevents a reduction in fight duration associated with the maintenance of a dominance relationship, an effect that may be due to a role of urine signals in carrying information about dominance. Urine could convey information about the identity of the winner or about the social status of the winner. This study does not allow discriminating between these possibilities of olfactory assessment in Norway lobsters. Studies of American lobsters and different crayfish species revealed opposite mechanisms between lobsters and crayfish. In

lobsters, hermit crabs and mantis shrimps dominance is based on individual recognition while in crayfish dominance appears to be based on status recognition (Atema & Steinbach, 2007). Social experience from previous fights (winner/loser effects) could have an additional effect on fighting performance. For example, individuals that have lost the first fight might lose confidence and reduce their aggression in subsequent fight. This could lead to the observed reduction in fight duration from first to second encounters in unrestrained Norway lobsters. However, abolishing urine-borne signals in the second encounter prevented the reduction in fight duration from first to second day. Second encounters were as long as first encounters in urine-blocked Norway lobsters. This suggests that the assessment of the opponent's urine signal is more important for the maintenance of dominance than the individuals' memory of its own social experience. However, winner/loser effects could be responsible for a change in urine signals that allows the Norway lobsters to assess the social status of the opponent (Bergman & Moore, 2003).

We had no reversal of dominance even when urine release was blocked and fights were relatively long. This bias in the outcome of fights may be explained by genuine differences in fighting abilities or fighting motivation between the combatants. If one animal is slightly stronger, this animal is expected to win both the first and the second fight even if it is not recognized as a winner. Alternatively, winner/loser effects, although not distinct enough to cause a reduction in fight duration, may have resulted in the same animal winning repeatedly albeit the disruption of chemical recognition.

Norway lobsters live in depth ranging from 20 to 800 m (Holthuis, 2006).

At the low light intensities associated with their crepuscular habit and usual habitat resolution of nephrops eyes is poor compared to other crepuscular crustaceans (Shelton & Gaten, 1996). It is expected that olfactory signals gain in importance as the utility of vision is reduced. In addition, chemical signals are very well suited to transfer detailed information about hormonal state of a conspecific which might be particularly important for assessment of social status or individual identity.

In conclusion, Norway lobsters, similar to other decapod crustaceans, have the ability to form lasting dominance relationships, which are probably based on olfactory assessment of the opponents' urine. In their natural environment, formation of a dominance hierarchy may reduce overall aggression when animals live at higher densities. These results provide first insight into the little known behaviour of a

commercially important species.

Future studies need to address how dominance in the Norway lobster relates to availability of shelter, access to food and reproductive success. A better knowledge of the behaviour of this species may help improving guidelines for sustainable fisheries of the Norway lobster.

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Paper II

Communal holding conditions and the effects of social hierarchy in Norway lobster, *Nephrops norvegicus*: dominance pheromones reduce aggression in groups of *Nephrops*

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Abstract

Many aquatic crustaceans have the ability to form dominance hierarchies, yet depending on the species different strategies are used. Forming dominance relations within a group leads to a reduction in aggressive encounters and therefore less injuries and deaths. This behaviour could be used for aquaculture purposes to maintain commercial species in communal tanks. In this study Norway lobsters, *Nephrops norvegicus* were kept in two different holding conditions; communal and individual holding conditions to test whether there is a difference in survival rate. Moreover, although it is known that *Nephrops* establish dominance between individuals, the strategy used is unknown. Therefore this study investigates the fighting behaviour of sized-matched male Norway lobsters in familiar (previously known opponents) and unfamiliar (unknown opponents) treatments. The survival rate in both holding conditions did not differ significantly. The fight durations in familiar and unfamiliar fights differed between the first day and second day however, no significant difference where found between the two treatments. When dominance odour was added to male encounters, the fight durations were significantly shorter compared to when adding fresh seawater. The results show that Norway lobsters can be kept in communal holding conditions and that they develop dominance relations using status recognition. The study also provides evidence that dominance odour plays an important role in regulating aggressive behaviour.

Keyword: communal holding, individual holding, familiar, unfamiliar, status recognition, dominance fights, chemical signals, *Nephrops*, Norway lobster

Introduction

Since the late 1980's the global fish catch has been declining (Pauly et al., 2002) and the human consumption is rising (Delgado et al., 2003). Unfortunately, the issue is not the high consumption by humans but the increasing fishing activity to satisfy the demand. This can lead to decreasing population size and in the worst case to extinction. Many species are assigned to risk categories such as the commercial species like bluefin tuna (*Thunnus thynnus*), Dusky Grouper (*Epinephelus marginatus*), Sea Bass (*Dicentrarchus labrax*) or Hake (*Merluccius merluccius*) (IUCN, 2011). In order to satisfy human seafood consumption, people began to start farming and culturing fish and shellfish species for over 2000 years (Swann, 1992). Aquatic animals such as salmon, trout (Laird, 1997), cod (Björnsson et al., 2001) some shrimp species (McIntosh and Fitzsimmons, 2003) and giant clam (Hart et al., 1998) are now farmed. In the last 15 years the global production of farmed fish and shellfish has more than doubled and approximately 300 aquatic species are cultivated worldwide and more species are studied (Jennings et al., 2001; Naylor et al., 2000). The number of species farmed is increasing as the global fish stocks are decreasing.

Farming is used as an alternative to fishing in order to support the natural population and to satisfy the food demand. However, farming animals is a huge challenge, as the natural environment of the animals has to be reproduced in captivity. If they are not kept in the best possible environment they will die or the stress levels will increase, which would reduce the quality of the end product. In many crustaceans stress induces a decrease in immunocompetence and are therefore more susceptible to disease (Truscott and White, 1994). In order to produce and keep good quality animals it is necessary to observe their natural environment. This requires detailed behaviour experiments. The main issue of keeping animals in captivity is the aggressive behaviour and thus the survival rate and quality/health of the animals. Studying animal behaviour enables fishermen, farmers and also the government to use the information as a tool to improve the fishing industry and moreover the management. Human fail to manage natural populations in a sustainable manner and actions are often taken too late to save declining population, for example when the cod and haddock population collapsed (Hutchings, 2000).

Successful cultures and farms have firstly collected basic information such as water quality, light intensity, and food source. The information will help to keep animals in good conditions for a long period of time.

Many animals including the Norway lobsters display aggressive behaviour and fighting occurs between individuals over limited resources such as food, space and mating opportunities (Bergman and Moore, 2003; Chapman and Rice, 1971; Dissanayake et al., 2009). Fighting can cause injuries, limb loss or even death, which is an issue when keeping them communally (Briffa and Sneddon, 2006, McVean, 1982, Norman and Jones, 1991, Smith and Hines, 1991). The level of aggressions in fights and the method of forming a dominance hierarchy are affected by intrinsic and extrinsic factors (Landau, 1951a, b; Moore and Bergman, 2005). Intrinsic factors are inherent physiological features, such as size, sex or reproductive status. The size of an animal is correlated with fighting ability in terms of physical prowess (i.e. its resource holding power (RHP) (Moore and Bergman, 2005; Parker, 1974). Larger animals usually dominate smaller ones and the subordinate animal usually positions itself as far as possible from the dominant animal (Lee and Fiedler, 1982). Extrinsic factors are environmental circumstances such as winning/losing history of individuals (Landau, 1951 a, b; Mesterton-Gibbons, 1999; McGregor and Peak, 2000; Moore and Bergman, 2005), resource availability or variability (Hazlett et al., 1975; Bergman and Moore, 2003), High injury and death rates can be avoided with individual holding conditions however, this method of keeping animals is very costly and time intensive. More equipment is necessary to keep them separately and therefore more space is needed. Preparing food for individuals and feeding them one by one is time consuming when operating in an industrial scale. Therefore, low cost and low time consumption can only be reached when keeping animals in communal holding conditions.

In various species including cockroaches (Ewing, 1972), lobsters (Fiedler, 1965), wasps (Pardi, 1984), hermit crabs (e.g., Hazlett, 1968), and species of crayfish (Bovbjerg, 1956) it was observed that they are able to build dominance hierarchies. Once dominance relationships are established, they are likely to be maintained in subsequent encounters without further prolonged assessment of individual abilities (Winston and Jacobson, 1978). Therefore, it generally leads to a reduction in the

frequency and intensity of fights (Wilson, 1975). A previous study of *Nephrops* showed that the fight duration and intensity between two individuals in dyadic fights decreases, suggesting a formation of dominance relationship (Katoh et al., 2008). If that was the case, the follow up question should be what are the mechanisms that these animals use to maintain the formation.

There are three possible mechanisms to maintain dominance formation: individual recognition, status recognition, or winner/loser effect (Mesterton-Gibbons and Dugatkin, 1995; Goessman et al., 2000; Hsu and Wolf, 2001; Gherardi and Daniels, 2003; Dugatkin and Earley, 2004). In some fish species a winner from a previous fight tends to win again and loser are more likely to lose in future fights, which is the winner and loser effect (Hsu and Wolf, 2001). The 'winner-loser effects' does not depend on sensory assessment of the opponent (Chase et al., 1994; Hsu and Wolf, 2001) but can produce divergence of hierarchical ranks: when winner effect is dominant, when an excess of loser effect is determined or when there is a balance of winner loser effects (Hock and Huber, 2005). However, depending on the species the winner-loser effect has different lasting affects (Bergman et al., 2003). In some species of crayfish (Seebacher and Wilson, 2007), hermit crabs (Gherardi and Atema, 2005; Gherardi et al., 2005; Gherardi and Tiedemann, 2004; Hazlett, 1969), mantis shrimps (Caldwell, 1985, 1979) and lobsters (Karavanich and Atema, 1998; Johnson and Atema, 2005) use individual recognition for maintaining dominance. These species have the ability to recognise conspecifics individually from previous encounters. The subordinate animal usually avoids the dominant animal, which leads to less fighting and maintaining dominance formation. Status recognition is used by other species of crayfish (Copp, 1986) and hermit crabs (Winston and Jacobson, 1978). In this mechanism the animals are able to recognise the status of an opponent from a previous encounter. They do not recognise who, as an individual they are meeting, however, they will know whether the opponent is dominant or subordinate compared to their own status. Most of the time the subordinate animals tend to avoid dominant individuals and therefore maintain a dominance hierarchy. It is often assumed that the formation of these hierarchies depends on learned recognition of dominants by subordinates (Bovbjerg, 1956) or learned recognition of individuals (Hazlett, 1969) as occurs in some vertebrate species (e.g. Bernstein and Gordon, 1980). The dominance hierarchies in a hermit crab (Winston and Jacobson, 1978), and

most crayfish (Copp, 1986; Johnson, 1977; Zulandt Schneider et al., 2001, Breithaupt and Eger, 2002) do not involve learned individual recognition but form on the basis of recognition of an 'aggressive state'. Moreover, in lobster (Atema and Steinbach, 2007) and mice (Hurst, 1990 a, b) it was shown that chemical signals play a role in individual and status recognition (Atema and Steinbach, 2007; Hurst, 1990 a, b).

Previous studies showed that in general chemical communication plays an important role in the social behaviour of many decapods crustaceans (Thiel and Breithaupt 2011). For example Little (1975; 1976) demonstrated that larvae crayfish use chemical cues to distinguish brooding from non-brooding females or that adult crayfish have the ability to recognise a stress pheromone released by agonistically interacting conspecifics (Thorp and Ammerman, 1978). Many studies on chemical communication in crustaceans however, have focused on the use of pheromones in reproductive behaviour (Dunham, 1978). Moreover, some species use chemical cues to distinguish conspecifics from other species (Tierney and Dunham, 1982) or distinguish males from females (Ameyaw-Akumfi and Hazlett, 1975). The study by Katoh et al., (2008) showed that Norway lobsters' urine plays a role in establishing and maintaining dominance hierarchy, however, there is no study on whether conspecific odour can manipulate the behaviour of *Nephrops*.

In general, the behaviour of animals in captivity can be very well associated with the behavioural patterns in the wild (Bergman and Moore, 2003; DeFran and Pryor, 1980). In order to improve holding conditions for crustaceans, it is important to understand the animals' social behaviour. The aim of this study is firstly to investigate whether there is a difference in survival rate between communal holding condition and individual holding condition. If there is no difference between the two holding conditions (e.g. similar survival rate) it can be assumed that *Nephrops* have the ability to form dominance relations in a group. If that is the case the second question in this study will be how *Nephrops* maintain the dominance hierarchy. Three mechanisms were explained previously and when status recognition occurs it will mean that a specific odour/chemical cue can most likely influence the behaviour of conspecifics. In this study three different odours were tested: dominant male odour; female odour and fresh seawater. In the previous study by Katoh et al., (2008) it was shown that males form a dominance hierarchy, therefore it is assumed that dominance male odour might influence fighting behaviour in conspecifics. In other species it was shown that

female odour/urine increases the fight duration (Sneddon et al., 2003), whether *Nephrops* male behaviour is affected will be tested. The fresh seawater is used as a control. At the end of the experiments it will be clear which methodology is an efficient way of holding *Nephrops*.

Methodology

Animals and housing conditions

Norway lobsters, *Nephrops norvegicus* (Linnaeus, 1758) were caught from Kirn and from Eyemouth (Scotland) by local fishermen using creel pots. After a transportation period of approximately 8 h the animals were kept individually in plastic boxes (3 litres). The water temperature was maintained constant at 10 ˚C. The animals were kept in a light/dark (12 h: 12 h) cycle, and the light intensity was reduced using neutral density Wratten Gel filters. They were fed with frozen, cooked prawn meat(*Pandalus borealis*) or worms (*Hediste diversicolor*) once a week; the leftovers were taken out the next day. After leftovers were taken out the water was partially exchanged in all boxes. Only animals with hard shells were used, carapace length sizes ranging from 35 to 51 mm carapace length (CL) (43.9 ± 0.49 mm, mean ± SE). The animals selected were healthy with all appendages intact and without any signs of disease.

Experimental treatment: two different holding conditions

After arrival in the lab, animals were exposed in fresh seawater to dispose, during the transport accumulated ammonium. After washing, they are introduced into a 800-l holding tank (5 x 1 x 0.5 m). The holding tank is divided in four compartments. In each of the two outer sections there is one protein skimmer and one bio-filter. The two middle compartments (each $1m^2$) differ by one having egg-grids dividing the section into 25 smaller compartments (each 40 cm^2) and the other section not having any dividers (Figure 1). Fifty animals were divided into two groups kept in different

conditions. One population of 25 *Nephrops* were separated in individual compartments, whilst the other twenty-five animals were not separated and could therefore interact with each other. In total 12 holding experiments were conducted; 10 were only males, one only females and one a mixed sex group. Both sexes were used for the holding experiments, as females showed different recognition mechanisms (see Paper 3), any differences between male and female holding behaviour would be particularly significant from an aquacultural point of view. As males and females had previously shown different recognition mechanisms different results were also expected in the holding experiments. The water quality and temperature was the same for all treatments. The animals are kept in this condition for one month.

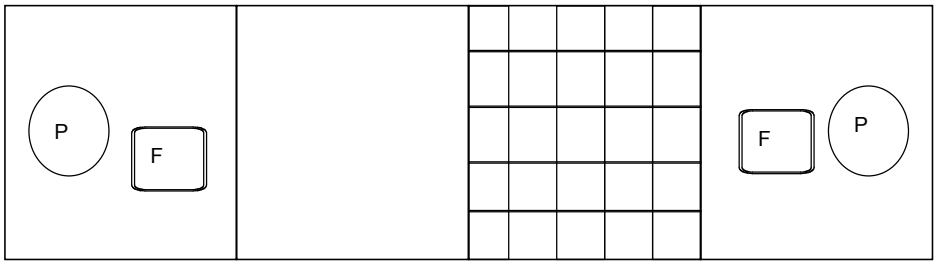

Figure 1. A schematic drawing of the holding tank. The two outer compartments ($1m^2$) were provided with a proteinskimmer (P) and biofilter (F). The two middle compartments ($1m^2$) are different by the right part having 25 smaller compartments (each 40 cm^2).

General procedure for dyadic contests

Animals selected for observations were caught between May and September. *Nephrops* individually isolated in plastic boxes (3 litres) for seven to ten days to ensure no physical, visual or chemical contact with other animals. To avoid any influence of size on the agonistic behaviour the carapace length of each pair did not differ by more than 5% (Karavanich & Atema, 1998). Observation of male pairs was made using a 70-l glass tank (38 × 61 × 30 cm) which had three sides darkened with a black sheet. Foothold for the animals was provided covering the bottom with 1cm of black sand (Aqua one, Decorative Gravel Black). All observations were carried out with only the tank illuminated with dim red light using a 25-W bulb suspended 36 cm above the surface of the water. The water temperature in the experimental tank was

between 10 and 12 °C. One of the two animals was marked with duct tape around the propodus of the chelipeds, to allow discrimination of the two oponents. The pair was introduced into the experimental tank on opposite sides with an opaque plastic sheet separating the two sides. The animals were given thirty minutes to acclimatize to the new environment. After this time, the divider was lifted and the animals were allowed to interact for 30 minutes. Animals were fought on two consecutive days with a 24 ± 2 h isolation period between encounters. We use the term "encounter" to refer to the 30 minutes time period animals were allowed to interact. "Fight" refers to the time periods when the animals are interacting. A separation time of 24 h has been used in previous studies of dominance in decapod crustaceans and has proved to be long enough for complete recovery between encounters (Breithaupt and Eger, 2002; Gherardi and Daniels, 2003; Johnson and Atema, 2005 Karavanich and Atema, 1998; Katoh et al., 2008; Zulandt Schneider et al., 2001). The encounters were recorded using a Sony digital video camcorder (DCR-TRV480E) with camera and observer in the dark. All encounters were given a code so the analyser did not know the identity of the treatment. Based on stereotypical agonistic behaviours (see Table 1) the animals were identified as winners or as losers. Animals were categorised as losers when it showed avoidance (level -1) or escape (-2) behaviour towards the winner and does not show any aggression exceeding level 3 for the remaining time of the interaction. Overall 142 animals (70 pairs, 14 pairs per treatment) were used.

Table 1 Definition of agonistic levels for fighting *N. norvegicus* (adapted from Atema & Voigt, 1995)

Level	Behaviour	Definition
-2	Fleeing	Walking backwards, walking away or turning away, tailflipping
−1	Avoidance	Walking around but avoiding opponent, body pressed to the ground
0	Separate	No activity
L	Separate	Locomotion, cleaning
1	Approach	Animals within reach of claws, facing approaching, turning towards, following
2	Touching	Some body parts (e.g., abdomen, pereopods) touch for extended time without any higher levels of aggression
3	Threat display	High on legs, meral spread (horizontally spread chelipeds without display physical contact)
4	Cheliped pushing	Combatants push each other face to face in meral spread position pushing
5	Wrestling	Smacking, pushing, antennal touching claw grabbing, punching

Experimental treatment: Individual or Status recognition

The Norway lobsters participated in two rounds of encounters. The first fights enabled *Nephrops* to assess the opponent. The second fights were designed as either familiar or unfamiliar treatment. In familiar treatments *Nephrops* encountered the same opponent that they fought in the first fight. In this case both the identity of the opponent and the status of the opponent have been encountered previously by the combatants. In the unfamiliar fights, both *Nephrops* had the same fight history as in the familiar treatment but had not previously encountered the opponent. In this case the individual identity of the opponent is unfamiliar but the opponent's status was not (Figure 2). In total fifteen sets of fights were run in each treatment. The *Nephrops* were used only once in the course of experiment.

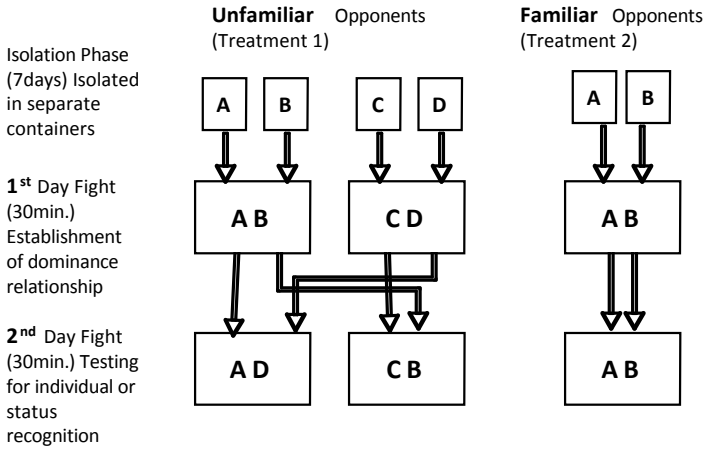

Figure 2. Schematic diagram showing the experimental fight set up. The letter A and C indicates individual with dominant status and, B and D signifies individuals with subordinate status. Animals were isolated for at least one week to eliminate any prior experience. A, B, C, and D represent *Nephrops* isolated in separate tanks during the isolation phase. In the familiar experiment animals fought their opponents from the first fight, while in the unfamiliar treatment animals' status had been encountered before but is unknown to the opponent. Arrows indicate the movement of *Nephrops* from treatment to treatment.

Experimental treatment: The effect of conspecific odour in agonistic interactions

The role of conspecific odours was tested in Norway lobsters agonistic encounters. Three treatments were tested and for each treatment 14 fights were conducted. In the first treatment a dominant male established in a previous fight were kept for 12h in the experimental tank to condition the water with dominant male odour. After the conditioning period the dominant male were replaced with two other males. They were allowed to interact immediately after they were introduced in to the tank. The experiment duration was 30 minutes. In the second treatment a female were kept in the experimental tank to condition the water with female odour for 12h. After that period the females were replaced with two males. The males were allowed to interact immediately. The experiment duration was 30 minutes. Lastly, no odour was added and a pair of males was allowed to interact for 30 minutes immediately after they were introduced to the tank. All males used for the fighting experiments were size matched. The experiments were recorded using a Sony digital video camcorder

(DCR-TRV480E). In this paper only unberried females (without eggs) were used for experiments therefore the word “female” indicates an unberried (without eggs) female.

Statistics

The survival rates in the communal holding condition and individual holding condition were analysed using parametric statistics since the data were normally distributed ($p < 0.05$; Kolmogorov-Smirnov test). The fight durations of first and second encounters in familiar and unfamiliar fights were independently evaluated for both experiments. Differences in fight duration between first and second fights and between the treatments were determined using two-way ANOVA tests. We regarded a significant decrease in fight duration from the first to the second encounter as evidence that dominance was maintained. No difference in fight duration indicates that dominance was not maintained (see also Karavanich & Atema, 1998). The effects of conspecific odours in agonistic interactions were determined by comparing the fight durations between the three treatments by using non-parametric statistics since the data were not normally distributed (One-way ANOVA test, $p < 0.05$). Moreover, to isolate the group or groups that differ from the others a multiple comparison procedure was used (Tukey Test, $p < 0.05$). Details of the statistical results can be found in the figure legends.

Results

Holding Experiment

In the two different holding conditions (Figure 1) where 25 animals were kept together and 25 animals kept individually in the same water condition, no significant differences were found in the survival rate ($p > 0.05$, T-test; $N = 600$, Figure 3). The survival rates in both holding conditions were high (79% in communal holding and 84% in individual holding conditions). Most of the animals used the shelter provided in the communal holding tank. Animals kept in the individual holding compartments burrowed into the substrate.

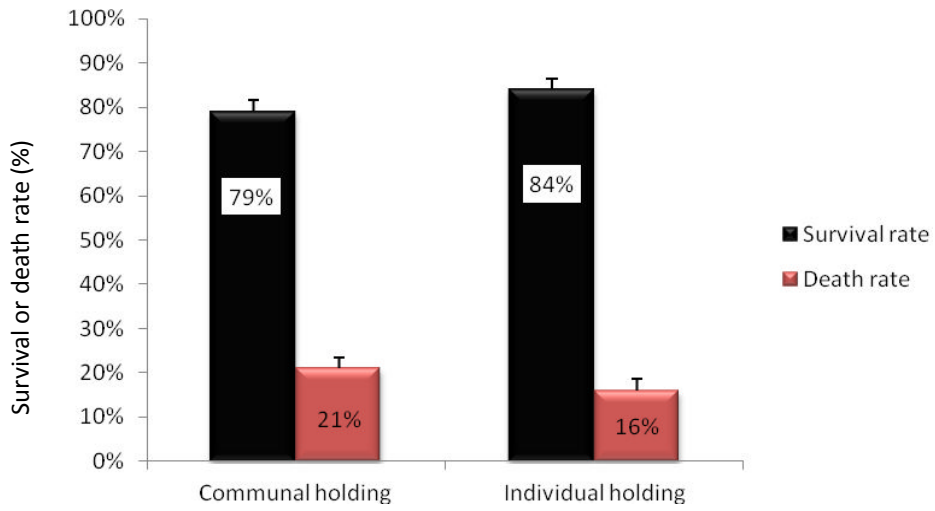

Figure 3. Comparing survival rates in two different holding conditions. There is no significant difference between the two holding conditions (means + S.E., t = 0.3, t-test; N = 12).

Individual or Status recognition in Nephrops

In both treatments familiar and unfamiliar experiments were conducted to investigate which recognition mechanisms *Nephrops* use. However, no significant difference was found in the fight durations between days and treatments (p = 0.698, Two way ANOVA; N = 56 pairs, Figure 4). In both treatments, first day fights were significantly longer compared to second day fights (p = 0.001, Tukey test; N = 28). There is no significant difference in fight durations between familiar and unfamiliar treatments (p = 0.52, Tukey test; N = 28,).

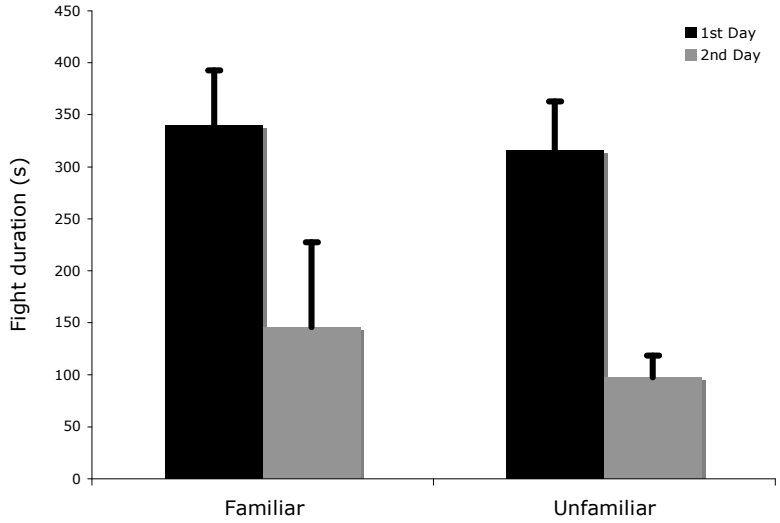

Figure 4. Fight durations. Fight durations (s) of familiar and unfamiliar size-matched *N. norvegicus* on two consecutive days (means + S.E., N = 14 pairs per treatment). Familiar treatment: A pair of size-matched male *Nephrops* fights on two consecutive days. Unfamiliar treatment: A pair of sized-matched males *Nephrops* fight on the first day, while on the second day they will fight an previously unknown opponents. The fight durations were shorter on the second day compared to the first day ($p < 0.01$,Two-Way ANOVA; N = 14 pairs). However, there is no interaction between the day and the treatment ($p > 0.05$,Two-Way ANOVA; N = 28).

The effect of conspecific odour in agonistic interactions

In this experiment three treatments were tested and for each experiment 14 male pairs were used. Conspecific odour and fight durations showed a significant interaction effect ($p = 0.003$, One-Way ANOVA; N = 42 pairs, Figure 5, Table 2). Adding dominant odour to a tank with two fighting males decreased the average fight duration significantly compared to the control, where no conspecific odour were added to the experiment ($p < 0.05$, Tukey post-hoc test; N = 28 pairs, Figure 5). However, when adding female odour no significant difference in fight durations were found between female odour and control ($p > 0.05$, Tukey test; N = 28 pairs) and also no significant difference were found between female odour treatment and dominant male odour treatment ($p > 0.05$, Tukey test; N = 28 pairs).

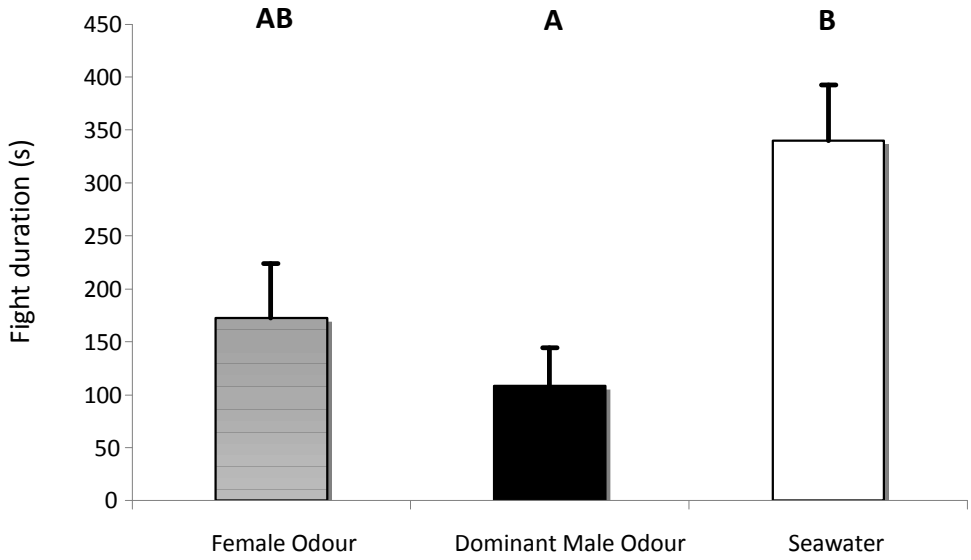

Figure 5. Fight durations. Fight durations (s) following the introduction of different stimuli: Female odour, intermoult female conditioned seawater; Male dominant odour, dominant male conditioned seawater odour; Seawater, unconditioned seawater, N = 14. Dominant male odour significantly shortens fight duration in Norway lobster, *N. norvegicus* ($p < 0.05$; One-Way ANOVA). Values are mean ± S.E. Different letters above column indicates significant differences ($p < 0.05$).

Table 2. Tukey's test applied to fight duration for multiple comparisons between the three treatments introducing different stimuli: Female = intermoult female odour; Male = dominant male odour; Control = water (q: display whether P is < 0.05 or < 0.01 for the pair compared. Large values of q indicate the difference of the two groups compared is statistically difference).

Comparison between fights	Difference of Ranks	q	P < 0.05
Female vs. Male	44.5	3.181	Not Significant
Male vs. Control	214	4.662	Significant
Control vs. Female	154	1.481	Not significant

Discussion

The results indicate several important key points about the aggressive behaviour and abilities of the Norway lobsters. In the holding experiments it was shown that there is no difference in the survival rate between communal holding and individual holding conditions. Contrary to expectation, competitions between individuals in communal

holding conditions did not result in injuries leading to high death rates. This suggests, that male *Nephrops* were either not fighting or established dominance hierarchies thereby considerably reducing aggression. Similar behaviour was also found in other crustaceans, such as crayfish and crabs (Bovbjerg, 1956; Gherardi and Daniels, 2003; Hazlett, 1968). In communal holding conditions, encounters between individuals occurred immediately after *Nephrops* were introduced into the tank. After few hours, Norway lobsters seemed to acclimatise to the new environment and established a dominance hierarchy and thus the fighting behaviour decreased. Most of the animals occupied artificial shelter or were located close to the corner with other *Nephrops*, seemingly to avoid open spaces. Rarely, it was found that Norway lobsters were injured or that limbs were missing (personal observations in the laboratory). These observations indicate that Norway lobsters are able to maintain dominance hierarchies in a group over a longer period and agree with the previous findings by Katoh et al. (2008). Once dominance relationships are established, they are likely to be maintained in subsequent encounters without further prolonged assessments of individual abilities (Winston and Jacobson, 1978) and leads to a reduction in the frequency and intensity of fights (Wilson, 1975).

For the individual holding method, more materials and equipments are necessary to build individual compartments. Moreover, feeding individuals and cleaning compartments takes a lot of time. For those reasons it is less time consuming and more cost effective, when *Nephrops* are kept in communal holding conditions compared to individual holing conditions. Most businesses have the aim to keep material and productions costs as low as possible. In the husbandry of Norway lobsters communal holding conditions would be therefore less time intensive and more cost effective method.

From the first experiment (Paper I, Katoh et al., 2008) it was shown that Norway lobsters form dominance hierarchies in a group and have the ability to maintain dominance. The mechanism behind maintaining dominance hierarchy however was not known. In this study it was tested which mechanisms Norway lobsters use to recognise conspecifics. Familiar and unfamiliar treatments were conducted and the results showed that the first day fights were significantly longer than the second day fights. The results could be explained by three different phenomena: winner-loser effect (Bergman et al., 2003; Chase et al., 1994; Hock and Huber, 2005), individual

recognition (Caldwell, 1979; Caldwell, 1985; Gherardi and Atema, 2005; Gherardi et al., 2005; Gherardi and Tiedemann, 2004; Hazlett, 1969; Johnson and Atema, 2005; Karavanich and Atema, 1998; Seebacher and Wilson, 2007) and status recognition (Atema and Steinbach, 2007; Copp, 1986; Johnson, 1977; Zulandt Schneider et al., 2001, Breithaupt and Eger, 2002). The winner loser effect is when the social experience influences the outcome of conflicts in Norway lobsters such as that winner are more likely to win again and loser will more likely lose again, even against different opponents. If the winner-loser effect was occurring in Norway lobsters it would be expected that the encounter duration are independent of the treatments. Moreover, this possibility can be excluded, as it was shown previously that blocking the nephropores on the second day did not shorten the fight durations (Katoh et al., 2008). If winner loser effect was occurring, it would be expected that despite blocking nephropores the second fight would be shorter compared to the first fight.

A second possibility is that individual recognition is occurring. Many other species use individual recognition to recognise mates and/or maintain stable dominance hierarchies, e.g. crayfish (Seebacher and Wilson, 2007), hermit crabs (Gherardi and Atema, 2005; Gherardi et al., 2005; Gherardi and Tiedemann, 2004; Hazlett, 1969), mantis shrimps *Gonodactylus festae* (Caldwell, 1979; Caldwell, 1985) and lobsters *Homarus americanus* (Karavanich and Atema, 1998; Johnson and Atema, 2005). Individual recognition is an important mechanism in dominance assessment in small groups where repeated aggressive encounters occur between individuals (Tibbetts and Dale, 2007). Should *Nephrops* have the ability to recognise an opponent based on the outcome of previous interactions, it would be expected that the second encounters are shorter in durations. This would indicate that individual recognition occurs. The same results were found in the current study however individual recognition could only occur in the familiar treatments. If Norway lobsters use individual recognition it would be expected to find a statistical difference between the fight duration of familiar and unfamiliar fights. No significant difference was found between familiar and unfamiliar fight durations, thus the possibility of individual recognition in this experiment can be ruled out. The last possible phenomenon is the status recognition. In this case if *Nephrops* have the ability to recognise an opponent by its status it would be expected that the second fights are shorter in duration and less intensive. Status recognition is likely to play an important role in the dominance establishment in larger groups where it is common that repeated interaction between unfamiliar

animals occur (Tibbetts and Dale, 2007). If status recognition were occurring in *Nephrops* the fight durations between familiar and unfamiliar would be expected to be similar. The result of the current study shows the same results and agrees with this conclusion. Further, status recognition is supported by the outcome that the unfamiliar fights are shorter compare to the familiar fights, indicating that the status recognition is utilised. In hermit crabs (Winston and Jacobson, 1978) or crayfish (Copp, 1986; Johnson, 1977; Zulandt-Schneider et al., 2001,) for example it is suggested that dominance orders is mediated by the recognition of aggressive state. A subordinate who recently established its status tends to not approach dominant individuals as often as naive animals (Copp, 1986). Results from Zulandt-Schneider et al. (2001) showed that crayfish also have a reduction in fight duration from the first to the second day however, did not differ in intensity between the two treatments, familiar and unfamiliar. Combining all the studies discussed together with the current study it can be concluded that status recognition is the mechanisms in maintaining dominance hierarchy. It is often assumed that the formation of these hierarchies depend on learned recognition of dominants by subordinates (Bovbjerg, 1956) or learned recognition of individuals (Hazlett, 1969) as occurs in the dominance orders of some vertebrate species (e.g. Allee et al., 1959; Bernstein and Gordon, 1980). In Norway lobsters it seems also that losers maintain the dominance hierarchy by reducing aggressive behaviour. The results of this study concluded that in Norway lobsters the loser recognise the status of a previous winner and thus a dominance hierarchy can be maintained over a period until changes occur, such as death, new arrivals or water conditions. Moreover, *Nephrops* frequently change their burrows and fight over burrows (Chapman and Rice, 1971) compared to American lobsters (*Homarus americanus*), which stay in the same areas and same shelter over longer time. In American lobsters (*H. americanus*) the chances are very high that they meet the same animal repeatedly where individual recognition is a beneficial strategy (Karavanich and Atema, 1998). However, in *Nephrops* status recognition is a good mechanism to avoid fights with dominant animals, as they meet many unknown opponents when they frequently change burrows (Chapman and Rice, 1971).

In many crustaceans it has been shown that chemical communications plays an important role (Thiel and Breithaupt, 2011). From a young age such as in larvae crayfish they use chemical cues to distinguish brooding from nonbrooding females

(Little, 1975; 1976) or adult crayfish reacted aggressively when exposed to water from a tank containing a male conspecific odour, but showed submissive behaviour to water from a tank containing female odour (Ameyaw-Akumfi and Hazlett, 1975).

This study was conducted to test, whether body odour of conspecifics can reduce aggression levels in other males. The study by Zulandt-Schneider et al. (2001) found that crayfish with urine present had shorter fight durations compare to encounters where urine release was blocked. In other species it was also shown that chemical signals play a role in individual and status recognition (Atema and Steinbach, 2007; Hurst, 1990 a, b). In Norway lobsters it was shown that the fight durations decreased from the first day to the second day. However, no significant difference in fight durations between first and second day was found when urine release was blocked on the second fight (Katoh et al., 2008), indicating that odour/chemical communication plays an important role in Norway lobsters.

After it was found that dominance is based on status recognition and not on individual recognition, adding a chemical, such as dominance odour would be an efficient procedure to even further reduce aggression in communal holding tanks. The result showed that although the dominant male was not present the odour alone was enough to manipulate the agonistic behaviour of two other males, by them decreasing fight durations.

Conclusion

In conclusion, Norway lobsters are able to build and maintain a dominance hierarchy in captivity. Therefore there is no necessity to keep *Nephrops* in individual compartments. The mechanism they are using is status recognition, where an individual has the ability to recognise the status of an opponent. The subordinates do not attempt to approach the dominant animal and avoid encounters. Chemical cues/odour showed to play an important role in decreasing fight durations between individuals. The source needed come from a dominant male to manipulate the agonistic behaviour of conspecific males. For future studies it is recommended to look at the component of chemicals in the urine of dominant male Norway lobsters. The

chemicals can then be added as a powder or liquid to reduce the aggressive behaviours in a group of Norway lobsters.

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Paper III

Do female Norway lobsters (*Nephrops norvegicus*) build dominance hierarchies? Or is it a male thing?

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Abstract

In many species the formation of dominance hierarchy results in a reduction of aggressive encounters and therefore a reduction in the death rate and the number of injuries. Previously it was found that male Norway lobsters, *Nephrops norvegicus*, have the ability to recognise opponents who they had previously fought and thus are able to build a dominance relationship with conspecifics. However, nothing is known about female agonistic behaviour: do they fight? If so, how do they fight? And, do they form dominance hierarchies? Females are expected to fight similarly to males to compete for food and shelter. This study investigates the difference in same-sex and mixed-sex fights of size-matched animals on two consecutive days. Following this, three aspects of the experiments were analysed; fight duration, fighting behaviour and the effect of claw size differences. The fighting duration of the female fights showed a trend to be longer on the second day compared to the mixed sex fights and male fights were the second day fights were significantly shorter. The fighting behaviour between males and females did not differ. The behaviour of the females was not affected whether being a loser or winner. The results also show that male have larger claw size compared to females and that claw size affects the outcome of the fight. The results suggest that females do fight, however the mechanisms of maintaining dominance hierarchies are more variable than in males.

Keywords: agonistic behaviour, dominance hierarchy, individual recognition, status recognition, Norway lobster, *Nephrops*.

Introduction

In the animal Kingdom aggressive conflicts often lead to the establishment of dominance hierarchies. The development of a social hierarchy generally leads to a reduction in the frequency and intensity of fights between individuals, within a group or a dense population (Drews, 1993; Wilson, 1975). Dominance hierarchies typically reflect differences in resource holding potential (RHP) between individuals (Parker, 1974). Correlates of RHP could be body size, weapon size, prior ownership, or energetic state (Dugatkin and Earley, 2004; Gherardi, 2006; Briffa and Sneddon, 2007). Dominance is generally established in dyadic fights and maintained in subsequent contests (Drews, 1993). Often dominant individuals have priority over food or shelter resources and access to mates over subordinate animals (Huntingford and Turner, 1987). There are three possible mechanisms to maintain dominance: individual recognition, assessment of social status, or winner/loser effect (Mesterton-Gibbons and Dugatkin, 1995; Goessman et al., 2000; Hsu and Wolf, 2001; Gherardi and Daniels, 2003; Dugatkin and Earley, 2004). Some species form linear hierarchies based on differences in confidence obtained through the previous winning/losing history of individuals (Alexander, 1961).

In animals, generally conflicts between individuals arise over limited resources such as space, food and mating opportunities. Factors influencing the fight outcome can be such as previous fight experience (Dunham, 1972), physical conditions (Ranta and Lindstrom 1992, 1993; Rutherford et al., 1995; Barki et al., 1997) or prior residence (Figler and Einhorn, 1983; Evans and Shehadi-Moacdieh; 1988, Peek et al., 1995). Depending on the species, there are injurious fighting (Batchelor and Briffa, 2010) and noninjurious fighting (Huntingford et al., 1995; Paye and Swanson, 1970; Petersen and Hardy, 1996; Sneddon et al., 1997).

In crustaceans body size plays an important role and often determines the outcome of fights (e.g., in American lobsters: Scrivener, 1971; freshwater prawns: Barki et al., 1992; swimming crabs: Huntingford et al., 1995; snapping shrimp: Hughes, 1996; crayfish: Schroeder and Huber, 2001; Bywater *et al*., 2008). In competitions between size-matched animals, social conditioning (i.e. winner/loser effect), as well as olfactory assessment of the opponents' identity and/or social status, has been found to

be important (Caldwell, 1979; Hsu and Wolf, 2001, Karavanich and Atema, 1998a, b; Goessman et al., 2000; Gherardi and Daniels, 2003; Obermeier and Schmitz, 2003; Moore and Bergman, 2005). The fighting ability of an opponent can be also assessed visually or through tactile interactions (Bruski and Dunham, 1987; Hughes, 1996). However, differences exist in the details of expression of the patterns and when and for how long animals display them during behavioural rituals (Nilsen et al., 2004).

Animal behaviour studies have been conducted mainly on agonistic behaviour in males (e.g. crayfish, Ahvenharju and Ruohonen, 2007; jumping spider, Faber and Baylis, 1993; lobsters, Karavanich and Atema, 1998b; mice, Ropartz, 1968; prawns, Barki et al., 1991; shore crabs, Sneddon et al., 2000). However, recent studies have revealed differences and similarities in morphology and behaviour in both, males and females. In European lobsters (*Homarus gammarus*) it was shown that female fights were more aggressive compared to male fights (Skog, 2009). When males differ from females such as in colour, size, presence or absence of body parts it is called sexual dimorphism (e.g. in birds, Owens and Hartley, 1998; crabs, Valiela et al., 1974, crayfish, Garvey and Stein, 1993). Often it is the case that the male is larger than the female and dominates the female but when the female is larger, she is usually dominant over the male (Gorlick, 1976; Payne and Swanson, 1970; Peeke et al., 1995). For example the chelipeds of many crustaceans including the Norway lobster is a conspicuous morphological feature (Lee, 1995). They are a multi-functional organ, used for foraging, agonistic interactions, competition for and handling of mates. In some species it was found that chelae contain chemosensory structures to recognise female odour source (Belanger and Moore, 2006). Some species, such as crayfish, show sexual dimorphism in chelae size. In this species the male has larger and heavier claws than the female (Stein, 1976) and the males seem to dominate females. Gender based dominance is particularly noticeable during the mating period, when males appear to overpower females (Stein, 1976).

Even when morphological differences do not exists between genders, the aggressiveness, fight duration and number of fights between males and females may vary (Skog, 2009; Adamo and Hoy, 1995; Swanson, 1974). Also the ability of recognition of conspecifics varies between sexes (Hughes, 1996; Skog, 2009). Fights can have an effect on the post-fight-behaviour. Males for example can be stressed

after a fight when they are defeated, while females show no sings of stress (Haller et al., 1999). However, females become stressed over social instability (Haller et al., 1999). Then there are also cases where gender does not play a role and one gender does not dominate over the other and only size matters; invariably the larger animal dominates over small ones (Evans and Shehadi-Moacdieh, 1988; Fielder, 1965). Several factors such as size, sex and the ability of recognition are factors to maintain dominance between individuals, within a group and in dense population. Moreover, it was shown that berried female American lobsters (*Homarus americaus*) are more aggressive compared to non-berried females. Indicating that the physiological change in the female affects the aggressive behaviour to maximise the survival of her offspring (Mello et al., 1999).

Female behaviour in *Nephrops* however has not been studied yet and, apart from a slightly wider tail than males and the fact that the first pair of pleopods are thicker in males, sexual dimorphism seems minimal. It is known that male Norway lobsters fight in the wild (Chapman and Rice, 1971) and in captivity (Katoh et al., 2008). In the field they have been observed to change burrows frequently (Chapman and Rice, 1971). The animals live at depths ranging from 20 to 800 m (Holthuis, 2006) and are well adapted to the low light environment by having large reniform reflecting superposition eyes (Shelton et al., 1985; Johnson et al., 2002). Chapman and Rice (1971) reported that Norway lobsters have a crepuscular habit and are rarely active during the day. They build burrows in sandy or muddy sediments, which can extend 20–30 cm below the surface and vary from simple burrows with one opening to more complex tunnel systems with more than one entrance (Howard, 1989). Although it was found that *Nephrops* larvae cohabit burrows with adult Norway lobsters (Chapman, 1980), adults live at densities from one animal in five square metres to as many as 4 animals per m^2 (Howard, 1989). Population density depends on the substrate and is generally higher in course sand than in fine mud (Chapman and Bailey, 1987). Growth is negatively correlated with population density and it has been suggested that at high population densities, competition for food could be a limiting factor (Tuck et al., 1997). These studies suggest that competition for food and shelter may have a strong affect on productivity of Norway lobster populations in the field.
The Norway lobster is one of the most commercially important European crustacean species (Scottish Government, 2006). However, knowledge of female Norway lobster

behaviour does not exist for this commercially important species. A better understanding of female behaviour may allow improved holding conditions in captivity and increases the prospective of culturing Norway lobster.

This study investigates the formation and maintenance of dominance in female size matched Norway lobsters. Previous studies showed that male Norway lobsters have the ability to establish dominance and that status recognition plays a role in the maintenance of dominance in *Nephrops* (Paper I, Katoh et al., 2008). Furthermore, this study compares female and male fight behaviours. It is hypothesised that females are less aggressive compared to males as was found in American lobsters (*Homarus americanus*) (Scrivener, 1972).

Methods

Animals and housing conditions

The Norway lobsters, *Nephrops norvegicus* (Linnaeus, 1758) were caught between September and October using lobster pots by local commercial fishermen in Dunoon, Scotland and were transported in a refrigerated van to the laboratory (University of Hull). Animals selected for observation were kept individually in plastic boxes (3 Litre). The water temperature was maintained constant at 10 °C. The animals were kept under dim red light. They were fed with 1g of frozen, cooked prawn meat (*Pandalus borealis*) or 1g of worms (*Nereis diversicolor*) once a week; the leftovers were taken out the next day. The water was exchanged once a week after they were fed. Only animals with hard shells were used, carapace length sizes ranging from 32 to 50 mm (41.5 ± 0.5 mm, mean ± SE). The animals selected were healthy with all appendages intact and without any signs of disease. The animals were given at least one week of acclimatization to laboratory conditions before being used in behavioural experiments.

General procedure for all contests

The animals selected for observation were isolated in individual plastic boxes for seven to ten days to ensure no physical, visual or chemical contact with other animals. To avoid size disparity influencing any agonistic behaviour, the carapace length of each pair did not differ by more than 5% (Karavanich and Atema, 1998a). Observation of pairs was made using a 70-l glass tank (38 × 61 × 30 cm) which had three sides darkened with a black sheet. All observations were carried out in the dark, with the tank illuminated by a dim red light using a 25Watt bulb suspended 36 cm above the surface of the water. The water temperature in the experimental tank was between 10 and 12°C. One of the two animals was marked with duct tape around the propodus of the chelipeds in order to allow easy identification. The pair was introduced into the experimental tank on opposite sides with an opaque plastic sheet separating the two sides. The animals were given thirty minutes to acclimatize to the new environment. After this time, the divider was lifted and the animals were allowed to interact for 30 min. Animals were fought on two consecutive days with a 24 ± 2 h isolation period between encounters. Fight refers to the time periods when the animals are interacting. A separation time of 24 h has been used in previous studies of dominance in decapod crustaceans and has proved to be long enough for a complete recovery between encounters (Karavanich and Atema, 1998a, b; Zulandt Schneider et al., 2001; Breithaupt and Eger, 2002; Gherardi and Daniels, 2003; Johnson and Atema, 2005). The encounters were recorded using a Sony digital video camcorder (DCR-TRV480E). All encounters were coded by date for the identification of the participants to aid in analysis of the fights at a later date. Based on stereotypical agonistic behaviours (see Table 1) the animals were identified as winners or losers. Overall 90 animals (45 pairs, 15 pairs per treatment) were used.

Table 1. Definition of agonistic levels for fighting *N. norvegicus* (adapted from Atema and Voigt, 1995).

Level	Behaviour	Definition
-2	Fleeing	Walking backwards, walking away or turning away, tailflipping
-1	Avoidance	Walking around but avoiding opponent, body pressed to the ground
0	Separate	No activity
L	Separate	Locomotion, cleaning
1	Approach	Animals within reach of claws, facing approaching, turning towards, following
2	Touching	Some body parts (e.g., abdomen, pleopods) touch for extended time without any higher levels of aggression
3	Threat display	High on legs, meral spread (horizontally spread chelipeds without physical contact)
4	Cheliped pushing	Combatants push each other face to face in meral spread position
5	Wrestling	Smacking, pushing, antennal touching claw grabbing, punching

Experimental treatment

Three experimental treatments were used. In the first treatment a female encountered another female. In the second treatment a male fought a female and in the last treatment a male encountered another male. All animals were allowed to use chemical communication to establish dominance on both encounters. Fight duration was recorded on the two consecutive days in 15 size-matched pairs of Norway lobsters. After the first fight the animals were returned to their plastic container and held in isolation for 24 ± 2 h. On the second day after the acclimatization period of 30 min, the pair was allowed to interact for another 30 minutes fight. In this paper only unberried females (without eggs) were used for experiments therefore the word "female" indicates an unberried (without eggs) female.

Analysis of behaviour

The videotapes were transferred to DVDs and analyzed on a PC. Fighting behaviour was analyzed at 5 seconds intervals. For each interval a behavioural category was assigned for both winner and loser (Table 1). Behaviours were analyzed for 30 min

after lifting the divider. If animals displayed more than one behaviour in one interval an overall level was assigned for that interval on the basis of the following ranking: levels 5, 4 and 3 outranked (>) level 2, 1, 0, −1 and −2; level 5 > 4 > 3 > 2 > 1; level −2 outranked level −1, and both level −2 and −1 outranked levels 2, 1 and 0. Fight duration was measured as a sum of the duration of individual bouts that occurred within each encounter. Bouts included aggressive behaviours of both combatants higher then level 3. A bout started when the combatants were within reach of the claws (level 1 or higher) and ended at the start of a separation of at least 15 s. The fight ended when one of the animals (the loser) showed avoidance (level −1) or escaped (−2) from the winner at the end of a bout and did not show any aggression exceeding level 3 for the remaining time of the interaction. In order to avoid potential observer bias, the DVD's were coded by a third person so that analysis was conducted without knowledge of the treatment.

Statistics

The fight duration differences were calculated for all three treatments and were analysed using a non-parametric test (Kruskal-Wallis test), as the data was not normally distributed ($p < 0.05$, Kolmogorov-Smirnov test). The fight duration of first and second encounters were independently evaluated for both experiments. Difference in duration between first and second fights in female vs. female and male vs. male experiments were determined using a Paired t-test. The mixed-sex fights (male vs. female) experiment failed the normality test therefore the Wilcoxon Signed Rank test was used. A significant decrease in fight duration from the first to the second encounter was regarded as evidence that dominance was maintained. No difference in fight duration indicates that dominance was not maintained (see also Karavanich and Atema, 1998a). Durations of offensive behaviours, defensive behaviours were analysed using non-parametric statistics since the data was not normally distributed (Kruskal-Wallis One Way Analysis of Variance on Ranks test). The fight durations were analysed using a parametric test (Paired t-test) and non-parametric statistics (Wilcoxon-Signed Rank test and Mann-Whitney Sum Rank test). The effects of loser or winner on the second day were tested using the Fisher Exact Test. The crusher claw size difference between male and female were tested using

ANCOVA. Moreover, whether claw size influences the outcome of the fights was tested using the logistic regression (see also Briffa and Elwood, 2010). For all conducted statistical test p values lower than 0.05 were regarded as significant. Details of the statistical results can be found in the figure legends.

Results

Fight duration

The mean fight durations between first and second day fights were compared. In female fights no difference was found between the first and the second day ($p = 0.505$, Paired t-test, N = 15 pairs; Figure 1). However, male fights ($p = 0.004$, Paired t-test, N = 15; Figure 1) and mixed sex fights ($p = 0.027$, Wilcoxon signed-rank test, N = 15 pairs; Figure 1) differed significantly between the first day and the second day. The fights on the first day were significantly longer compared to the fights on the second day.

It was found that there was a significant difference in 'fight duration differences' (difference between 1st day fight duration and 2nd day fight duration) between all three treatments ($p = 0.015$, Kruskal-Wallis test, N = 45 pairs; Figure 1). When comparing the 'fight duration differences' pairwise using a post-hoc test, a significant difference was found between female fights and male fights ($p < 0.05$, Tukey test, N = 30 pairs; Table 1). However, the 'fight duration differences' between female fights and mixed sex fights ($p > 0.05$, Tukey test, N = 30 pairs; Table 1) and between mixed sex fights and male fights ($p > 0.05$, Tukey test, N = 30 pairs; Table 1) showed no significant differences.

The mean fight durations between all three treatments were compared on the first day and second day. The mean fight durations on the first day were not significantly different between the three treatments ($p = 0.21$, Kruskal-Wallis test, N = 45 pairs; Figure 1), but when comparing the mean fight durations between all three treatments on the second day a significant difference was found ($p = 0.04$, Kruskal-Wallis test, N = 45 pairs; Figure 1). Furthermore, the post-hoc test showed there was a significant

difference in the mean fight durations on the second day between female and mixed sex fights ($p < 0.05$, Tukey test, N = 30 pairs; Table 2). However, the post-hoc test showed that there was no significant difference in the mean fight durations on the second day between female fights and male fights ($p > 0.05$, Tukey test, N = 30 pairs; Table 2) and between male fights and mixed sex fights ($p > 0.05$, Tukey test, N = 30 pairs; Table 2).

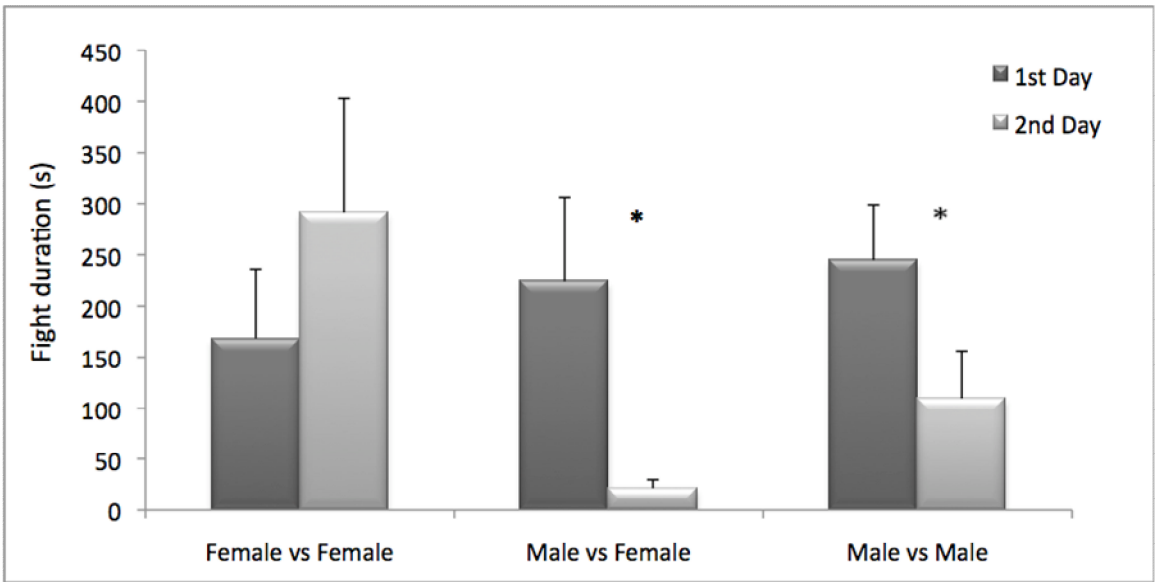

Figure 1. Fight duration. Mean fight duration (s) of same sex fights and mixed sex fights on two consecutive days (mean ± standard error of the mean). In each treatment the pairs (N= 15) met on two consecutive days. Asterisks denote significant difference between 1st day and 2nd Day, $p < 0.01$ (Paired t-test and Wilcoxon signed-rank test).

Table 1. Tukey's test applied to 1st day fight duration differences for multiple comparisons between the three treatments: female fights, mixed sex fights and male fights (q = test value).

Comparison between fights	Difference of Ranks	q	P < 0.05
Female fights vs. Male fights	198.5	3.902	Significant
Male fights vs. Mixed sex fights	44.5	0.875	Not significant
Female fights vs. Mixed sex fights	154	3.027	Not significant

Table 2. Tukey's test applied to 2nd day fight durations for multiple comparisons between the three treatments: female fights, mixed sex fights and male fights (q = test value).

Comparison between fights	Difference of Ranks	q	P < 0.05
Female fights vs. Male fights	96	1.887	Not significant
Male fights vs. Mixed sex fights	79.5	1.563	Not significant
Female fights vs. Mixed sex fights	175.5	3.45	Significant

Behaviour of winner and loser

In all three treatments the total duration of both the winners and losers offensive (level 4, 5) and defensive (level, -2, -1) behaviour was compared (Figure 2-5.). The time the winners spent exhibiting offensive behaviour differed significantly across treatments on the first day ($p = 0.013$, Kruskal-Wallis test, $N = 15$; Figure 2) and second day ($p = 0{,}016$, Kruskal-Wallis test, $N = 15$; Figure 2). The duration of offensive behaviour on the first day of the winners differed only between female fights and male fights ($p < 0.05$, Tukey Test, $N = 30$ pairs; Table 3) but no significant difference were found between male fights and mixed sex fights and between female fights and mixed sex fights ($p > 0.05$, Tukey Test, $N = 30$ pairs each; Table 4).

When comparing the offensive behaviour from the first day to the second day, although the winner showed less offensive behaviour on the second day compared to the first day, no significant difference was found ($p = 0.903$, Signed Rank test, $N = 15$; Figure 2).

The defensive behaviour of the winners on the first day ($p = 0.005$, Kruskal-Wallis test; Figure 3) and on the second day ($p = 0.046$, Kruskal-Wallis test; Figure 3) between the three treatments differed significantly.

The losers of male fights spend significantly more time behaving offensively on the first day compared to the second day ($p = 0.024$, Paired t-test, $N = 15$; Figure 4). On the other hand, the losers of female fights increased the durations displaying offensive

behaviour from the first day to the second day, however there was no significant difference ($p = 0.401$, Wilcoxon signed-rank test, $N = 15$; Figure 4).

In the male and female fights the losers spend a similar amount of time displaying defensive behaviour on the two consecutive days (Male fights: $p = 0.136$, Paired t-test, $N = 15$ and Female fights: $p = 0.305$, Paired t-test, $N = 15$; Figure 5). Yet, in the mixed sex fights the losers increased the duration of defensive behaviour significantly compared to the first day ($p = < 0.001$,Signed Rank test, $N = 15$; Figure 5).

There was no significant difference between the duration of defensive behaviour in female and male losers on the first day ($p = 0.407$,Mann-Whitney rank-sum test; $N = 15$). Furthermore, there was no significant difference between the duration of defensive behaviour in female and male losers in the mixed sex fights on the second day ($p = 0.460$, Mann-Whitney rank-sum test; $N = 13$). Moreover, males who lost the fights did not change the duration displaying defensive behaviour from the first day to the second day ($p = 0.082$; Mann Whitney rank-sum test, $N = 11$). Only the losers increased the duration of defensive behaviour significantly from the first day to the second day ($p = 0.0016$; Mann Whitney rank-sum test, $N = 17$).

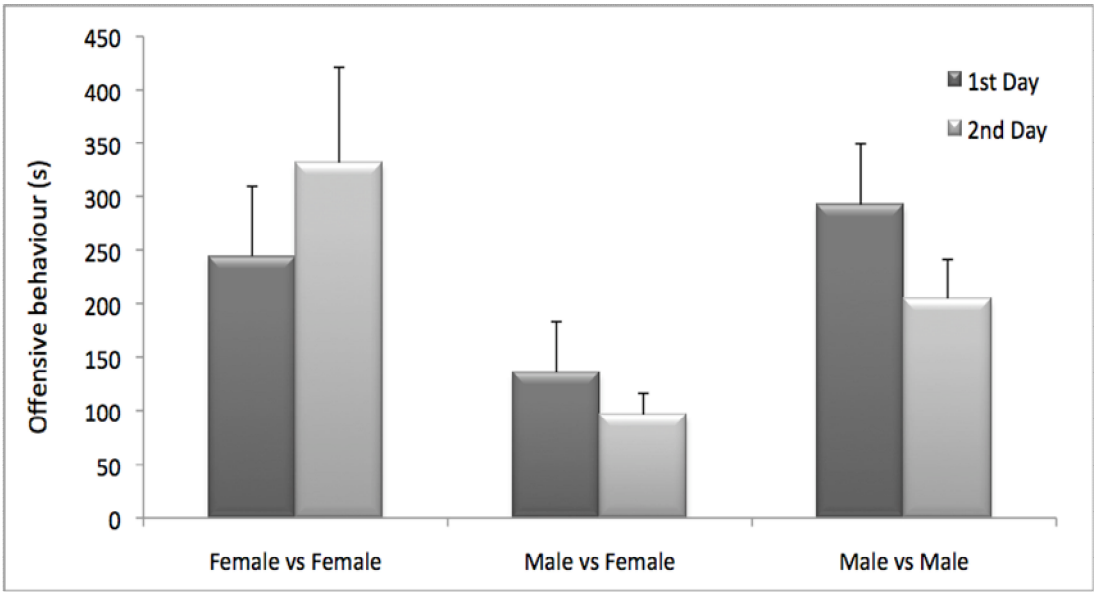

Figure 2. Winner's offensive behaviours (levels 4 and 5). Comparison of the mean duration winners spent exhibiting offensive behaviour (seconds) between the three treatments in *N. norvegicus* on two consecutive days (mean + SE, N = 15). There is a significant difference between the three treatments on the first day (p = 0.013; Kruskal-Wallis test) and on the second day (p = 0,016; Kruskal-Wallis test).

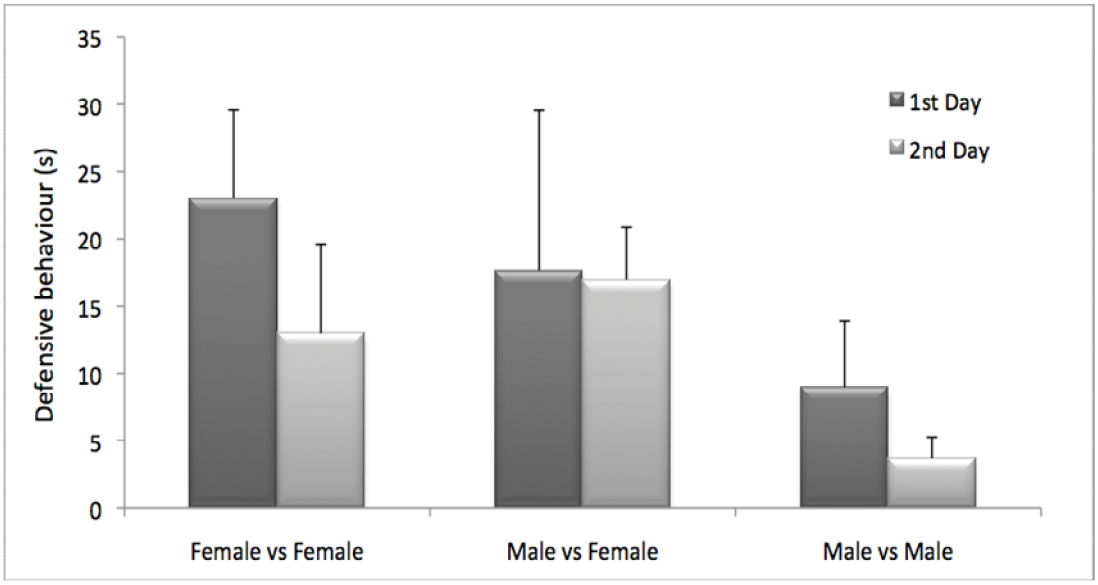

Figure 3. Winner's defensive behaviours (levels, -2 and -1). Comparison of mean duration winners spent exhibiting defensive behaviour (seconds) between all treatments (female vs. female, male vs. female, males vs. male) in *N. norvegicus* on two consecutive days (mean + SE, N = 15 pairs). There is a significant difference between all three treatments on the first day (p = 0.046; Kruskal-Wallis test) and on the second day (p = 0.005; Kruskal-Wallis test).

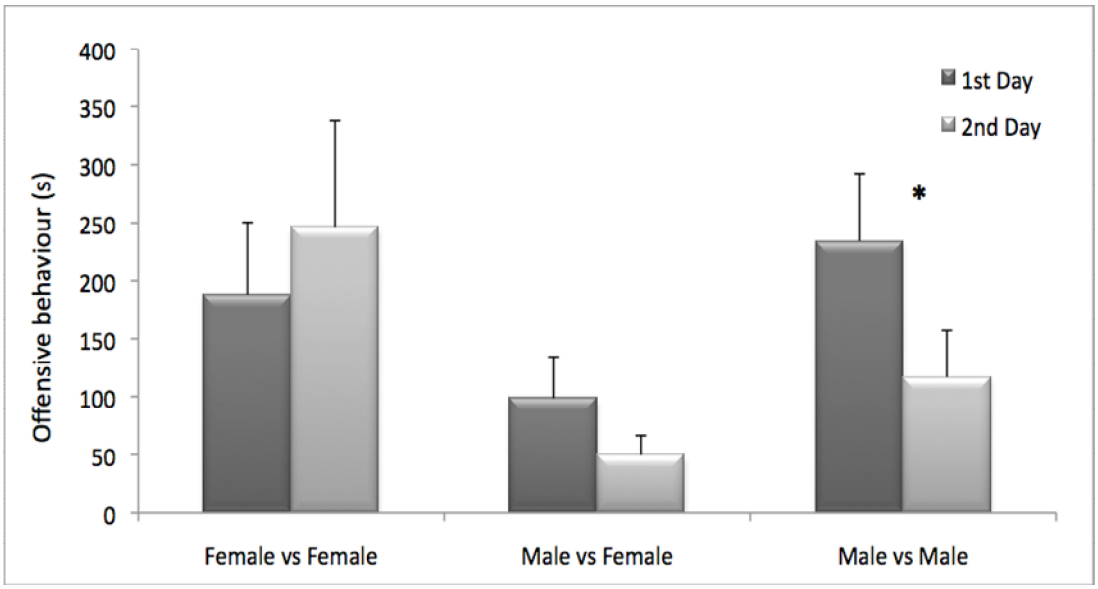

Figure 4. Loser's offensive behaviours (levels 4 and 5). Comparison of mean duration losers spent exhibiting offensive behaviour (seconds) between all three treatments (female fights, mixed sex fights, male fights) in *N. norvegicus* on two consecutive days (mean + SE, N = 15 pairs). There is no significant difference on the first day between all three treatments (p = 0.071; Kruskal-Wallis test) but there is a significant difference on the second day (p = 0,007; Kruskal-Wallis test).

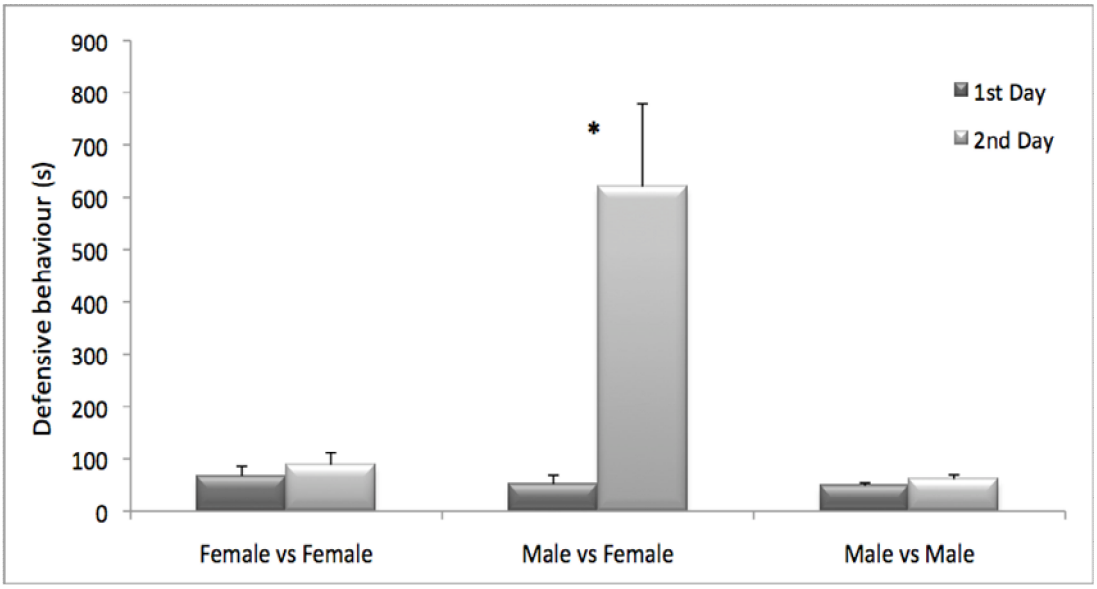

Figure 5. Loser's defensive behaviours (levels -2 and -1). Comparison of mean duration losers spent exhibiting defensive behaviour (seconds) between all three treatments (female fights, mixed sex fights, male fights) in *N. norvegicus* on two consecutive days (mean + SE, N = 15). Although there is no significant difference between the three treatments on the first day ($p = 0.3$; Kruskal-Wallis test), there is a significant difference between the three treatments on the second day ($p = < 0{,}001$; Kruskal-Wallis test). The asterisk indicates the significant difference between the first and the second day in mixed sex fights ($p < 0.005$; Mann Whitney rank-sum test).

Table 3. Tukey's test applied to offensive behaviour duration differences for multiple comparisons between the three treatments on the first day: female fights, mixed sex fights and male fights (q = test value).

Comparison between fights	Difference of Ranks	q	P < 0.05
Female fights vs. Male fights	208	4.089	Significant
Male fights vs. Mixed sex fights	72.5	1.425	Not significant
Female fights vs. Mixed sex fights	135.5	2.664	Not significant

Table 4. Tukey's test applied to offensive behaviour duration differences for multiple comparisons between the three treatments on the second day: female fights, mixed sex fights and male fights (q = test value).

Comparison between fights	Difference of Ranks	q	P < 0.05
Female fights vs. Male fights	195.5	3.843	Significant
Male fights vs. Mixed sex fights	41.5	0.816	Not significant
Female fights vs. Mixed sex fights	154	3.027	Not significant

Changes of winners on the second day

In male fights, the winner on the first day always won on the second day ($p < 0.001$, Fisher exact test, N = 15 pairs; Table 3) whereas in female fights, eight of the winners from the first day became losers on the second day ($p = 0.643$, Fisher Exact Test, N = 15; Table 3). In mixed sex fights, there were only two cases, where the winner from the first day became the loser on the second day. A significant difference was found when comparing the number of animals who remained winners on the two consecutive days between the treatments ($p = 0.001$,Chi-square test, N = 15; Table 3). Moreover, a significant difference was found between the number of winners who became losers between female fights and male fights ($p = 0.002$, Fisher Exact Test, N = 30 pairs; Table 3) and female fights and mixed sex fights ($p = 0.05$, Fisher Exact Test, N = 30 pairs; Table 3). However, no significant difference was found when comparing mixed sex fights and male fights ($p = 1$, Fisher Exact Test, N = 15; Table 3).

Table 3. Numbers of winners and losers on the first day and second day. The number of winners from the first day fight, who became losers on the second day fight.

	Female Fights	Mixed sex fights	Males Fights
Winner Female 1st Day	15	6	0
Winner Female 2nd Day	15	5	0
Winner Male 1st Day	0	9	15
Winner Male 2nd Day	0	8	15
Winner become losers from the 1st Day to the 2nd Day and vice versa	8	2	0

The effect of claw size on fight outcomes

There is a tendency that claw size has an effect on the outcome of the fights (Table 4). Depending on the sex and fight day the effect of the claw size on the fight outcome varies. In male fights, the bigger the claw the higher the possibility to win the fight on the first day fights (Wald value = 4.7, Logistic regression, $p = 0.03$; N = 15 pairs).

Yet, on the second day fight the claw size has no effect on the outcome of the fights (Wald value = 1.84, Logistic regression, p = 0.175; N = 15 pairs). In female fights claw size does not play a role on the first day (Wald value = 0.77, Logistic regression, p = 0.38; N = 15 pairs) nor on the second day (Wald value = 1.86, Logistic regression, p = 0.195, N = 15 pairs). It was shown that males have in general larger crusher claws compared to females ($p < 0.001$, Paired t-test; N = 30).

Table 4. Number of winners. The relative claw size of winners in female fights, mixed sex fights and male fights in Norway lobster, *Nephrops norvegicus*.

Claw Size of winner relative to loser	Female Fight	Mixed Sex Fight	Male Fight	Total
Larger	10	9	11	30
Smaller	5	6	4	15
Total	15	15	15	45

Discussion

This study shows that agonistic behaviour of female Norway lobsters does not differ from that of their male counterparts. However, females in general have smaller claws than males and chelae size determines the outcome of the fights. Yet, size matched female *N. norvegicus* do not establish and maintain dominance hierarchies through dyadic fights. However, female losers appear to recognize dominant males and show longer defensive behaviour on the second day compared to the first day. Furthermore, the duration of offensive behaviour differed between the sexes. In the female fights, both the winners and losers showed longer offensive behaviour on the second day compare to the first day. More than 50% of the winners on the first day become losers on the second day.

Fight duration

In competitions between size-matched animals, fights tend to be longer and involve more potentially injurious behaviour than those between disparate animals (Smith et al., 1994). On second day encounters, social conditioning, as well as olfactory

assessment of the opponents' identity and/or social status, was found to be important (Caldwell, 1979; Karavanich and Atema, 1998a, b; Goessman et al., 2000; Gherardi and Daniels, 2003; Obermeier and Schmitz, 2003a; Moore and Bergman, 2005). The internal hormone level can alter as a result of a fight experience and influence the outcome of a second fight (Huber and Delago, 1998; Goessman et al., 2000; Daws et al., 2002; Bergman and Moore, 2003, 2005). Firstly, this study found no difference between male and female fighting method. However, the fight duration on the second day differed between female fights and that of male and mixed fights. Some animals such as female crabs show less intensity in terms of content or length (Thorpe et al., 1994), which was not the case in female *Nephrops*. Although, there is no significant difference in the female fight durations between the first and second day, however Figure 1 shows that there is a tendency that second day fights are longer than first day fights. This shows that no dominance hierarchy was established in female fights. Male fights and mixed-sex fights in *Nephrops* on the other hand, demonstrated by shorter second day fight durations (Figure 1.) an establishment of dominance between the individuals.

The results indicate that female *Nephrops* do not recognise other females encountered previously in the same way as males do. Female fights have the tendency to be longer on the second day compared to the first day. However, male fights are shorter on the second day and it was previously shown that they have the ability to recognise the opponents chemically (Katoh et al., 2008). When considering only the fight durations from the first day to the second day it could be assumed that female *Nephrops* lack the ability to recognise previously encountered individuals either chemically or visually. That would be unexpected, as females in other lobster species, e.g. European lobsters, have the ability to recognise conspecifics (Skog, 2009). Yet, the assumption that females do not have the ability of recognition other factors must induce fights in female *Nephrops*. In other species, agonistic encounters are affected by the environment (Bergman and Moore, 2003) and changing social circumstances (Ahvenharju and Ruohonen, 2007; Patullo et al., 2009). However, neither the environment nor the social circumstances changed for the Norway lobsters used for the experiments, therefore this cannot be the cause of the female agonistic behaviour. Although all *Nephrops* were kept in the same conditions, motivational differences

(perhaps limited resources, such as food or space) might influence the behaviour of female *Nephrops* during the fights.

It can be argued that, since berried females spend most of their time during the incubation period in their burrows (Bell et al., 2006) and males generally move around more outside the burrows to search for mating partners and therefore have the capacity to deal with competition quicker and more efficiently compared to the females. Although this sounds reasonable, it is not actually known whether it is the male or the female *Nephrops* who initiates copulation. Moreover, it is important to mention that berried females are also caught in lobster pots. This indicates that berried females leave the burrows to forage, since they are attracted to the bait in the lobster pots. Despite this, in their natural environment females might not encounter other females as often as males do. If so, they may be able to manage to avoid conflicts. However, the purpose of this study was to help to understand the behaviour of female *Nephrops* in captivity, and the results indicate that keeping females individually reduces stress and increase their quality (see Paper II).

Behaviour of winner and loser

Female *Nephrops* did not show significant differences between the two consecutive days in their behaviour whether or not they were losers or winners. Although there was no significant difference in the fight duration between the first and second day, it was unexpected to find no significant differences between the behaviour of the winner and loser over the two days, when considering the agonistic behaviour of male *Nephrops*. This indicates that female *Nephrops* lack the ability to recognise dominant female conspecifics.

On the other hand, in mixed sex fights the loser showed significantly longer defensive behaviour on the second day. In this case the loser determines the outcome of the fight, as there is no behaviour difference in the winner. In other species the gender of the animal will determine the agonistic behaviour, such as in the Mongolian Gerbil, where the female shows high aggressiveness and the male never attacks the female (Swanson, 1974). In the fights with male *Nephrops* the loser also determines the outcome of the fight by reducing the duration of offensive behaviour. Haller et al.

(1999) showed that male rats become stressed when defeated. If this is the case with male *Nephrops*, then this combined with the fact that they are able to recognise their previously encountered opponent may have influenced the behaviour on the second day.

Changes of winners on the second day

In female fights over 50% of the winners become loser on the second day. While, in mixed-sex fights the winner and loser changed in only in two cases. In male fights the winner of the first day remained the winner on the second day. Indicate that females may be also capable of chemical recognition as it was previously shown in males (chapter 1, Katoh et al., 2008). However, as previously mentioned a possible explanation could be that female losers have the ability to remember previous fights better than winners. Thus the female losers could have the potential to defeat the previous encountered opponent. This hypothesis can be strengthened by the tendency of increasing offensive behaviour of the losers on the second day and moreover the high number of winners becoming losers on the second day.

Males, on the other hand show recognition ability remaining winners on both days. In the mixed sex fights the ability of the male reduces the possible change in the outcome of a fight on the second day. Only in one mixed-sex fight did a male become a loser on the second day (possible fighting motivation in the male was lacking). Out of fifteen fights, females won six fights on the first day and five on the second day. However, no clear gender dominance within Norway lobsters was found, the tendency of male dominance is present, like in other species where one sex dominates over the other (Payne and Swanson, 1970; Stein, 1976).

The effect of claw size on fight outcomes

In many crustaceans, size is an important factor that affects the winning or losing of aggressive encounters between individuals e.g. crayfish (Pavey and Fiedler, 2009), mantis shrimp (Caldwell and Dingle, 1979), prawns (Evans and Shehadi-Moacdieh, 1988), swimming crabs (Huntingford et al. 1995). Hughes (1996) found that for female snapping shrimps, chelae size is important in aggressive interaction

independent of the body size. With respect to *Nephrops*, the result shows that claw size does play a role in determining the winner or loser of a fight. Moreover, sexual dimorphism was found in the claw size of *Nephrops*. Male fights were won by *Nephrops* with larger claws, a third of the female fights were won by *Nephrops* with smaller claws. Female fights are unusual in being reversible and do not show the typical dominance relationship as known from males. This suggests that other factors may influence the fight outcome.

One aspect that was not tested in this study is the claw strength. Gabbanini et al. (2006) showed that chelae strength is the main factor influencing the outcome of a contest between female crabs. While in male crabs the chelae size is the important factor in a fight (Gabbanini et al., 2006). It could be considered that in female *Nephrops*, claw strength might influence the outcome of a fight, however, considering that more than 50% of the winners of the first day went on to losers on the second day, it is unlikely that any consistent factors such as strength could have had a significant effect on the outcome of the female fights.

Conclusion

Until now, agonistic behaviour of female Norway lobsters was unknown and has been assumed to be similar to male *Nephrops* and other lobster species. This study demonstrates that there are several significant differences between female and male *Nephrops* behaviour and other closely related species. Although there are no differences in fighting rituals of female and male Norway lobsters, females do not seem to have the same ability of recognition as males. Females appear to recognise dominant males, yet a missing female dominance signal may be responsible for the difference in dominance fights. Furthermore, claw size has a significant affect on the outcome of the fight. Further studies may find similar behaviour in other species, which will help interpretations of female *Nephrops* behaviour.

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Paper IV

Mating behaviour and evidence for female sex pheromones in Norway lobster (*Nephrops norvegicus*)

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Abstract

Many aquatic organisms use different mating strategies and chemical cues to conduct courtship. The understanding of mating behaviour can help improving fishing management and thus the sustainability of wild populations. This study focuses on mating behaviour, mating strategies and the possible importance of female chemical cues during the breading season in captivity. Norway lobsters generally mate after the female has moulted. Male-female interactions were investigated at different conditions using intermoult and postmoult females. This study gives a detailed description of *Nephrops* mating behaviours. The results show that postmoult mating and intermoult mating occur in *Nephrops*. The results suggest that female postmoult odour plays an important role in inducing mating behaviour in males. However, the results also suggest that intermoult females are able to produce sex specific pheromones to reduce male aggression and to induce mating behaviour in males.

Keywords: mating, moult, odour, male, female, Norway lobster,

Introduction

The seafood demand is rapidly increasing, and the importance of collecting information on animal behaviour is growing. The few research about Norway lobsters may be the inaccurate/outdated information that claims the Norway lobster fishing industry is sustainable (Smith, 2010). Decreasing catch and decreasing size of individual *Nephrops* is an indication that *Nephrops* fishing is no longer sustainable, as indicated by Cameron (2010). As a result, the growing concerns of fishermen and processors have served to renew interest and research into *Nephrops*. The understanding of mating behaviour is especially important in order to improve the

management of the fishing industry (Kamio et al., 2003). Moreover, the study of mating behaviour can lead to alternative methods of cultivation to avoid overfishing, e.g. successful copulation in captivity or even artificial insemination. It is unknown, what percentage of females achieve successful fertilisation after copulation; females with fertilised eggs can be kept in captivity, or released back into their natural habitat with the aim of increasing the number of wild population.

Crustaceans moult primarily to grow in size, yet is also known that many crustacean species mate after the female has moulted. During the moulting process the exoskeletal membrane between the thorax and abdomen ruptures and the animal withdraws from the old cuticle (Whale and Fogarty, 2006). When the female reaches maturity (between the age of 3 and 4 years in *N. norvegicus*) mating takes place shortly after moulting when the female is still in a soft-shell (postmoult) stage, and the male is in a hard-shell (intermoult) stage (Bell et al., 2006).

The diversity of mating behaviour has been verified in crustaceans. Different signals such as, visual, tactile or chemical signals or a combination of signals play a role in attracting mating partners. Depending on the species, the male or female initiates mating behaviour. In some species it was observed that females initiate mating by approaching the male (Lipcius et al., 1983); visiting the males' shelter (Cowan, 1991) or backing under the males' body (Jivoff and Hines, 1998). In general, females are selective in terms of size. This is seen when a female shore crab approaches and performs courtship behaviour to the largest available male (Sneddon et al., 2003). Female American lobsters visit males' shelters to assess the males' potential in order to choose a mating partner. After an American lobster female has chosen a male, she will form a bond with the male by repeatedly visiting his shelter before mating occurs (Cowan, 1991). In some species when the female is in the pre-moult stage, she will back under the males' body to initiate his pre-copulatory mate guarding behaviour. The guarding behaviour of the male will protect the female from predators and other males during the moult stage, when the female is weak and vulnerable (Jivoff and Hines, 1998).

In species where females search for mates, males use more complex mate attraction signals than in species where the males search for a mating partner (DeRivera and

Vehrencamp, 2001). Females produce and release sex pheromones during pre-moult, moult and post-moult stages, which seem to play an important role in manipulating male behaviour. Males change their behaviour by displaying sexual attraction or protection towards females who release sex pheromones at different moult stages (Kamiguchi, 1972).

In crustaceans it is known that there are three different mating strategies. Firstly, intermoult mating; here the mating takes place when the female is in intermoult stage, i.e. the exoskeleton is hard (e.g. crayfish; Berry and Breithaupt, 2008). Secondly, there is postmoult mating; here the male copulates with a female while she is in the soft-shell stage (e.g. many Brachyuran crabs) (Christy, 1987). Finally, there are species where males copulate with females in both intermoult and postmoult stages (e.g. European lobster, *Homarus gammarus*) (Skog, 2009). Moreover, depending on the male guarding strategy, females can mate with more than one male. Streiff *et al*. (2004) established the occurrence of multiple paternity in wild brood of Norway lobsters, *N. norvegicus*. Single paternity can be explained by behavioural mechanisms called postcopulatory guarding. In this situation, the male keeps other males away from the female for the short period of time during which the female is receptive. Such behaviour has been observed in other Nephropidae, such as the American lobster, *Homarus americanus* (Atema et al., 1979).

Female clawed lobsters have been found to have specialised internal sperm-storage organs, where sperm may be stored for up to three years before use (Waddy and Aiken 1986; Talbot and Helluy, 1995). Similarly, tanner crab *Chionoecetes bairdi* sperm has been found to be viable for up to 2 years of storage in a female's spermathecae, where sperm from different years and mates is known to accumulate (Paul, 1984; Webb, 2009). This gives the indication that spermatophores can be retained throughout the moult process and that females have the possibility to fertilise their eggs without copulating (Paul, 1984, Webb, 2009). In tropical and deep-water lobsters, where the population density is low and consequently the male-female encounter rate, the provision to store sperm for extended periods can be particularly important (MacDiarmid and Sainte-Marie, 2006). Some males have the ability to discriminate between mature and immature females and moreover between

inseminated and uninseminated females, which may be determinant factors for the mating success (Waddy and Aiken, 1990).

Knowledge of mating behaviour is important to establish the extent to which crustaceans use chemical communication and of its role in mating strategies (Streiff et al., 2004). In many crustaceans chemical cues play an important role in conspecific communication (Bushmann and Atema, 1996, 1997, 2000; Bamber and Naylor, 1997; Breithaupt and Atema, 2000; Thiel and Breithaupt, 2011; Zulandt-Schneider and Moore, 2000). Chemical cues are used to recognise conspecifics (Tierney and Dunham, 1982; Wyatt 2011) and to distinguish between males and females (Ameyaw-Akumfi and Hazlett, 1975; Hay, 2011). Males and females can perceive chemicals released from their own and the other species (Gherardi and Tiedemann, 2006), but are only attracted to the chemicals of conspecific member of the opposite sex (Tierney and Dunham, 1982). The presence of female pheromones can cause changes in intensity and duration of fight between males. Fights were longer and more intense when female pheromones were present (Sneddon et al., 2003). Conspecific male odours can cause aggressive reactions in other males, while males receiving conspecific female odour show subdued behaviour. It is thought that crustaceans can avoid dangerous situations by recognising stress pheromones produced by conspecific animals (Thorp and Ammerman, 1978).

Chemical courtship signals have been studied since the 1950's. This started with the discovery of the silk moth, *Bombyx mori*, sex pheromone (Schneider, 1957, Butenandt et al., 1959). Since, sex pheromones have also been reported in lobsters (Atema and Engstrom, 1971; Atema et al., 1979; Dunham, 1979; Atema and Steinbach 2007; Aggio and Derby, 2011), crabs (Ryan, 1966; Eales, 1974; Gleeson, 1980; Kamio et al., 2002, 2003; Kamio and Derby, 2011), and shrimps (Kamiguchi, 1972; Caskey et al., 2009; Bauer, 2011). Sex pheromones can be species specific and may contribute to reproductive isolation amongst sympatric species (Ryan, 1966; Eales, 1974; Kamio and Derby, 2011). So far, sex pheromones have been found to be components of a crustaceans moult body odour (Atema and Cowan, 1986; Bauer, 2011) and urine (Berry and Breithaupt, 2010). Some females release sex pheromones only during a specific period, such as the breading season (Stebbing et al., 2003). However, in some cases the odour of a mature female is enough to induce searching and chasing

behaviour in males even in the absence of the female (Yano et al., 1988). Moreover, it was also found that female olfaction is crucial for intermoult mating. There might be a possibility that in some animals, female sex pheromones can be present during the entire female moult cycle and not only at the time of moulting (Skog, 2009). Crustaceans use chemical cues from an early age, for example crayfish larvae distinguish brooding from non-brooding females by using chemical cues (Little, 1975, 1976).

In crustaceans most of the work on chemical communication has focused on the use of pheromones in reproductive behaviour (Dunham, 1978). However, the sex pheromones of many species of crustaceans remain unknown. The objective of this work is to achieve mating in Norway lobsters (*Nephrops norvegicus*) in captivity. When mating occurs, the next step is to describe courtship behaviour in *Nephrops* and to investigate whether chemical cues from females are involved. It is further explored whether moult related female sex pheromones may play a role in courtship, being it is known that male *Nephrops* only mate with postmoult/soft-shell females (Farmer, 1974). The general interest of this approach concerns both ex situ and in situ management of crustacean populations (Streiff et al., 2004).

Material and Methods

Animals

Nephrops were caught between July and August using lobster pots by local commercial fishermen in Dunoon, Scotland and were transported in a refrigerated van to the laboratory (Hull University). Animals selected for observation were kept individually in plastic boxes for at least seven days, to ensure no physical, visual or chemical contact with other animals. Whether the females moulted were examined twice daily. The animals were given at least one week of acclimatization to laboratory conditions before being used in behavioural experiments.

Norway lobsters were maintained at 10 °C, in a 12h dark/light cycle and fed with worms (*Hediste diversicolor)* once a week. The males were marked with black duct tape on the top of their carapace or they had a black band around their claw in order to

allow easy identification between male and female. No animals were injured during the experiment apart from one moulted female, which casted off one claw during the experiment.

General procedure

Forty-eight pairs of Norway lobsters, ranging in size from 31 to 45 mm in carapace length (CL) (40.19 ± 0.38mm, mean ± SE) were used for observation. All observations were carried out with only the tank illuminated with dim red light using a 25 Watt bulb suspended 36 cm above the surface of the water. The water temperature in the experiment tank was between 10˚ and 12˚C. Salinity ranged from 35 to 38 ‰. To avoid any influence of size on the agonistic behaviour the carapace length of each pair did not differ by more than 5%. Observation of the pair was made using a 30 litre glass tank (45.5 x 25.5 x 25.5 cm), which had three sides darkened with a black sheet. Foothold for the animals was provided covering the bottom with 1cm of black sand (Aqua one, Decorative Gravel Black). The animals were allowed to interact for 12h and were filmed and recorded (compressing 12 hours real time on a three hours tape) with a time-lapse videocassette Recorder (Sony SVT-124P).

Postmoult Mating (PFW+PF: Postmoult female conditioned water + Postmoult female)

Freshly moulted females were only used within three days from the day they moulted. After the third day those soft shell females were not used for experiments. A freshly moulted female was introduced first and was left for 12 ± 1h to condition the water. After the conditioning period the moulted female was replaced with another freshly moulted female and a male was introduced to the same tank as the new female. After both animals were introduced to the tank the recording started. In total 48 animals were used. 16 freshly moulted females to condition the water and 16 moulted females and 16 intermoult males for the experiment.

Intermoult mating in postmoult conditioned water (PFW+IF: Postmoult female water + Intermoult female)

A freshly moulted female (from the day of moult up to the third day) was introduced into the experiment tank and left for 12 ± 1h to condition the water. After the 12h the moulted female was removed and replaced with an intermoult female and an intermoult male. The pair was allowed to interact for 12h. In total 48 animals were used. (Freshly moulted female N = 16, intermoult female N = 16 and intermoult male N = 16).

Intermoult mating (IFW+IF: Intermoult female water + Intermoult female)

An intermoult female was introduced into the experiment tank and left for 12 ± 1h to condition the water. After 12h another intermoult female and a male were introduced into the experiment tank and were allowed to interact for 12h. In total 48 animals were used, 16 intermoult females to condition the water and 16 intermoult females and 16 intermoult males for the experiment.

Video analysis

The mating behaviours of *Nephrops* were analysed in all interaction by an observer blind to the treatments. This was achieved by having a third person providing new numbers to both the DVDs and recorded interactions prior to analysis. All behaviours were noted and categorized in order to compare the behaviours between all experiments. Mating success was determined by whether or not the male performed thrusting with his abdomen. Mating attempt was described as grabbing the female from behind and trying to turn her around, and further copulation behaviour e.g. thrusting behaviour will not follow. The latency to onset of mating was calculated from when the animals were introduced to the start of mating behaviour 2 (mounting; see table 1). The mating duration started from the behaviour mounting to when the animals were separated.

Statistical analyses

Once data had been collected SigmaPlot (SigmaPlot 11.0 2008) were used to analyse the data. After the copulation behaviour was defined the numbers of matings or mating attempts in the three different experiment conditions were observed. In the experiments three categories of mating were observed; mating, attempted mating and no mating. The total number of matings and mating attempts were added. The groups 'matings + attempted matings' and 'non-matings' were compared using the Fisher's exact test. It was also recorded how long the *Nephrops* mate in minutes in each experiment (mating duration). The average for each experiment condition was calculated and then compared between all treatments using One-Way ANOVA test. Before comparing the results the data were tested for normality using the Shapiro-Wilk test. The latency of copulation was quantified in seconds and the data were compared using the One-Way ANOVA test.

Results

Mating behaviour in Norway lobsters (*Nephrops norvegicus*) can be divided into 6 stages. These stages took place within a period of 28 seconds to 6.40 minutes. Six different behaviours were observed in 16 matings (Table 1).

Table 1. Definition of courtship levels for mating *N. norvegicus* (adapted from Skog, 2009)

Level	Behaviour	Definition
1	Male Approach	The male approaches the female by walking towards her from the front (N = 6), the side (N = 2) or from the back (N = 8)
2	Mount	The male climbs onto the females' carapace, from behind or from the side when they are parallel to each other, using his pereiopods. The female is usually passive during mounting and does not move unless she refuses to mate. In the latter case, the female tries to escape, which occurred 8 times out of 24 mounting attempts
3	Turn	The male turns the female using his walking legs (pereiopods) onto her back (N = 6) or onto the side (N = 6). During this procedure the male often holds a claw or antenna of the female with one cheliped (N = 11). In some cases there was no reason for the male to turn the female, since in 4 out of 16 matings the female turned onto her back herself while the male tried to climb on her
4	Positioning	The male positions himself on top of the female (N = 6 out of 16 matings) so the ventral-to-ventral and face-to-face position can be maintained and the male gonopods are closest to the female seminal receptacle. This stage is skipped if the male has turned the female to the side directly instead of turning her onto her back
5	Rolling	The males who turned females on their backs and positioned themselves on top, will now turn with the females to the side while the hold on to the female with their pereiopods. Usually at this point the males' claw lets go of the females' claw or antenna. The female are in a torpedo shape with outstretched chelipeds
6	Thrusting	The male moves his abdomen rapidly, while the uropods at the telson open and close (Indication of spermatophore transfer and thus mating success (Skog, 2009))

Postmoult Mating (PFW+PF: Postmoult conditioned female water + Postmoult female)

In the 16 postmoult experiments there were 10 matings and 3 mating attempts (Figure 1). A mating attempt is when the male tries to copulate with the female by turning her body around (behaviours 1, 2 and 3) but is unsuccessful (lack of behaviours 4, 5 and 6). There is a significant difference in the ratio of 'matings + mating attempts' and 'non-matings' between postmoult treatment and intermoult treatment ($p = 0.001$; Fisher's exact test, Figure 1). However, there was no significant difference between the postmoult treatment and the postmoult conditioned treatment ($p = 0.433$; Fisher's exact test, Figure 1). The average duration of copulation was 2:14 minutes (Figure 2). The average latency from the start of the experiment to beginning of the mounting was 123.93 seconds (Figure 3).

Apart from one pair, each pair mated only once. One female did autotomize her claw during mating without any clear reason; the male was in contact with the female,

however did not hold her claw. After the copulation, the male and the female usually went separate ways.

Intermoult mating in postmoult conditioned water (PFW+IF: Postmoult female water + Intermoult female)

In the postmoult conditioned water experiments, out of 16 interactions there were 4 matings and 6 mating attempts (Figure 1). There is no significant difference between 'PFW+IF' and postmoult treatment 'PFW+PF' ($p = 0.433$; Fisher's exact test, Figure 1) when comparing the ratio of 'matings + mating attempts' and 'non-matings'. However, there was a significant difference between the postmoult conditioned treatment and intermoult treatment ($p = 0.029$; Fisher's exact test, Figure 1) when comparing the ratio of 'matings + mating attempts' and 'non-matings'.
The average duration of copulation was 3:47 minutes (Figure 2). The average duration from when the experiment started until the beginning of mounting was 246 seconds (Figure 3). Most pairs mated only once. However, one paired was observed to mate twice within the 12h±1 h experiment period. After the copulation, the male and the female usually went separate ways.

Intermoult mating (IFW+IF: Intermoult female water + Intermoult female)

Intermoult matings has been described in Norway lobsters (Figure 1). Two of the 16 pairs mated. One male tried to mate with an intermoult female, however she managed to escape during the mounting process. No moulting odour was present in the tank during this experiment. The pairs who mated, copulated only once during the experiment. However, the male who attempted to mate with the female approached her five times.

There was a significant difference between the ratio of 'matings + mating attempts' and 'non-matings' between postmoult matings (PFW+PF) and intermoult matings (IFW+IF) ($p = 0.004$; Fisher's exact test, Figure 1). However, there is no significant difference between the ratio of 'matings + mating attempts' and 'non-matings' between postmoult matings (PFW+PF) and intermoult matings with postmoult

conditioned water (PFW+IF) (p = 0.433 Fisher's exact test, Figure 1). Yet, there is a significant difference between the ratio of 'matings + mating attempts' and 'non-matings' between postmoult conditioned water (PFW+IF) and intermoult mating (IFW+IF) (p = 0.029; Fisher's exact test, Figure 1).

The pairs in this experiments had the longest average copulation duration with 4:43 minutes (Figure 2). Moreover, the males took the longest time to approach the female and initiate copulation, 306 seconds (Figure 3).

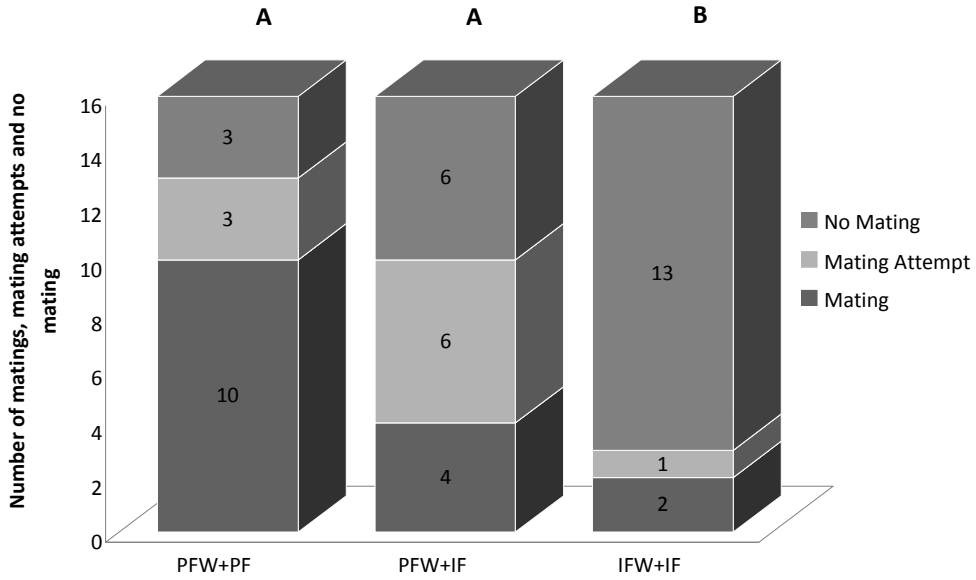

Figure 1. The total number of 'matings', 'mating attempts' and 'non matings' in *N. norvegicus* male-female pairs (N = 16) exposed to different treatments: PFW, postmoult female water; PF, postmoult female; IFW, intermoult female water; IF, intermoult female. The male was always in the intermoult stage. The letters indicate differences between the treatments. Columns with different letters are significantly different from each other with respect to the ratio between 'matings + mating attempts' and 'no-matings' ($p < 0.05$; Fisher Exact test). Different letters above column indicates significant differences ($p < 0.05$).

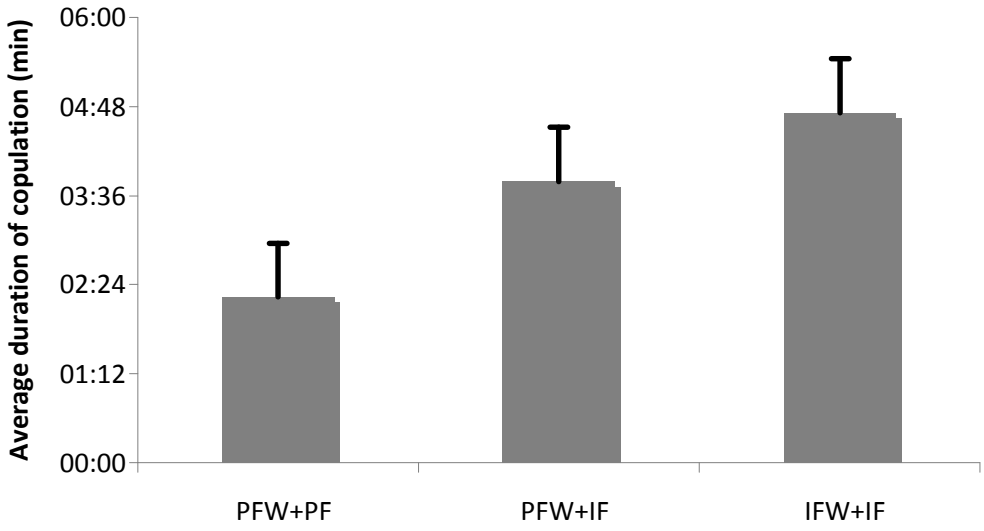

Figure 2. The mean duration pairs spent copulating in all three treatments. Values are mean times (min.) ± S.E. PFW, postmoult female water; PF, postmoult female; IFW, intermoult female water; IF, intermoult female. The male was always in the intermoult stage. Values are not significantly different ($F_{2,13}$ = 3.499, P=0.061, One-Way ANOVA; N = 16).

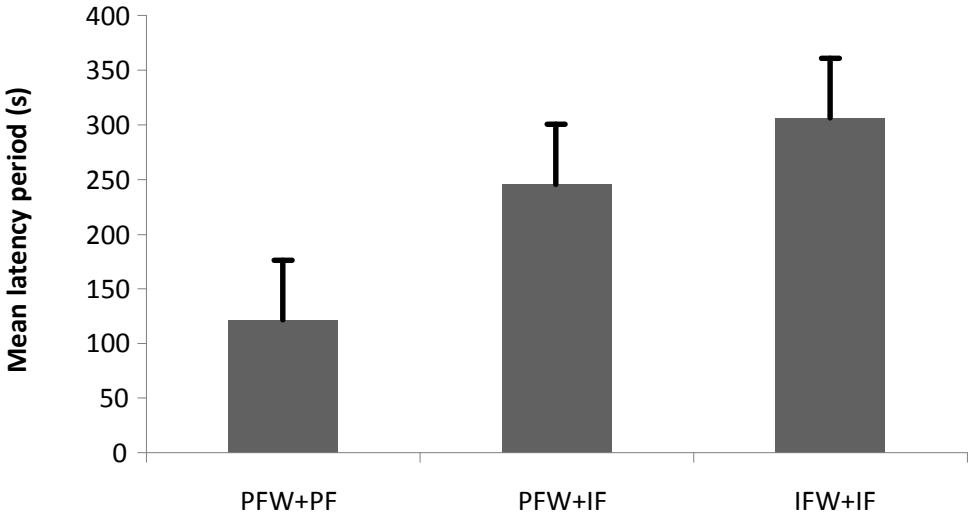

Figure 3. Latency to mating. The mean duration male *Nephrops* took to begin mounting the females during all three treatments: PFW, postmoult female water; PF, postmoult female; IFW, intermoult female water; IF, intermoult female. The male was always in the intermoult stage. Values are mean times (min.) ± S.E. (p = 0.333, Kruskal-Wallis test; N = 26 pairs).

Discussion

Since Farmers' study in 1974, no one has looked at mating behaviour in *Nephrops* ever since. In this study, 16 complete matings were observed. In this study, six distinct mating stages were observed, approaching, mounting, turning, positioning, rolling and thrusting. Farmers' study defined the different behavioural stages of Norway lobsters' mating behaviour. In the current study the male did not show "exciting manners" in the presence of a freshly moulted female. This is where the body of the male is raised very high and he walks on the tips of the fully extended pereiopods, as described in Farmer (1974). Males lowered their chelipeds, indicating low aggression levels towards the female during mating season. Moreover, digging behaviour in the substrate with the perieopods and chelipeds were shown in both, males and females, but it was not observed that the third maxillipeds were involved in the digging activity. Some males and females spend time cleaning immediately after copulation, which might indicate that it is part of the mating strategy. However, this is difficult to determine for certain. Farmer (1974) described that when the male reaches the female they stroke them with their antennae for 2 – 20 minutes. This behaviour was not observed in any mating or mating attempts in this study. As mentioned in the results, the male does not have a specific approaching strategy; he can approach from the front, side or back and does not necessarily straddle the female from the rear. Instead the male will try to climb on the female with his pereiopods. Then, as observed by Farmer (1974), the male will turn the female on her back and adjusts position until the thelycum is opposite his first pair of pleopods. However, whether the third maxillipeds were involved in this process is doubtful. In the turning process, the male used one of his chelipeds to hold one of the females' claws or antennae. This behaviour was not mentioned in Farmers' study (1974). Usually after the male turned the female on her back or on to the side, he will let go of the claw or antenna, which he was holding. The observation by Farmer (1974), that the male curled his telson around that of the female was not observed in this study. No comments can be made on whether the first two pairs of pleopods of the male were thrusting forward and forcing the tips of the first pair into the thelycum of the female. The reason is the pairs were in such position during the copulation that those movements were not observable. It was seen that the male rapidly flexed his abdomen and the uropods opened and closed, like a fan. Yet, whether the male forced spermatophore into the

thelycum of the female by sliding the appendices masculinae along the grooves on the inside of the first pair of pleopods is unclear. After the "penetration/thrusting" the pair separated and showed no further interest in each other. In two cases, the pairs mated twice during the experiment. The observation of double mating in this experiment gives indication that females will mate more than once on occasions. In the Gosselin et al. study (2005), it was found that female American lobsters (*Homarus americanus*) had broods that were sired usually by two or more males. Unlike American lobsters (Atema *et al*., 1979), male *Nephrops* did not show guarding behaviour, in particular carrying females or burying them in the substrate.

Do pheromones play a role in Nephrops mating?

This is the first study to examine chemical communication during mating in Norway lobsters. While previous studies have shown that urine communication is necessary for the maintenance of dominance (chapter 1, Katoh et al., 2008), this study goes further by showing that female moult odours effects mating success. The highest numbers of successful matings were achieved when the water was conditioned with a freshly moulted female, which was then exchanged with another freshly moulted female. Out of sixteen experiments ten pairs mated and three males attempted mating. In the second treatment where the water was conditioned with a freshly moulted female and was replaced with an intermoult female; ten out of sixteen males tried to mate with their female, with only four being successful. This outcome shows that the odour of moulted female contains sex-specific substances, which induces mating behaviour in males. Similar outcomes have been observed in lobster courtship behaviour (Atema and Cowan, 1986; Hughes and Matthiessen, 1962). Moreover, during the mating process the male showed no aggression to females. The possible reason for this is that the female odours may suppress male aggression towards females (Cowan, 1991).

It is questionable whether female *Nephrops* have any choice in the mating process. In shore crabs (*Carcinus maenas*) for example, the female approaches and performs courtship to the largest male, demonstrating that female shore crabs may be selective in terms of size (Sneddon et al., 2003). Moreover, in American lobsters and in spiny lobsters it was shown that premoult females choose a mating partner by repeatedly

visiting the shelter of the chosen mate (Cowan, 1991, Lipcius, *et al.* 1983). However, similar behaviour from female *Nephrops* was not observed. In the first treatment where the female was freshly moulted, only two females managed to escape the mating attempt of their male *Nephrops*. The vulnerable and fragile condition of the moulted females indicates that they do not have a choice whether they want to mate or not. On the other hand, it could also been that a female's willingness to mate may be higher when she is freshly moulted. Female blue crabs, demonstrated a willingness to mate by backing under the males body to initiate precopulatory mate guarding (Jivoff and Hines, 1998). Yet, the moulted female *Nephrops* showed no such willingness. Furthermore, the male *Nephrops* did not show any form of guarding behaviour towards the moulted females. In the experiments where the water was conditioned with a freshly moulted female, which was then replaced with an intermoult female, only four matings were successful. Six males attempted to mate with their female, three of which tried twice to mate with their intermoult female; but in all six cases the female managed to escape. The male behaviour indicates that the moulted female odour contains sex-specific components, which caused their high interest in the intermoult females. This observation is strengthened by the fact that, in the treatment when the water was conditioned with an intermoult female which was then replaced with another intermoult female, the interest of the males in their females was very limited. Only two matings were successful and one male tried to mate several times without success.

The results show the importance of female moulting odour, since males only showed high interest in intermoult females when the odour of a moulted female was present. Therefore, male behaviour can be modified with female odour. However, when the female is in an intermoult stage, successful mating can only occur if she conscience. These findings indicate that either, females in the moult stage cannot choose their mating partners, but females in the intermoult stage can. Or that, female in the moult stage are more receptive to mate in advances then females in the intermoult stage.

Which mating strategy do Norway lobsters use?

This study presents the first description of intermoult mating in the Norway lobster *N. norvegicus*, and reports that all behaviours seen in intermoult mating were also

observed in postmoult mating. The traditional believe is that male Norway lobsters only mate with females within a few days after moulting (Farmer, 1974) has therefore been proved wrong. Although it seems that moult odour from females play an important role in inducing mating behaviour in male *Nephrops*, there may also be other cues that induce mating behaviour while the female is in the intermoult stage. Those pheromones were also found in mature female signal crayfish (*Pacifastacus leniusculus*) and spiny lobsters **(***Panulirus homarus*). It was found that the male crayfish spent more time handling the air-stone when the water is conditioned with a mature female (Stebbing, 2003), and that male spiny lobsters, *Panulirus homarus*, are only attracted to sexually mature females (Berry, 1971). In American lobster s(*Homarus americanus*) (Atema and Steinbach, 2007) and European lobsters (*Homarus gammarus*) (Skog, 2009) intermoult mating is an alternative mating strategy to postmoult mating. It is a likely strategy for females that failed to mate or receive spermatophores during the moult stage, or did not received enough sperm (Waddy and Aiken, 1990, 1991; Atema and Steinbach, 2007). It is suggested that lobster females are able to produce sex specific pheromones through out the year, which reduce the male lobsters' aggression and induce mating behaviour (Bushmann and Atema, 1994, 1997; Skog, 2009). Similarly mature female *Nephrops*, may release a sex pheromone that signals their sexual maturity to male *Nephrops*, and as a result induces mating behaviour. There is a possibility that the sex pheromone is in the urine as was examined in crayfish (Berry and Breithaupt, 2010). Female *Nephrops* in the intermoult stage may be able to control the release of urine containing sex pheromones, and therefore control the induction of mating behaviour in males. This would explain the low mating success rate in the third treatment.

Conclusion

Female moult odour plays an important role in initiating Norway lobster mating. However, it was also found that males have the ability to mate with females in the intermoult stages, indicating sex pheromone productions by the female through the whole moult cycle. Considering the economic importance of this species and the benefit it would have if a sex pheromone were known and available, this is the first study that suggests the presence of sex pheromones in the Norway lobster, *N.*

norvegicus. Further studies are recommended to investigate whether the chemical cues are urine-borne and to identify the molecular structure of the sex pheromone in *Nephrops*. From the compounds a powder or liquid can be produced which can be added to a tank with males and females to induce copulation. When culturing *Nephrops*, this would give an opportunity to control mating and possibly mating success.

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