Master of Science in Aquaculture Biology

Shelter seeking and competitive behavior in hatchery reared juvenile European lobster (*Homarus gammarus*) exposed to predator odors.

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Abstract

Releases of hatchery reared juvenile European lobster have been performed throughout Europe since the 19th century. However, poor survival in the wild reduces the success of both stock enhancement programs and large-scale sea-ranching. Predation on released naïve juveniles is substantial and presumably caused by behavioral deficiencies due to an impoverished rearing environment. Lobsters can recognize and discriminate between different chemical cues and modulate their behavior accordingly. This is assumed to also be true for odors related to potential predators. In the present study, shelter-seeking and competitive behavior was investigated after a series of exposures to a mix of predator odors. Odor exposure was hypothesized to serve as a form of habitat enrichment, acting as an induced predation pressure increasing the lobsters' motivation to find and compete for shelter.

Three experiments were conducted in test-environments of gradually increasing complexity. Behavioral assays on individual lobsters in a small and relatively low-complexity test-environment indicated no effects of odor exposure on shelter-seeking or general activity level. In contrast, when allowing for intraspecific competition in the same environment, lobsters exposed to predator odors were significantly more adept at gaining dominance, evident as superior ability to win agonistic interactions. The winners also spent more time in shelter, less time on roaming and displayed less freezing behavior than the losing opponent. Interestingly, interactions were initiated by naïve lobsters significantly more often than lobsters that had experienced predator odors. When performing a small-scale simulated release in a more comprehensive and diverse semi-natural environment, naïve lobsters initially performed better than those exposed to predator odors. However, the exposed lobsters significantly increased shelter occupancy as the experiment progressed over a 35-day period, whereas the opposite was true for naïve lobsters. The results suggest that there is a long-lasting effect of prolonged odor exposure, but it is only manifested as altered behavior in the presence of environmental triggers. Reasons for the apparent long term effects are discussed, and it is suggested that prolonged exposure to predator odors may either positively influence behavioral plasticity through chemical enhancement, or elevate the lobsters' underlying fright-related motivation in competing for shelter. Further studies are needed to confirm an effect of odor exposure in a more realistic release setting, ideally performed as a small-scale release in natural lobster habitats with predators present to assess natural predator-avoiding behavior.

Contents

1. Introduction

Background

The European lobster (*Homarus gammarus*) is historically an important and economically valuable species, and has supported a coastal fishery in southern- and western Norway since the 17th century (Boeck 1869; Appelöf 1909, in Borthen et al. 1998). Official records for landed catch are available back to the 1820s. Landings fluctuated between roughly 250 and 1.000 metric tonnes/year, peaking at 1300 mt around 1932, up until the early 1960s when the stock collapsed (Tveite 1991; Borthen et al. 1998; Ottermo et al. 2007) and landings decreased from 700-900 mt/year in the 1950s to less than 30 mt/year in the late 1980s. Even though landed catch have increased moderately to 44-62 mt/year in 2005-2011 (Directorate of Fisheries statistics), the stock remains in critical condition compared to historical biomass estimates. This apparent lack of intrinsic stock recovery, despite both reduced fishing effort (Ottermo et al. 2007) and the implementation of strict management legislation (Agnalt 2008), provides an incentive to replenish the stock using hatchery reared juveniles as is done with a wide variety of other marine invertebrates and fish species (Munro & Bell 1997; Bell et al. 2005). Releases of postlarval European lobster have been attempted on numerous occasions since the 19th century but have largely been abandoned due to ambivalent stock enhancement effects (Nicosia & Lavalli 1999). During the last 25 years, refined stock enhancement programs focusing on release of older hatchery-reared juveniles have been initiated in Germany, France, Ireland, the United Kingdom, and Norway (Bannister & Addison 1998; Nicosia & Lavalli 1999; Gendron 1997; Agnalt 2008; Schmalenbach et al. 2011) as a response to overfishing or natural recruitment failure.

The motivation to develop methods for successful release of lobster juveniles goes beyond stock replenishment. Sea-ranching of hatchery reared juveniles is an attractive commercial concept, and especially so in Norway given the favorable legislation providing exclusive rights to harvest lobsters within licensed areas (Agnalt 2008). The European lobster is a rather stationary species, and released juveniles rarely venture far from their "home" area (Bannister & Addison 1998; Moland et al. 2011; Schmalenbach et al. 2011), which in combination with exclusive harvesting rights should allow for high recapture rates. However, low survival and slow growth may limit the success of both stock enhancement programs and large-scale lobster sea-ranching, although the potential to enhance a depleted local stock have been shown (Agnalt et al. 1999; Schmalenbach et al. 2011).

The behavior and ecology of juvenile European lobster in its natural environment is poorly understood (Howard & Bennett 1979; Mercer et al. 2001), much due to the cryptic nature of small juveniles in the size range of 5-40 mm carapace length (CL) also called the *early benthic phase* (EBP, definition for American lobster *Homarus americanus*, in Wahle & Steneck 1992), and this gap in current knowledge restricts the ability to assess actual survival and growth of released hatchery reared juveniles (van der Meeren 2005). EBP juveniles have so far not been found in the wild (Mercer et al. 2001) and essentially all information on this part of the life cycle is attained from laboratory- and experimental field studies (Berrill 1974; Howard & Bennett 1979; van der Meeren 1991; 1993; 2000; 2001; Agnalt et al. 1999; Linnane et al. 2000; Ball et al. 2001; Mercer et al. 2001; Jørstad et al. 2001). There is a better ecological understanding of other decapod crustaceans, e.g. American lobster, crayfish (*Orconectes* spp, *Astacus* spp) and spiny lobsters (*Panulirus* spp, *Palinurus* spp), with more comprehensive data from laboratory- and field work available on their juvenile and adult life stages. However, common for EBP lobsters is their presumed dependence on shelters and vulnerability to predation, where homarid lobsters in particular seem restricted to structural refugia in early ontogeny (Barshaw & Lavalli 1988; Wahle & Steneck 1992; Wahle 1992; Cobb & Wahle 1994; Linnane et al. 2000; van der Meeren 2001).

Post-release survival

Mortality from predation accounts for a considerable part of the total loss in a release situation, and mainly occurs shortly after deployment into the environment (Wahle & Steneck 1992; Ball et al. 2001; Mercer et al. 2001) with more than 10% estimated loss observed within the first hour (van der Meeren 2000). Since survival generally increases when juveniles are provided with appropriate cover (Barshaw & Lavalli 1988; Wahle & Steneck 1992; Linnane et al. 2000; Ball et al. 2001; Mercer et al. 2001; Diaz et al. 2005), the strength of their shelter-seeking behavior; their ability to rapidly find and settle in shelter upon release, is crucial for subsequent survival. Furthermore, their competitive behavior, i.e. capability to take possession of and defend a limited resource in competition with both conspesifics (Peeke et al. 1998; Spanier et al. 1998; Paille et al. 2002) and other crustacean species (Koponen 2003; Rossong et al. 2006) sharing the same microhabitats, is important to retain protection especially in scarcity of shelters (van der Meeren 2005). Consequently, a strong shelter-seeking behavior and high competitive ability will result in instant fitness benefits for small lobsters until they reach a size less vulnerable to predation (Wahle 1992; Spanier et al. 1998).

Notwithstanding the ability of naïve juveniles to find shelter in experimental settings (Howard & Bennett 1979; van der Meeren 1993; 2001; Linnane et al. 2000), survival from release programs has generally been low. The highest cumulative recapture in a large scale European release program was 6.2 percent over a span of 10 years at Kvitsøy, Rogaland (Agnalt 2008). A likely explanation is that hatchery reared juveniles lack the behavioral repertoire required to cope with a novel and highly fluctuating natural environment (van der Meeren 2000; 2005; Salvanes & Braithwaite 2006; Fernö et al. 2011).

Producing lobsters fit for release

Hatchery reared animals must possess or learn certain basic skills to survive in the wild (Olla et al. 1998; van der Meeren 2005; Fernö et al. 2011), e.g. the ability to catch food, avoid predation and compete for limited resources. The rearing conditions in intensive hatcheries, where lobsters are kept individually in compartments offering few physical or chemical stimuli except for feeding, are clearly different from the wild marine environment. As a consequence, morphological and behavioral deficits possibly rendering the juveniles unsuited for release have been described for lobster as well as for a number of invertebrates and domesticated fish species (Svåsand et al. 1998; van der Meeren 2005; Bell et al. 2005; Salvanes & Braithwaite 2006). Lobster juveniles have thus suffered high mortalities due to predation from several species (van der Meeren 2000) assumed to be caused by lack of appropriate anti-predator behavior.

In the present study, individually reared naïve juveniles were exposed to water from tanks holding three potential predator species in an attempt to “train” the juveniles in becoming more fit for release. Communal rearing in spatially complex environments can be applied in attempts to reduce behavioral deficits such as poor predator-avoiding- and maladaptive aggressive behaviors (Jørstad et al. 2001; Salvanes & Braithwaite 2005). Although communal rearing may be space demanding and give variable survival rates depending on the developmental stage, initial size and stocking density (see e.g. Linnane et al. 2000; Jørstad et al. 2001; 2009, Kristiansen et al. 2004), there is potential to stimulate development of a more natural behavioral repertoire as shown for Atlantic cod (*Gadhus morhua*) (Salvanes & Braithwaite 2005). Similarly, introduction of predator odors to the rearing environment will represent a form of habitat enrichment in offering novel chemical rather than spatial stimuli to otherwise sensory deprived animals.

It is well established that aquatic species can be aversively conditioned to predator odor, e.g. through pairing with conspesific alarm cues (e.g. Olla & Davis 1989; Brown & Smith 1997; Berejikian et al. 1998; Ferrari et al. 2006). However, for such conditioning to show an effect the stimuli must be reintroduced in the test-environments. At the time of release, in a marine environment characterized by highly fluctuating biochemical composition, we do not know if predator odors are present above background levels, and effort is in fact made to release juveniles in periods where predators are less active (van der Meeren 2000). Hence, lobsters should be "trained" to increase their shelter-seeking and competitive behavior irrespective of whether predator odors or alarm cues are present or not at the exact time and place of release. Some level of predator recognition- and avoidance are presumably highly preserved through evolution as innate abilities (Stein & Magnuson 1976; Wahle 1992; Boudreau et al. 1993; Olla et al. 1998; Berejikian et al. 2003; Vilhunen & Hirvonen 2003; Hawkins et al. 2004). Thus, habituation should be highly specific towards predator-related cues (Hemmi & Merkle 2009; Raderschall et al. 2011), and prolonged exposure to predator odors presumably increase rather than suppress awareness towards any risk-resembling environmental disturbances.

The role of chemical cues

An abundance of studies on decapod species have established that chemical cues can modulate behavior and association with the perceived presence of conspecifics (Boudreau et al. 1993a; Karavanich & Atema 1998; Nevitt et al. 2000), prey (Derby & Atema; 1981; Daniel & Bayer 1987), specific habitats or shelter-providing substrata (Boudreau et al. 1993a; 1993b; Briones-Fourzán 2008; Horner et al. 2006; 2008), and potential predators (Wahle 1992; Appelberg et al. 1993; Boudreau et al. 1993a; Briones-Fourzán 2009; Gristina et al. 2011). Chemical cues are mainly perceived either by the olfactory or gustatory sensory system (Derby & Sorensen 2008), but olfaction mediates more complex behaviors than gustation (Atema 1977, cited in Derby & Sorensen 2008). Decapods are also able to differentiate between complex odor mixtures and their single components (Zimmer-Faust 1987; Daniel & Derby 1988). However, documentation is scarce concerning the olfactory capacity and the presence of chemically mediated behavior in European lobster. This is especially true for EBP lobsters, although the work of Nilsen (2007) indicated an ability to modify behavior after exposure to predator odor. Alberts-Hubatsch et al. (2011) also showed that newly settled juveniles could differentiate between odor plumes. All the same, given the similarities in anatomy and physiology for clawed lobsters it is exceedingly likely that olfaction is of similar importance for the European lobster.

Aims of the study

In this study, a series of experiments were performed to assess the effect of exposure to predator odors on the shelter-seeking- and competitive behavior of naïve European lobster juveniles (~12-16 mm CL). In the first experiment it was investigated whether basic individual behavior was affected in a relatively low-complexity environment with bottom substrate and a shelter but no other tactile, visual or chemical stimuli. Juveniles have a predisposition for an early life in shelter and for substrate manipulation (Howard & Bennett 1979; Botero & Atema 1982; Wahle & Steneck 1992; Van der Meeren 1993; 2001; Wickins et al. 1996; James-Pirri & Cobb 1999; Linnane et al. 2000), and the goal was to investigate whether predator odor could affect predator-avoiding and protective behaviors such as shelter-seeking, activity level, and digging activity.

In a second experiment focusing on intraspecific interactions, lobsters exposed and naïve to predator odors were paired together and had to compete for shelter. This represented a more complex situation since agonistic interactions are presumably important when competing for limited resources (van der Meeren 2005). If exposure to predator odors can improve the competitive behavior of hatchery reared juvenile lobsters beyond that of naïve individuals this may increase their survival upon release.

The long-term effect of exposure to predator odors is critical, since potential improvements in lobster performance are only transient if the improved behavior is not retained. In a third experiment, exposed and naïve lobsters were released into a semi-natural habitat after a simulated transport stage and had to compete for a limited number of shelters. It was investigated whether lobster performance changed over time post-release, and if so, whether the change could be explained by the prolonged exposure to predator odors. This experiment represented an up-scaling of the small-scale interaction experiment, further enhancing the habitat complexity by allowing for multiple agonistic interactions and competition for shelters in a more comprehensive arena offering a diverse set of environmental impulses.

Exposure to predator odors was hypothesized to serve as an artificially induced predation pressure increasing the lobsters' motivation to find and defend shelter when introduced to unfamiliar environments of increasing complexity. A perceived elevated predation risk has been shown to reinforce the association of naïve juveniles with shelter (Stein & Magnuson 1976; Wahle 1992; Wahle & Steneck 1992; Castro & Cobb 2005; Oliver et al. 2008; Brionez-Fourzán 2009; Gristina et al. 2011), and to influence shelter preference as lobsters may settle more rapidly but less selectively under the perceived presence of a predator (Boudreau et al. 1993b; Gristina et al. 2009). Exposed lobsters were thus predicted to find shelter more rapidly and have a stronger association with the shelter compared with naïve lobsters. Furthermore, odor exposure was predicted to raise the lobsters’ motivation in competition for shelter when this represented a limited resource, and thus result in a stronger agonistic response towards conspecifics.

2. Materials and methods

2.1 Experimental animals

2.1.1 Lobster juveniles

A total of 170 juveniles of European lobster were purchased from the commercial lobster hatchery of Norwegian Lobster Farm AS (NLF) at Kvitsøy, Rogaland (59°24’09”N 05°24’09”E). They were approximately six months old, and had been hatched and reared in a temperature range of 19-21°C, with post-settlement rearing confined to single celled compartments deprived of environmental stimuli. All animals originated from the same production batch hatched late in 2010. As they originated from numerous locally wild-caught berried females, unidentified maternal effects (Huntingford 2004) were considered negligible and any differences compared with local wild stock should be attributable to the rearing conditions (Moberg et al. 2011). Lobsters had been fed commercially available lobster feed (patented by NLF, manufactured by Nofima), but specific details on light- and feeding regimes prior to the experimental period are unknown.

Lobsters were transported to Bergen in an ice-chilled polystyrene container and brought to the Institute of Marine Research’s (IMR) wet-lab facility at Parisvatnet, Øygarden (60°37’45”N 04°48’07”E). Mortality and claw loss during transport was low, with 1.18 and 2.35 percent transport loss, respectively. The carapace length (CL), corresponding to the distance between the posterior rim of the eye socket and the dorsal posterior margin of the cephalothorax, was measured with Vernier calipers to the nearest 0.1 mm below (Table 1). Lobsters were then placed in rectangular single celled compartments (4x9x5 cm, Fig. 1) with numerous compartments on two larger units, and randomly assigned to one out of four experimental treatments (Table 1). The compartments consisted of white PVC walls and a plastic mesh bottom to secure water exchange in each compartment. Each unit was placed in 1m^2 holding tanks with water at ambient temperature (~9.0-13.0°C, Appendix 1). Lobsters were given 21 days of acclimation to the lower temperatures while preparing the experimental infrastructure and treatment protocols. Light regime was fixed to L:12/D:12 for all groups, with lights on between 08.00 and 20.00 until the 1st of June, when the correct light regime for the experimental period was introduced (L:18/D:6 for treatments A and B, L:6/D:18 for treatments C and D, Table 1). Lobsters were allowed further acclimation until the start of Experiment 1 on the 8th of June.

Table 1: Overview of the main experimental treatments. "Exposed" lobsters received odor exposure and seawater in fixed intervals, while "Naïve" lobsters received only seawater. Light regime was imposed on the 1st of June. There was a significant difference in size (CL; mean ± SD) between Summer simulations (A and B) and Winter simulations (C and D) ($-2.90 \geq t \geq -5.67$, $0.022 \geq p > 0.001$) where the Summer simulations were significantly larger, but no differences were found within Summer or Winter simulations (A vs B: $t= -1.624$, $p= 0.37$; C vs D: $t= -1.229$, $p= 0.61$, respectively). Consult Fig. 2 for a complete overview of the experimental setup.

Experimental treatment	No. of lobsters	CL (mm) mean±SD	Predator odor	Light regime	Tank no.	Water volume (l)	Water flow (l/min)
A (Summer simulation)	40	14,36 ± 1,50	Exposed	L:18/D:6	5	300	9.2-10.2
B (Summer simulation)	42	13,95 ± 1,47	Naïve	L:18/D:6	2	300	9.2-10.2
C (Winter simulation)	40	13,09 ± 1,23	Exposed	L:6/D:18	4	300	9.2-10.2
D (Winter simulation)	40	12,71 ± 1,04	Naïve	L:6/D:18	3	300	9.2-10.2

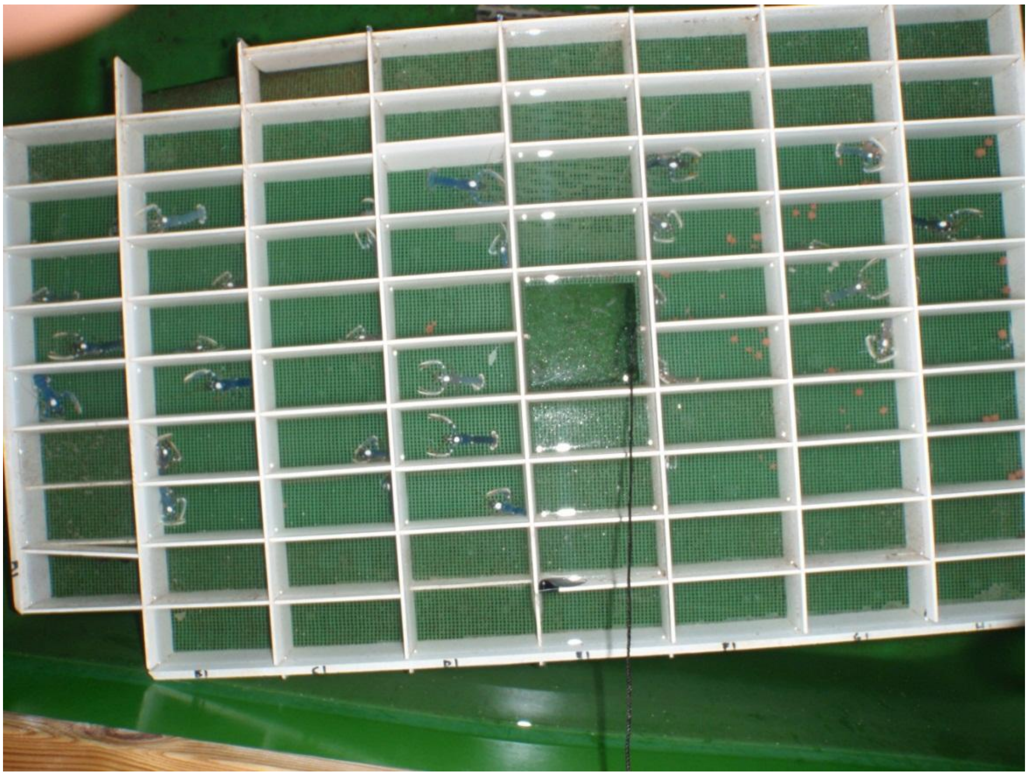

Figure 1: Cell unit with numerous single cells containing lobsters (Photo: H. Trengereid).

From arrival on the 18th of May until the 10th of July lobsters were held in the indoor wet-lab facility and manually fed 2-3 pellets of commercial lobster feed every fourth day. Feed was never distributed while lobsters were exposed to predator odors. Mortality in this period was low and related either to problems with ecdysis or as a result of cannibalism when large juveniles managed to enter the compartments of smaller ones. Dead lobsters were removed continuously, and single celled compartments and holding tanks cleaned in weekly intervals. Since both injury and molting can affect behavior (Lipcius & Hernkind 1982; Juanes & Smith 1995) lobsters that lacked claws or had recently molted were removed from the experiment and stored in tank 1 (Fig. 2). Twelve lobsters died or were removed in the period between the 8th and 29th of June. Only apparently healthy intermolt juveniles were used in this study, and claw dimorphism was evident in all lobsters. Determination of the sex and molt-stage in small juveniles is not possible without sacrificing the animal (Agnalt IMR, pers. comm.), and was not done since lobsters were needed in later experiments.

2.1.2 Predators

Predators were caught east of Øygarden on the 2nd and 3rd of June. Green crabs (*Carcinus maenas*) were captured with common Måløy pots, while cod (*Gadus morhua*) and Ballan wrasse (*Labrus berggylta*) were captured in trammel nets. Great care was taken to avoid significant shell-loss or gill damage to the fish when loosening them from the trammel nets. Each species were kept in separate tanks (Table 2), and three boulders were placed in the cod and wrasse tanks to add some habitat complexity. The wrasse tank also contained black plastic strips as artificial seaweed. Temperature was continuously recorded with an automatic data logger (EL-USB-1-PRO), and O_2 levels measured once a day (Oxyguard Handy Polaris 2 calibrated to 33 ppt). The water held ambient temperature (9.0-13.0°C, Appendix 1), and the oxygen saturation was stable, ranging from 90 to 100 percent saturation over a time span of 55 days for all tanks containing animals. Predators were fed *ad libitum* with pelleted fish feed once per day (22.00) (Skretting Amber Neptune 1300 mixed with raw paenaid shrimp and then frozen). Predator tanks were cleaned of feces and excess feed once per day.

Table 2: Details on the predators used in this study. Light regime for predators were L:14/D:10, with lights on between 10.00-00.00. *Carapace width. NA= not available. Consult Fig.2 for overview.

Predator Species	No. of animals	Mean size(cm)	Mean weight(g)	Tank no.	Water volume (l)	Water flow (l/min)	Diel and seasonal activity
Atlantic cod (*Gadhus morhua*)	3	47.0	1172	6	190-200	8.8-11.2	All day, year-round
Ballan wrasse (*Labrus berggylta*)	5	24.2	284	7	190-200	6.8-10.0	Diurnal, mainly summer
Green crab (*Carcinus maenas*)	7	7.7*	NA	8	45-55	4.4-7.2	Nocturnal, year round

Three predator species were used since no study concerning differential species-specific predation has been performed on EBP European lobsters, thus it was not possible to predict which predator would have the best potential to elicit a response in behavior. Therefore, odors from known predators in several field studies were chosen (Wahle & Steneck 1992; van der Meeren 2000; Ball et al. 2001; Mercer et al. 2001). Also, there are diel and seasonal differences in the activity pattern of the three predators (Table 2, van der Meeren 2000; Ball et al. 2001), this being important as lobsters were exposed to predator odors both during the light (treatment A) and dark phase (treatment C, Table 1) simulating light conditions in the summer (August) and winter (December), respectively. To add further authenticity to the simulated predation pressure, the selected predators are naturally foraging in a range of typical release habitats (cobble, gravel, sand) (van der Meeren 2000; Ball et al. 2001; Mercer et al. 2001).

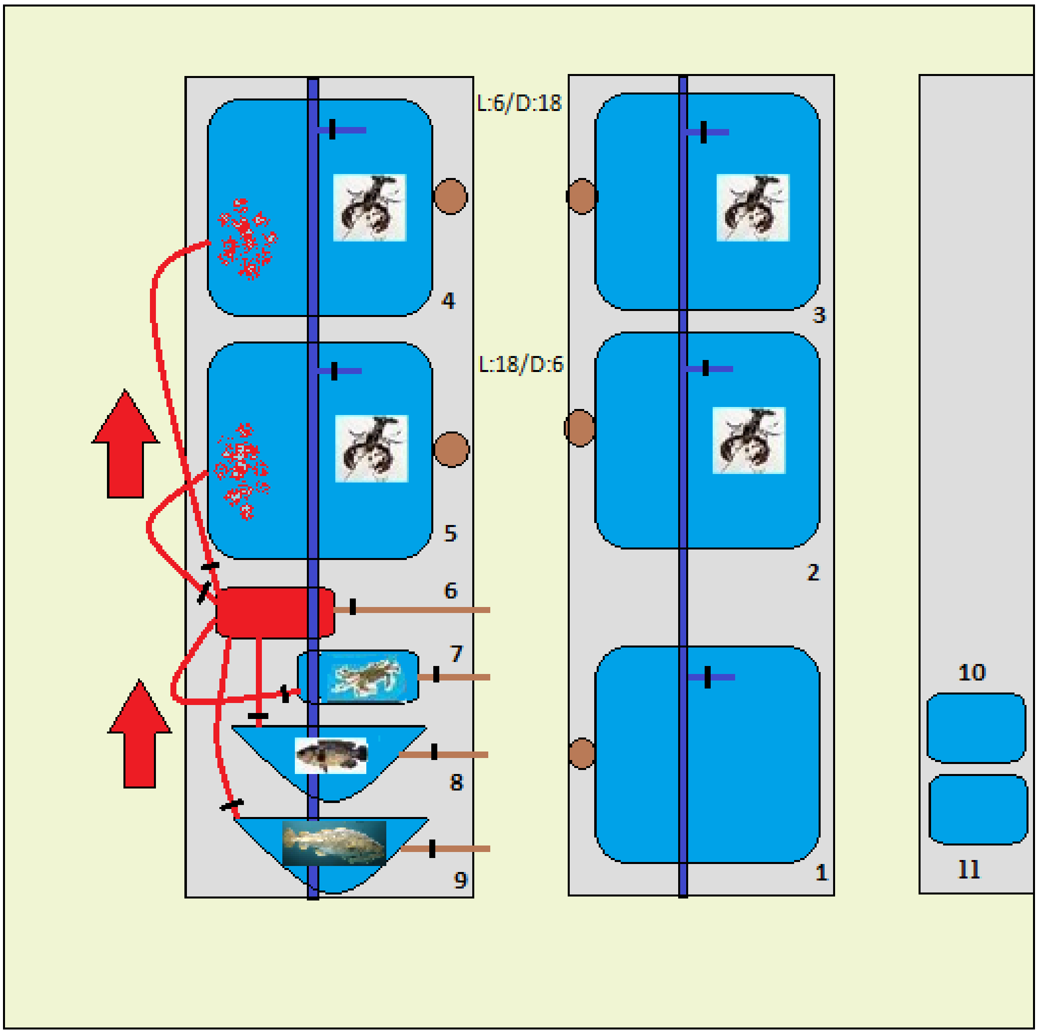

Figure 2: Overview of experimental setup (wet-lab facility). Red color represents predator water and its direction, brown = drains, dark blue = water intake through pipes from a common reservoir. Experimental tanks are numbered 1 through 10. Tank 1: Miscellaneous. Tanks 2 to 5: Lobster tanks. Tank 6: Collection tank for predator water. Tanks 7-9: Predator tanks. Tanks 10 and 11: Observation units. Light regime for tanks 2 and 5 are L:18/D:6, and L:6/D:18 for tanks 3 and 4.

2.2 Experimental infrastructure and odor exposure

The experimental system consisted of four 1m^2 tanks with lobsters (2-5, Figs. 2 and 3), one elevated holding tank for each predator (7-9, Figs. 2 and 3), and one tank for collection of predator water (6, Figs. 2 and 3). Two 50 liter trays were used as observation units for behavioral assays (10, Figs. 2 and 4), and one backup tank was installed for miscellaneous use and storage of damaged lobsters (1, Fig.2). All tanks containing animals were set up as flow-through systems with water inflow by gravity from a common reservoir. Water from three meters depth (west of Nautnes, approximately 60^{o}37’42”N 04^{o}47’36”E) passed through a macro-grid to remove large debris, followed by drum-filtration (20 µm) before reaching the reservoir. This provided the system with one common water quality. Globe valves allowed for fine-tuning of water inflow at the tank level, but periodically unstable pressure in the pipes supplying water to the system gave some fluctuations in water inflow (Table 1 & 2).

Lobster treatment tanks (4 and 5, Fig.2) received water containing either a mix of all predator odors or fresh seawater in fixed intervals (Table 3), while the control tanks (2 and 3, Fig.2) had only continuous inflow of seawater. Preliminary trials indicated that while short exposure times seemed to have no effect on shelter-seeking, whereas long pulses indicated some effects (Appendix 5). In addition, habituation (Hinde 1966) has been shown to occur rapidly after exposure to stimuli of short duration in repeated intervals (Daniel & Derby 1988). Consequently, predator odor was delivered as approximately 15 hour long pulses at a rate of 9.0-11.5 l/min, with no pulse intermittency. In the nine hour period between odor pulses no predator water was led into the lobster treatment tanks, but circulation of fresh seawater in both predator and lobster tanks was maintained.

Exposure to predator odors was confined to the light phase for treatment A and dark phase for treatment C (Table 3). To assess the effect of odor exposure alone, it was important that lobsters did not learn to associate predator odor with handling (i.e. aversively conditioned to handling). Therefore, odor exposure ended approximately one hour before starting the observation trials to avoid a link between predator odors and handling procedures. Naïve lobsters (treatments B and D) followed the same light regime and test protocol but did not receive olfactory stimuli. Since a realistic release would generally have been performed in daylight, all observations were carried out in the light phase. The photoperiod was not interrupted for any of the treatments since the shift from darkness to light took gradually place through a 30 min crepuscular period to avoid abrupt changes in light conditions (Table 3).

Figure 3: Tank setup (flow through system). **Upper:** Summer (left) and Winter (right) lobster tanks. Inflow regulated with globe valves and volume fixed to 300 l through a lateral standpipe. Lights controlled by automatic timers. **Lower:** Predator tanks and collection tank for predator water. From the left: Elevated tanks for cod, wrasse and crab. Water was descending by gravity to the collection tank, and further into lobster treatment tanks (see also Fig. 2) (Photo: H. Trengereid).

Table 3: Experimental treatment protocol. Predator odor was delivered in 15 hour long pulses. See Fig. 2 for tank identification. NA= No odor exposure.

Experimental treatment	Tank	Light regime	Light phase	Dark phase	Start odor exposure	End odor exposure	Start testing
A (Summer simulation)	5	L:18/D:6	06.00 - 00.00	00.00 - 06.00	06.30	21.30	22.30
B (Summer simulation)	2	L:18/D:6	04.00 - 22.00	22.00 - 04.00	NA	NA	20.00
C (Winter simulation)	4	L:6/D:18	15.30 - 21.30	21.30 - 15.30	00.00	15.00	16.00
D (Winter simulation)	3	L:6/D:18	17.30 - 23.30	23.30 - 17.30	NA	NA	18.00

Preliminary observations indicated that exposure in the light versus dark phase would yield different results for shelter-seeking behavior (Appendix 5). In addition, van der Meeren (2000) found that survival differed between seasons and recommended early-winter releases. To assess whether odor exposure in the light ("summer simulations") and dark ("winter simulations") phase would affect behavior differently, photoperiod was included as a co-effect on exposure to predator odors. Lobsters were thus subjected to light regimes simulating conditions in August and December. In order to reverse the photoperiod for winter simulations (C and D, Table 3) but still keep the room illuminated as required for observation, a permanent framework covered with black opaque plastic was built over the designated winter tanks (3 and 4, Fig. 2; Fig. 4). Lamps were positioned approximately 50 cm above each lobster tank, giving light intensities in the range of 120-160 lux at the water surface (measured with Lutron LX-101 luxmeter). The lamps were connected to automatic timers and light dimmers enabling a precise photoperiodic control and a gradual shift in light conditions.

The observation units used for observation of lobsters were made in two 50 liter (50x40x25 cm) transparent plastic containers (Fig. 4) each filled with approximately 25 liters of water at ambient temperature. Bottom substrate was four liters of washed shell sand, and two cobble stones represented a simple three-dimensional shelter with one semi-flattened stone supported against a small brick creating a crevice. Cobble stones were selected since juveniles prefer cobble-providing habitats (Wahle & Steneck 1992; Cobb & Wahle 1994; Linnane et al. 2000). The shelter was placed about 5 cm from the tank-wall to prevent lobsters from accidentally encountering shelter when roaming along the tank-walls. Light intensity at the water surface ranged between 115 to 130 lux. A camera (480 TVL-PAL) was mounted approximately 30 cm above the water surface and connected by hardwire to a computer in an adjacent room. Geovision GV-1120 surveillance hardware was used for video recordings.

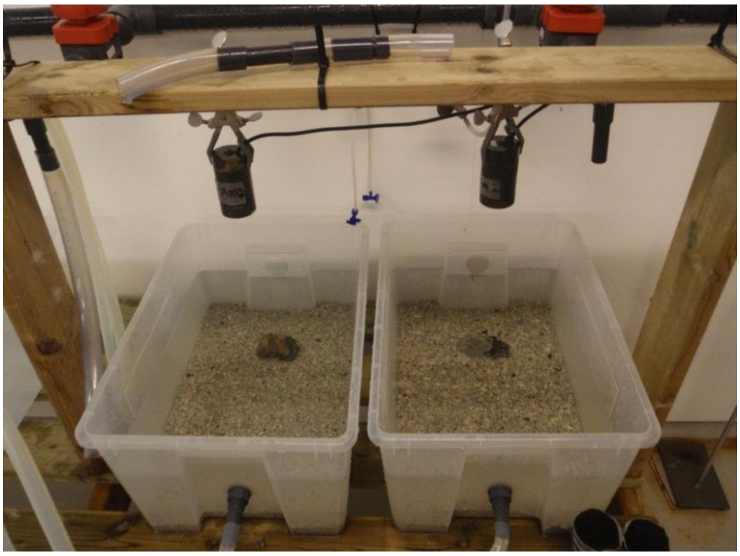

Figure 4: Observation units used for behavioral observations in Experiment 1 and 2. Shelters placed at the far end, while lobsters were introduced at the opposite side. (Photo: H. Trengereid).

2.3 Experiment 1; Individual behavior after prolonged exposure to predator odors

To investigate effects of odor exposure on the basic behavior in absence of environmental disturbances, individual lobsters were observed in a relatively low-complexity environment absent of tactile, visual and chemical stimuli above background levels, except for bottom substrate and a simple three-dimensional shelter. Emphasis was put on predator-avoiding and protective behaviors such as shelter-seeking, activity level, and digging ability. Each lobster was observed four times (after 4, 8, 12 and 16 days in treatment) since it was unknown if or when the effects of odor exposure would alter behavior, and whether the effects would intensify or diminish according to duration of exposure.

Throughout this text, treatments A and B will be referred to as “summer simulations”, while treatments C and D are referred to as “winter simulations”. This is because treatments differed based on light regime (Table 1 & 3) simulating light conditions in the summer (L:18/D:6) and winter (L:6/D:18), respectively. In addition, lobsters exposed to predator odors are referred to as “Exposed”, and lobsters not exposed to odor referred to as “Naïve”.

2.3.1 Experimental design

Experiment 1 was performed in the indoor wet-lab facility and lasted from the 8th to 27th of June. Forty juveniles were randomly allocated to each treatment (Table 1 and 3). Since it was not possible to observe 160 lobsters on a daily basis, each treatment was divided into four subgroups (n=10). In this way, the daily number of observations was reduced from 160 (40x4) to 40 (10x4), where all four treatments were represented by one subgroup each day (Fig. 5).

Since behavioral assays were scheduled after 4, 8, 12 and 16 days of exposure, it was important that all lobsters were exposed to equal durations of predator odors. Keeping in mind that only one subgroup from each treatment was observed daily, the subgroups had to be introduced to the treatment in consecutive order, and they were numbered 1 through 4 (Fig. 5). To illustrate, Subgroup 1 (A1, B1, C1 and D1) was introduced on 08.06 and observed the first time on 12.06, after four successive days in treatment. The next observation on Subgroup 1 was then performed on 16.06 after eight days in treatment, and so on. The remaining subgroups were transferred the following three days with one day time-lag (Fig. 5). Thus, on 11.06 all lobsters had been relocated from the large units to smaller ones, and upon the first observation trial the following days they had all received four days of odor exposure (Fig. 5).

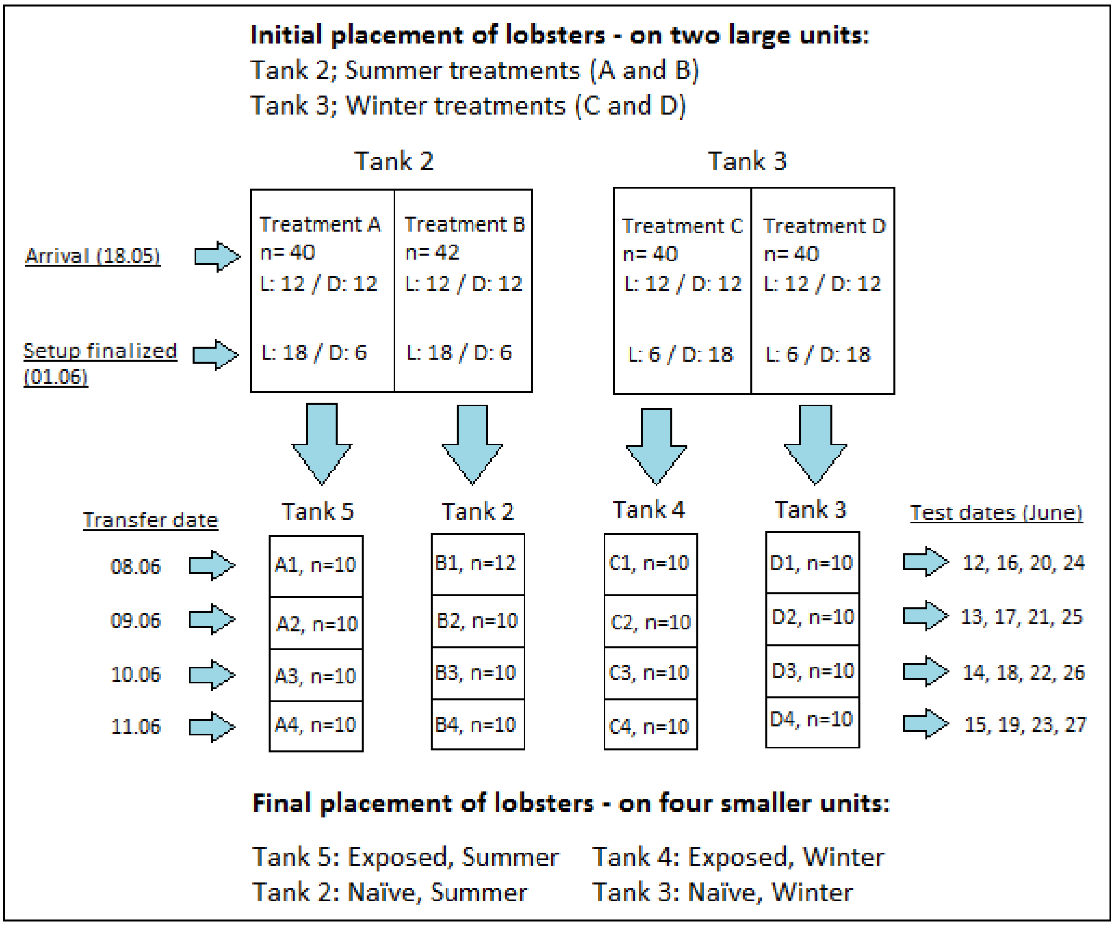

Figure 5: Events prior to start of Experiment 1: Lobsters arrived on 18.05, and were initially placed in two tanks on two large units. The experimental infrastructure was built between 18.05 – 01.06, and when this was finalized on 01.06, the correct light regime could be introduced. Four days before start of testing (08.06), one subgroup from each treatment was transferred to the smaller units. Remaining subgroups were transferred during the following 3 days in the same way, so on 11.06 the large units were empty and removed. One smaller unit (shown in Fig. 1) remained in tanks 2, 3, 4 and 5, with the treatments now separated into four subgroups.

2.3.2 Behavioral assays

To assess whether there was any changes in behavior according to the duration of exposure (number of days) behavioral assays were repeated every fourth day. Thus, each lobster was observed four times in a 16 day time-span. In the three day intervals between each trial lobsters were not handled, with feeding being the only direct external stimuli apart from minor unavoidable disturbances when other subgroups were tested.

Two lobsters from the same treatment (and subgroup) were removed from their single celled compartments and placed in separate acclimation chambers made from black PVC pipe sealed in one end by fine black mesh, thus preventing visual stimulation when moving lobsters to the observation units. Lobsters were submerged individually in the observation units opposite from the shelter and restrained in the chamber for one minute before starting the assay. After starting the video recording session, the containers were carefully removed and the observer moved to an adjacent room to prevent any visual disturbance. As field studies have shown that predation usually occurs within 15 minutes (van der Meeren 2000; Ball et al. 2001; Mercer et al. 2001), the lobsters were allowed to move freely within the arena for exactly 15 minutes, after which they were placed in the chamber and returned to their single celled compartments. After each assay the shelter stones were reset to their original position, and any entrances or other structures made by the lobsters were destroyed. The water was changed between testing of each treatment (Table 3) and the substratum and shelter stones washed in running water for approximately 30 minutes.

During the 15 minute observation period the behavior of the lobster could be grouped into several categories (Table 4, Fig. 6). Due to small lobster size and limited video quality, other behaviors as e.g. antennule flicking and pleopod fanning (Atema & Voigt 1995) could not be adequately distinguished in this setting. Pleopod fanning was occasionally observed either briefly when the lobster was digging in the tank corners or indirectly as outflow of debris at the shelter entrance, but it was not possible to record accurately since lobsters mainly exhibited this behavior when covered by the shelter.

Descriptions in Table 4 cover the complete span of behavioral categories that could readily be observed in this setting. Categories were defined based on preliminary observations of lobsters in a similar environment (Appendix 5), and represented a way of dissecting behavior into observable units (Huber & Kravitz 1995) that could be analyzed separately. Classification of adaptive/maladaptive behavior was based on its presumed importance in nature, where behaviors related to predator-avoidance, like shelter-seeking (rapidly find shelter), a strong association with the shelter once found (to accept shelter and dwell within it during the day) and shelter manipulation (construction of entrances at the openings and a tunnel system) were considered adaptive. Although the test-environment restricted tunnel building, lobsters spent time to excavate a burrow and build entrances. According to Berrill (1974) and Botero & Atema (1982) EBP lobsters have a preference for tunnel building in association with stones and shelter-providing objects. The observed burrowing behavior presumably represented attempts of tunnel building and was regarded as adaptive.

Table 4: Behavioral categories present in individual observations. Time-to-event is recorded when a certain behavior is observed the first time. The duration of a behavior is the total time spent exhibiting that behavior. Frequency is the number of times a specific behavior is displayed.

Behavioral categories	Type of event	Description
Time to find shelter	Time-to-event	Time between release from chamber and the first complete entering into shelter, defined as disappearance of the telson under shelter when entering head-first, or the rostrum when backing into shelter.
Shelter dwelling	Duration	Time spent inside shelter, including time when a lobster was observed digging inside the shelter.
Cheliped shuffling	Frequency	After entering a shelter lobsters usually started digging entrances at the opening of the shelter, mainly by shuffling out substrate with their claws (cheliped shuffling), and to create a burrow in which they placed themselves.
Roaming	Duration	Time spent moving around the test arena, typically walking along the edges of the test arena and occasionally in the center. Walking speed was highly variable.
Climbing	Frequency	Lobsters were climbing on the walls in the test arena. A climb attempt was defined as a minimum of half the lobster's body up against the wall with the claws stretched upward.
Digging outside	Duration	Lobsters were observed to dig in either one of the tanks' corners, after which it often placed itself in the burrow. Outside digging behavior was recorded when it lasted for 10 seconds or longer.
Freezing	Duration	Lobsters remain motionless in one position for 20 seconds or longer. Lobsters readily walked and stopped in short intervals, but these stops rarely exceeded 10 seconds.

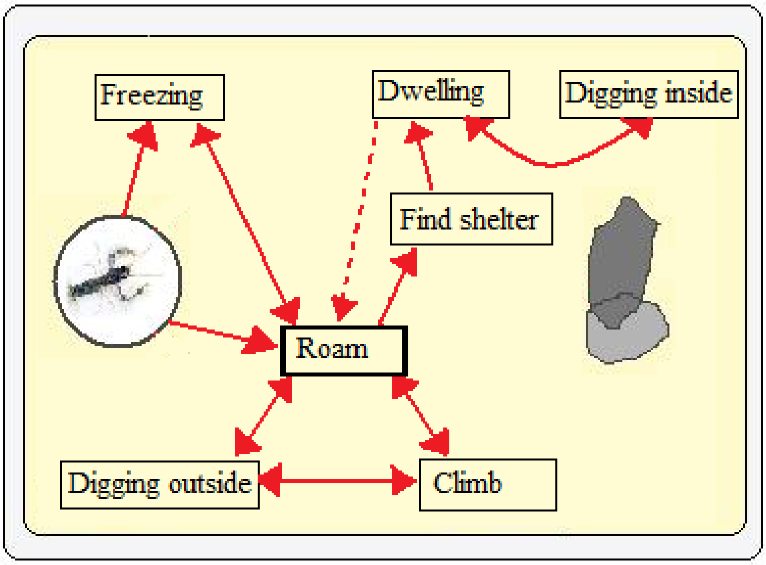

Figure 6: Observation unit with depiction of the observed behavior. See Table 4 for complete description of the behavioral categories. Arrows indicate the observed shifts in behavior. After finding shelter, the lobster would generally either remain in it (accept) or leave shortly or after ≤3 minutes.

In the preliminary trials essentially all lobsters located the shelter at some point, but numerous lobsters refrained from entering, or even attempting to do so, and would either roam around for a prolonged amount of time, display freezing behavior, or start to excavate a burrow in one of the tank corners. Prolonged roaming, sometimes referred to as "exploration" (Mehrtens et al. 2005), increase conspicuousness as exposure in open areas can attract predators (van der Meeren 1993; 2000). In addition, lobsters are mainly nocturnally active both in the wild and in experimental settings and stay in shelter during the day (Lawton 1987; Karnofsky et al. 1989; Mehrtens et al. 2005). Consequently, roaming was considered a maladaptive behavior. Conversely, freezing is a well-documented predator-avoiding strategy effective in avoiding visual predators (Johns & Mann 1987; Vilhunen & Hirvonen 2003; Zhao et al. 2006), and given that some lobsters were exposed to predator odors freezing would generally be considered adaptive. Nevertheless, in this particular setting, where preliminary trials showed that both Exposed and Naïve lobsters displayed freezing behavior, and considering that shelter was readily available within a confined area with no competition, it was not regarded as adaptive. Moreover, lobsters have an innate preference to excavate the substrate, also in habitats like shell-sand or mud where no pre-existing shelters are present (Howard & Bennett 1979; Botero & Atema 1982; Pottle & Elner 1982). In preliminary trials, digging behavior outside shelter was readily observed and most likely the initial phase of an attempt to build a burrow or tunnel acting as shelter. However, as the construction of shelter in these substrates takes several hours (Howard & Bennett 1979; Botero & Atema 1982) this behavior was not considered adaptive when lobsters had access to a suitable pre-made shelter. Climbing the tank-walls can be viewed as stereotypic behavior induced by confinement in a small and enclosed test-environment, as can the apparent preference to roam along the tank edges, which may also be reinforced by the positive thigmotaxis (Botero & Atema 1982) present in lobster juveniles. These behaviors are considered maladaptive in an experimental setting as they may displace predator-avoiding behaviors.

2.4 Experiment 2; Effects of prolonged odor exposure on intra-specific interactions

To study the effects of odor exposure on intra-specific interactions, Exposed and Naïve lobsters were paired together to compete for one shelter. The inclusion of environmental disturbances in the form of interactions and direct competition enhanced the habitat complexity.

2.4.1 Experimental design

The study proceeded directly into the second experiment that was performed on the 28th and 29th of June. The experimental design was similar to Experiment 1 concerning tank setup and lobsters remained in the same experimental treatments (see section 2.2 and Table 3), but slight modifications were made to the treatment-protocol to accommodate the need to trial Exposed and Naïve lobsters together.

Lobsters were size matched to avoid size dependent bias (Atema & Voigt 1995; Vye et al. 1997) (CL, mean ± SD; treatment A: 14.1±1.6 versus treatment B: 13.9±1.5 (t= 0.41, p= 0.98); treatment C: 13.0±1.1 versus treatment D: 12.8±1.2 (t= 0.44, p= 0.97)). Both the Lobsters were marked with solvent free correction fluid on the dorsal carapace (PRITT), a method also used by Peeke et al. (2000) without any reported harmful effects. Both Exposed and Naïve lobsters were marked to prevent possible confounding effects from the procedure when only marking one group. A cotton swab was used to wipe the carapace dry, after which the lobster was marked with one or two points/lines and the fluid hardened. Individuals were marked at the group level three days before the interaction assays were performed. Lobsters were exclusively paired within the summer and winter simulations (Table 3) to isolate the effect of predator odors, and light regime was included as co-effect to investigate whether the behavior differed based on photoperiod.

2.4.2 Behavioral assays

The test procedures were identical to the single trials performed in Experiment 1 (see section 2.3.2), with the exception that one Exposed and one Naïve lobster was placed together in each observation unit, and given 20 minutes to settle and interact. Interaction tests were only performed once for each lobster after a total of 20 days in treatment. Since interactions have been shown to include chemical signaling, at least for male adult lobsters (Karavanich & Atema 1998), the water was changed between each trial and the shelter stones scrubbed in running water.

Additional behavioral categories besides those for Individual behaviors described in Table 4 were defined for analysis of the Interactions (Table 5). These categories were with some modifications based on Atema & Voigt (1995), Huber & Kravitz (1995) and Gherardi et al. (2010). The focus was on agonistic interactions and behavior related to shelter and general activity level, as the intention was to determine the effect of exposure to predator odors on competitive behavior. Dominance is established through aggressive encounters (Huber & Kravitz 1995; Karavanich & Atema 1998; Skog 2009), but also the possession and defense of shelter can represent a measure of social dominance (Peeke et al. 2000). Therefore, a distinction was made between Interactions, which entailed aggressive or submissive displays or physical contact, and Individual behaviors where lobsters did not visibly interact. The behavior of both opponents was analyzed to quantify each of the behaviors listed below (Table 5), after which lobsters were categorized as either dominant or submissive.

The Interaction categories were scored from -2 to +2, with 0.5 point increments, in order to obtain an aggression score. For each trial, scores from all agonistic interactions were summarized for both participants. Certain behaviors were clearly more powerful indicators than others, thus direct interactions were scored based on their apparent strength relative to the opponent. Categories were classified as either aggressive or submissive, e.g. various forms of approach or threats versus several forms of retreat. Generally, aggressive displays or encounters were observed shortly after introduction to the observation unit.

An approach of one or both opponents towards each other represented the most subtle form of aggressive behavior (+0.5), followed by threat displays ("meral spread", +1). These could be exhibited by both opponents before a dominance relationship had been established, and typically either escalated to full-contact fighting or the retreat of one or both opponents. In addition, "meral spread" was observed frequently after one lobster had clearly gained dominance, in situations where the opponent approached or was in its close vicinity, resulting in some form of retreat by the opponent. Lunge attacks could be seen as a faster and more motivated approach/attack towards an opponent, often with claws stretched upwards and outwards (+1.5), which always ended up in either full-contact fights or the retreat of one individual. The lunging lobster was sometimes observed to chase a retreating opponent with its claws outstretched (+1.5). The most powerful aggressive behaviors were those of the "fight" category, which encompassed all actions resembling punches and/or strike-like claw movements directed towards the opponent (+2 per strike). Lobsters could be seen in violent full-contact fights, where they both displayed "fight" behavior. These encounters were generally of short to moderate duration (≤ 3 min), and the least fierce lobster tended to eventually retreat to the opposite side of the tank.

Table 5: Basis for calculation of dominance score in interaction trials. The categories were partly based on Atema and Voigt (1995), Huber and Kravitz (1995) and Gherardi et al. (2010). Nature of behavior describes whether it is considered positive/aggressive, negative/submissive or neutral in order to establish dominance. Instant events are recorded each time a certain behavior is observed, except for "Find shelter" where it is registered when the lobster first enters shelter. Duration is the total time spent exhibiting that behavior. The score represents the value of each observation.

Behavioral category	Nature of behavior	Description of behavior	Type of event	Value
Individual behaviors				
Find shelter	Positive	Whether a lobster finds shelter. See Table 4	Instant	+1
Shelter occupancy	Positive	Time spent inside shelter - dwelling, digging or engaged in interactions with an intruder.	Duration	+0.5/min
Leave shelter	Negative	Whether a lobster leaves the shelter unprovoked.	Instant	- 0,5
Roaming	Negative	Time spent roaming around the tank. Time spent digging outside, freezing or interacting is subtracted to attain the correct duration.	Duration	- 0.25/min
Digging outside	Neutral	Digging in one of the tank corners. Both aggressive and submissive lobsters dug outside the shelter.	Duration	0/min
Freezing	Neutral	A lobster remained motionless for more than 20 seconds.	Duration	0/min
Interactions				
Approach	Aggressive	Slow/moderate advance towards an opponent.	Instant	+0.5
Threat	Aggressive	Aggressive claw display (meral spread) claws stretched outwards and/or upwards) in the vicinity of opponent.	Instant	+1
Lunge attack	Aggressive	Rapid advance/attack against opponent with claws outstretched.	Instant	+1,5
Chase	Aggressive	Pursuit of a retreating opponent.	Instant	+1,5
Fight	Aggressive	Pull, push, punch, grab or strike towards an opponent in an attempt to displace or harm it.	Instant	+2
Evict	Aggressive	A lobster takes over the shelter without resistance from the lobster holding shelter, or alternatively pulls it out by force.	Instant	+2
Holding	Aggressive/ Submissive	One or both claws of opponents interlocked, followed by the retreat of one opponent. Positive score to the winner, negative to the loser.	Instant	+1/-1
Fight in shelter	Aggressive/ Submissive	Fight to gain control of shelter where opponents stand head-first inside the shelter, occasionally for a prolonged amount of time, followed by the retreat of one opponent. Positive score to the winner, negative to the loser.	Instant	+1/-1
Retreat	Submissive	A lobster moves or turns away from an opponent.	Instant	-1
Rapid retreat	Submissive	A lobster moves quickly away, either by walking rapidly or beating the tail once, e.g. when startled or held by an opponent.	Instant	-1,5
Evicted	Submissive	Leaves the shelter without a fight, or is forcefully pulled out by the intruder.	Instant	-2
Tailflip escape	Submissive	Multiple contractions of the abdomen to quickly propel a lobster away from the opponent.	Instant	-2

Three categories were mutually exclusive, in that they had a readily recognizable winner and loser. When opponents had one or both claws inter-locked, occasionally for a prolonged amount of time, making it difficult to observe each individual action it was recorded as "holding". Eventually, the retreating lobster was given a negative score and the winner given a positive score (±1, Table. 5). Similarly, when opponents were standing head-first into shelter from opposite sides, either because an intruder had challenged the resident lobster or if both entered simultaneously, their movements could not be observed. This was recorded as "fighting in shelter", and lobsters were scored as for "holding" (±1, Table. 5). Finally, if a resident lobster was challenged and subsequently gave up shelter without defending it, or if the intruding lobster pulled it out by force, the intruder had evicted the resident opponent. This clearly represented strong dominance/subordinate behaviors and were scored accordingly (±2, Table 5). After these interactions the loser generally retreated away from its opponent.

Submissive behaviors consisted of various forms of retreat. The mildest form of retreat (avoiding or moving away, -1) could be seen in both opponents before dominance had been established. After one lobster had gained dominance, however, the submissive individual generally retreated upon each subsequent approach without physical contact being observed. Threat displays was also observed to trigger a retreat from the submissive individual. A rapid retreat (-1.5), classified as either walking rapidly/"running" away from an opponent or beating the tail once to escape the grasp of a claw or when startled from behind, was typically displayed by submissive lobsters when they were lunged upon or chased. The most powerful indicator of sub-dominance was tail-flip escapes (-2), which was generally observed either after violent full-contact fights or if a submissive lobster was attacked, and may be considered a last resort escape to avoid serious injury.

Individual behaviors were included to describe activities when there were no interactions, and as for Interactions they were summarized to produce an "individual score". These categories were focused mainly on behavior related to shelter, as the acquisition of shelter is essential for juvenile lobsters, and also a sign of social dominance (Peeke et al. 2000). Here, behaviors were also scored based on their presumed significance in nature. Thus, lobsters were given a positive score when finding shelter (+1) and for remaining in it/defending it from an intruder (+1 per minute) and a negative score if they voluntarily left the shelter to roam around the tank (-1). Digging activity inside shelter was not recorded since lobsters showed highly variable digging behavior that seemed much less motivated than in individual observations.

They were primarily observed to either dwell (lie still) inside or guarding the entrances as the opponent frequently roamed around and approached the shelter.

Roaming, digging outside shelter and freezing behavior were included as a measure of the general activity level. Roaming around the tank not attempting to take possession of shelter was given a negative score since high activity levels increase conspicuousness (van der Meeren 1993; 2000). Digging behavior displayed outside shelter was rarely observed and considered neutral as both aggressive and submissive individuals showed this behavior. Freezing were clearly more common in submissive lobsters that tended to display prolonged freezing after repeatedly losing fights. However, they were already penalized for being submissive by a low aggression score, and freezing may be viewed as the least negative behavior of a submissive lobster in an experimental arena, denied both protection from shelter and a possibility of escape. Outside digging and freezing contributed to the indirect score only by adjusting the time allocated to roaming, i.e. they both had a weak positive effect.

2.5 Experiment 3; Long-term effect of exposure to predator odors

Exposed and Naïve lobsters were released into a semi-natural habitat after a simulated transport stage, and allowed 35 days to compete for a limited number of shelters. It was investigated whether lobster performance changed over time post-release, and if so, whether the change could be explained by the prolonged exposure to predator odors. This experiment represented an up-scaling of the small-scale interaction experiment, further enhancing the habitat complexity.

2.5.1 Experimental design

The small-scale simulated release experiment was performed outdoors from the 10th of July to 14th of August, with two $4 m^2$ (2x2 m) tanks used as test arenas. Bottom substrate was approximately 45 liters of coarse shell sand, and 30 single great scallop (*Pecten maximus*) shells were used as shelters (shell diameter, mean ± SD: 11.98 cm ± 1.58). To reduce water turbidity the sand was washed for approximately 24 hours to remove the finest grained particles. Water volume was then fixed to 1600 liters through a central standpipe covered with thin plastic mesh to prevent escapes, with a constant water flow of approximately 14 l/min. Water quality and origin was equal to tanks in the wet-lab, and ambient temperature was continuously recorded with an automatic data logger (10.0-18.0°C, Appendix 1). Small meshed black nets were stretched out over the tanks to reduce direct exposure to sunlight, and limit large amount of instant freshwater input from precipitation. Both light regime and light intensity at the tank-surface varied naturally, with the photoperiod for Parisvatnet ranging from approximately L:21.5/D:2.5 on the 10th of July to L:17.5/D:6.5 on the 14th of August (sky calendar). Light intensity on a cloudless summer day with no precipitation or exposure to direct sunlight was in the range of 8000-12000 lux at the water surface beneath the nets. The light intensity has a wider range depending on weather conditions, but 8000-12000 lux was representative for the light intensity in a variety of weather conditions.

To promote competition the shelter-to-lobster ratio was selected to 0.5. When limiting the number of available shelters Exposed and Naïve lobsters were forced to compete for a limited resource, as in the earlier smaller scale interaction experiments. To investigate whether any of the groups were superior in their shelter-seeking- and competitive behavior, and if post-release performance changed over time, samplings were performed three times during the 35 day experiment; on the 16th and 31st of July and the 14th of August, corresponding to 6, 21 and 35 days after release.

The treatment protocol was modified to some extent (Table 6) after the end of Experiment 2 (30th of June), mainly to accommodate regular working hours for the staff at Parisvatnet. Animals did not experience handling or other outer stimuli except for feeding every fourth day until the 9th of July, when exposure to predator odors ceased and lobsters included in the release experiment were individually marked with visible implant elastomer (VIE) tags (Uglem et al. 1996; Clark & Kershner 2006). Liquid elastomer was injected with a hypodermic needle (0.55 mm) in the ventrolateral musculature around the fifth segment of the tail. Care was taken not to damage surrounding tissue and to prevent leaching when the syringe was retracted from the tissue. This method was chosen due to the long duration of the experiment as elastomer marks remain visible even after several molts when administered correctly, and mortality from the marking procedure is low (Uglem et al. 1996; Clark & Kershner 2006). After marking, lobsters were returned to their single compartments for approximately 24 hours before initiation of the simulated release. According to Clark & Kershner (2006) there are no significant aversive effects of VIE tagging, thus 24 h acclimation was considered sufficient. A total of 120 lobsters were supposed to be included in the release, but 12 additional lobsters were marked to account for post-procedure- and transport mortality. However, no mortality had occurred 24 h after tag deployment. As a precautionary measure, lobsters were not subjected to predator odors the last day before release to avoid associative learning between the tagging procedure and predator odor. To standardize photoperiod the lights were turned on at midnight (approximately 8 hours before transport) for all treatments. Lobsters were size matched in both tanks (CL, mean ± SD; treatment A: 14.2±1.6 versus treatment B: 14.0±1.4 (t= -0.67, p= 0.91); treatment C: 13.0±1.2 versus treatment D: 12.9±1.1 (t= -0.39, p= 0.98))

Table 6: Experimental treatment protocol from 30th of June until 9th of July. Packing and release was done on the 10th of July. The treatment protocol remained the same but was shifted in time. NA = no exposure.

Treatment	Light regime	Light phase	Dark phase	Start odor exp	End odor exp	Packing/ Transport	Release time	Tank No.	Number released	Tag
A	L:18/D:6	14.30 - 08.30	08.30 - 14.30	15.30	07.30	08.30-12.30	12.30-13.00	2	30	Orange
B	L:18/D:6	14.30 - 08.30	08.30 - 14.30	NA	NA	08.30-12.30	12.30-13.00	2	30	Green
C	L:6/D:18	08.30 - 14.30	14.30 - 08.30	15.30	07.30	09.00-13.00	13.00-13.30	1	30	Orange
D	L:6/D:18	08.30 - 14.30	14.30 - 08.30	NA	NA	09.00-13.00	13.00-13.30	1	30	Green

2.5.2 Simulated release

To simulate a release situation a transport stage was included. A total of 132 marked lobsters were placed in two polystyrene containers, and separated by multiple layers of moist newspaper over a bottom layer of ice. The containers were sealed and placed in a dark room, where they were disturbed, i.e. lifted up and carried around for 5 min, roughly every 30 minutes to simulate a realistic transport. After approximately four hours, 60 lobsters were released in each tank by gently dropping them at the water surface allowing them to settle as in a large scale release. After release the lobsters were left undisturbed for 35 days except for handling related to sampling procedures. Feeding continued every fourth day (120-180 pellets per tank).

Each sampling began with the collection of lobsters observed outside the shelters, before the scallop shells were carefully turned over to collect lobsters inside. All collected lobsters were temporarily stored in a floating unit of single-celled compartments. After a thorough search, the shelters were returned to approximately the same location and lobsters dispersed at the surface to settle again. The number of lobsters found inside or outside of shelter and the number of claws on each lobster were registered at the group level. Lobsters that were not found after careful searching were classified as dead and assigned to the correct group by back-calculation. To uphold competition in the tanks, shelters were removed corresponding to the number of dead lobsters to maintain shelter to lobster ratio at 0.5.

When performing the simulated release, it was decided to keep summer simulations (A and B) and winter simulations (C and D) separated (Table 6). The alternatives were to either include all four treatments in both tanks (15 juveniles from each treatment) or keep them separated (30 Exposed and 30 Naïve lobsters in each tank separated on the basis of prior light regime). The latter alternative was chosen for two reasons: Lobsters in the Summer simulations were significantly larger in size (CL) than those in Winter simulations, whereas there was no difference within Summer or Winter simulations. Size was a potential source of bias (Atema & Voigt 1995), and larger individuals had been observed to kill or severely damage smaller ones. In addition, given the large variation in lobster performance, sample size was considered important. A design where two treatments were present in each tank in fairly high numbers would simplify the experimental design, and was also assumed to increase the goodness of fit for predictive models. Consequently, it was decided to separate the groups based on experience with predator odors.

2.6 Statistical analysis

Statistical analysis for all experiments was performed using R® v. 2.15.0 (R Development Core Team 2012). For the complete R-syntax see Appendix 2. All raw data are included in Appendix 4.

Mean size (CL) was compared using analysis of variance (ANOVA) by the lm function in R, with treatment as the predictor variable. This result is included here as it has consequences for other models. There was a significant size difference ($F= 13.52$, $p<0.0001$) between treatments so a Tukey HSD multiple comparisons tests was performed to assess which treatments that differed. There was a significant difference between Summer (A and B) and Winter (C and D) simulations ($-2.90 \geq t \geq -5.67$, $0.022 \geq p > 0.001$), where Summer simulations were significantly larger, but no differences was found within Summer ($t= -1.624$, $p= 0.37$) or Winter ($t= -1.229$, $p= 0.61$) simulations, respectively (see also Table 1). Consequently, CL was included as a covariate in subsequent statistical models.

2.6.1 - Experiment 1: Individual observations

Tank effects were considered negligible since lobster holding tanks were completely similar except for experimental treatment, and lobsters did not interact with each other. Rather they were separated in single cells and were considered independent observations. However, as repeated measurements were made on the same individuals, a first order correlation structure was added to the models when possible to account for autocorrelation in the data.

The two main explanatory variables; odor exposure and photoperiod, were arranged in a 2x2 factorial design giving a total of four different treatments. For all models in Experiment 1, both experimental treatment (A, B, C or D) and the number of days in treatment (NDT: 4, 8, 12 or 16 days) were included as predictor variables. The response variable was thus the only variable that changed between each analysis. Furthermore, for all behaviors analyzed in Experiment 1, the NDT variable was treated as a continuous predictor as individual lobsters were followed on four successive points in time, which was considered sufficient to reveal any overall patterns resulting from number of days in treatment. Additionally, contrast analyses were performed on all models in Experiment 1 to extract model parameters for each level of treatment. In this way, one could compare behavior between separate treatments.

Due to its configuration as time-to-event data, mean time spent to find shelter was analyzed using a survival model with censoring (Crawley 2007). The inclusion of censoring was essential since there was a considerable amount of lobsters that did not find shelter in the 900 sec (15 min) available, and by either removing them from the analysis (mean skewed toward 0 sec) or recording 900 sec as their time-to-shelter (mean skewed towards 900 sec), one would create severely biased estimates (Crawley 2007). Therefore, the survival model includes a binary censoring variable determining whether shelter was found. The analysis was performed by the survreg function in R.

The general activity level comprised several behaviors; shelter dwelling, roaming, digging activity inside shelter and climbing activity. The data for these four behaviors were Box-Cox transformed to account for non-normality and analyzed with a linear mixed effect model by the lme function in R (Pinheiro and Bates 2000). To account for repeated observations of individual lobsters, I assumed a first-order autocorrelation structure and specified the intercept of individual lobsters as a random effects factor. Digging outside of shelter and freezing behavior were not analyzed statistically due to low sample size.

2.6.2 - Experiment 2: Interactions

Dominance was transformed to a binary response variable based on the aggression- and individual scores, where the highest scoring lobster in a pair was termed dominant (1) and the lowest scoring lobster submissive (0). Observations were not independent since lobsters were tested together in pairs, thus dominance was analyzed with a glmm, where the model estimated probability of gaining dominance for Exposed and Naïve lobsters. The intercept of each lobster pair was considered a random effects factor. Due to size differences Summer (A and B) and Winter (C and D) simulations were paired exclusively against each other. Interactions were only performed once for each lobster, so NDT was not included as a predictor. Thus, the two main predictor variables were odor exposure and photoperiod. CL was included as a covariate to control for the effect of size.

It was also analyzed whether the individual that first initiated an Interaction depended on odor exposure or photoperiod. This relationship was analyzed with a glmm as described for analysis of dominance. To assess whether the Individual behaviors was influenced by the outcome of Interactions, data on time spent occupying shelter, roaming and freezing were Box-Cox transformed to account for non-normality and analyzed with a lme. The outcome of Interactions (winner or loser) was the predictor variable, and CL was included as a covariate. The intercept of each lobster pair was considered a random effects factor. The same analysis was performed on the summarized Individual score based on the values in Table 5, but these were not Box-Cox transformed before analysis.

2.6.3 - Experiment 3: Simulated release

The number of lobsters found inside or outside of shelter constituted the binary response variable termed “shelter occupancy”. Since the Summer (A and B) and Winter (C and D) simulations were significantly different in size it was not possible to include all four treatments in both tanks without creating size dependent bias (Atema & Voigt 1995). As a consequence, one Exposed and one Naïve group was released in separate tanks, and tank number was included as a random effects factor. Odor exposure and number of days in treatment (NDT, i.e. days post-release) were included as the main predictors (fixed effects), and the probability of “shelter occupancy” was estimated for Exposed and Naïve lobsters using a glmm. Since samplings were only performed three times during the 35 day period, and the fact that they were situated far from each other in time, the NDT variable was treated as a categorical predictor. Similar analyses were performed to compare probability of injury and mortality depending on exposure to odors and with NDT.

3. Results

3.1 Experiment 1 – Individual observations

Each lobster was observed four times in Experiment 1; after 4, 8, 12 and 16 days in treatment. After release into the observation unit lobsters generally displayed the same patterns of behavior, but the individual variation was large within each treatment and no consistent effects of exposure to predator odors were found. In total, shelter was found in 87% of the trials (528 of 608), but shelter dwelling could vary from 20 seconds to 14 minutes.

Irrespective of experimental treatment the lobsters would generally either; i) rapidly find shelter, dig entrances and show a minimum of activity in the open, ii) not enter shelter, "pace" around in open areas and climb along the walls, or iii) walk very slowly, enter shelter after several minutes and not dig inside shelter. These behavioral syndromes were stable over time, in that they were observed in each trial, but individual lobsters could show completely different behaviors from one trial to the next.

Shelter seeking

Time to find shelter was recorded at the first complete entering into shelter (see Table 4). There was no significant effect of the interaction between treatment and number of days in treatment (NDT henceforth) on mean time-to-shelter (survival analysis; Deviance= 6.41, df= 598, p= 0.093, Fig. 7). This indicated that any changes in time-to-shelter over time did not differ between treatments. It is evident that the proportion that failed to find shelter differed between the four test-days, but there was no systematic increase or decrease in time to find shelter (Fig. 7). Similarly, no overall effect was observed from NDT (Deviance= 0.24, df= 604, p= 0.623), or from the treatment alone (Deviance= 1.23, df= 601, p= 0.746). There was a negative effect of size (CL) on the time spent to find shelter within each treatment (Deviance= 6.99, df= 605, p= 0.008), implying that the motivation to find shelter was higher in small individuals. Raw data are included in Appendix 4.

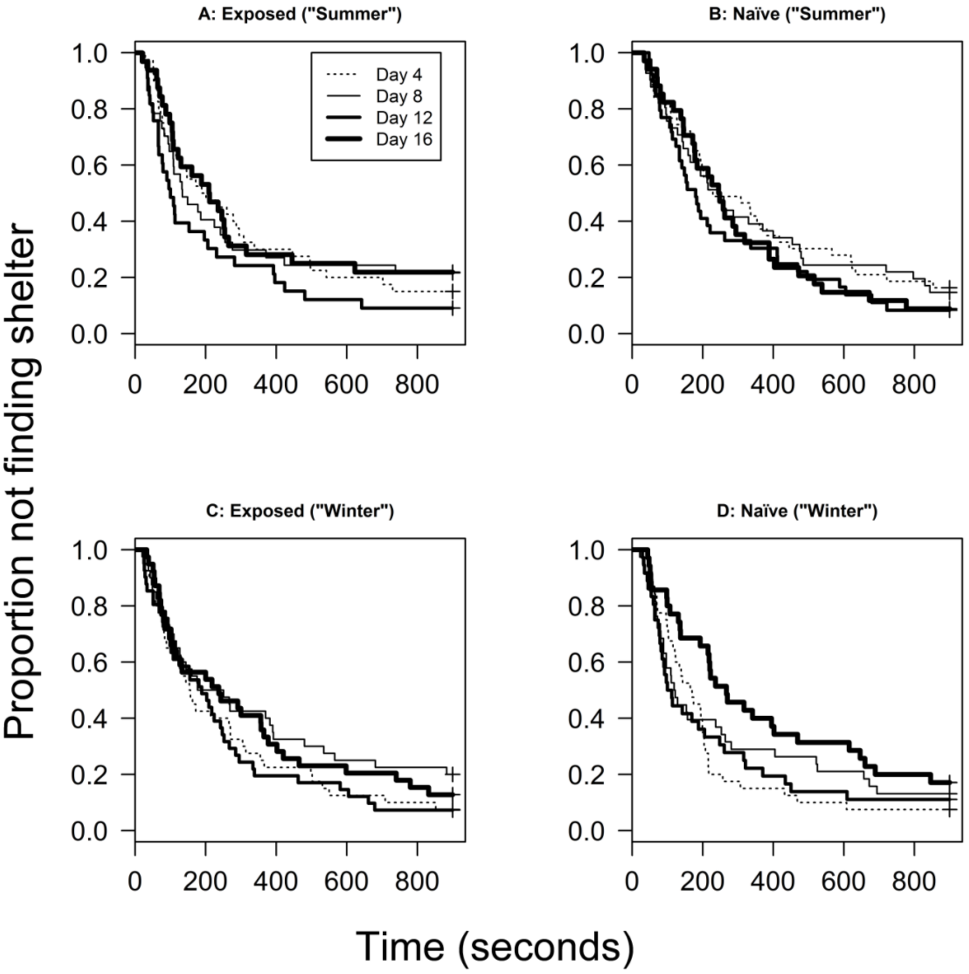

Figure 7: Survival plots with time-to-event curves. The curves are depicting time-to-shelter and the proportion that did not find shelter during the 15 minute trials. Censoring of lobsters that did not find shelter is indicated by a cross at the far right of the curve.

General behavior

The remaining behavioral categories analyzed were shelter dwelling, roaming, digging activity inside shelter (cheliped shuffling) and climbing activity (Table 4). Digging behavior outside shelter and freezing were only recorded in 97 (16.0%) and 76 (12.5%) out of 608 trials, respectively. These behaviors were not analyzed statistically due to few observations, but were used to adjust the roaming category. The raw data are included in Appendix 4.

Although the development over time could differ between treatments for some of these behaviors, the mean differences between treatments were small and infrequent (Appendix 3). In addition, the individual variation within each treatment was extensive. Due to this variation, measures on the general behavior have very limited biological significance, which also becomes evident when comparing between treatments in figures 8 to 11.

Shelter dwelling:

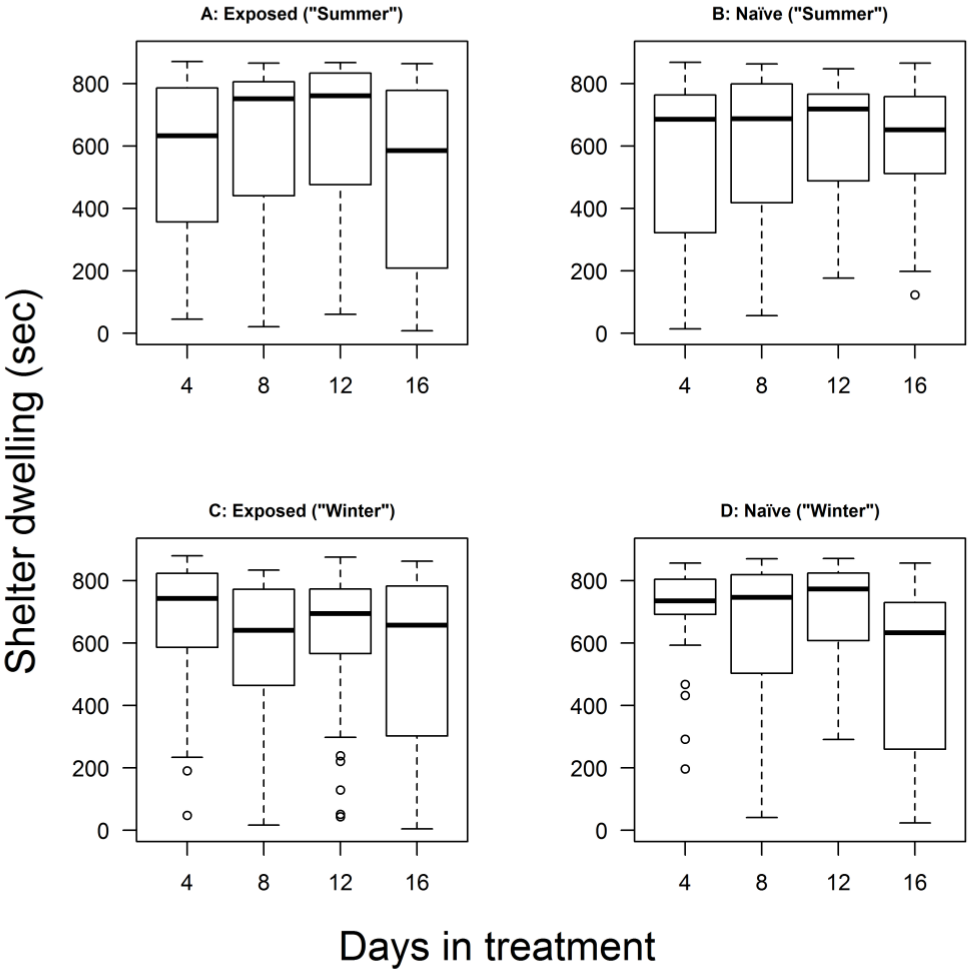

Figure 8: Time spent in shelter. Plotted are the raw data. The values depicted here are standard box-and-whiskers plot in R with median and first and third quartile. Whiskers represent minimum and maximum values except for outliers (1.5 times the length of the box away from the box) marked by an open circle.

There was a significant interaction between treatment and NDT on mean time spent dwelling in shelter (lme: F= 3.65, p= 0.013, Fig. 8), which implied that dwelling differed between the treatments with time. This was mainly due to a different development between Naïve Summer (B) and Naïve Winter (D) lobsters over time (t= -3.26, p= 0.001, Fig. 8), where the Naïve Winter lobsters (D) reduced shelter dwelling compared to Naïve Summer lobsters (B).

The significant change in dwelling with time came from a marked decrease at day 16 (Fig. 8). There was no apparent reason for this decrease, and it may be considered spurious. Since the development beyond day 16 was not recorded, conclusions will not be drawn based on these data. There were few differences in mean response over time between.

Roaming:

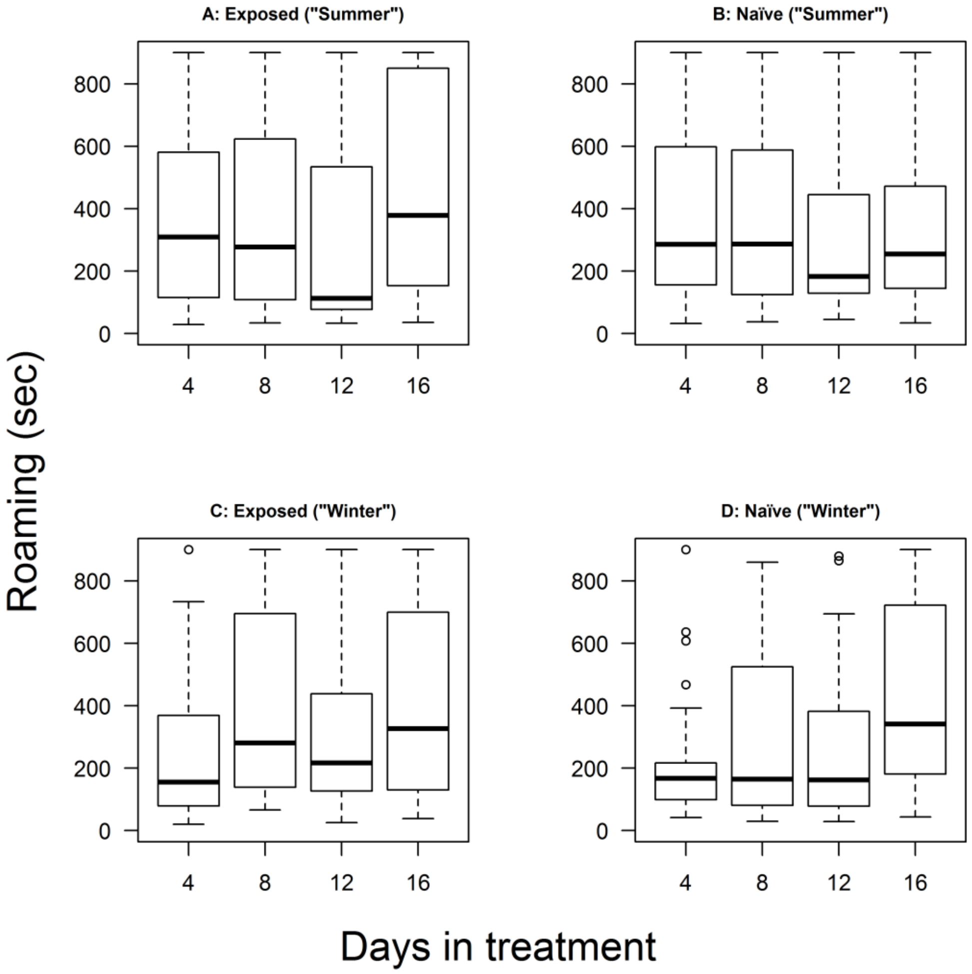

Figure 9: Time spent roaming around the tank. This included the time when lobsters were walking, climbing or moving-stopping rapidly in short intervals. Plotted are the raw data. For the values depicted see Fig. 8.

There was a significant interaction between treatment and NDT on mean time spent roaming (lme: F= 3.28, p= 0.021, Fig. 9), implying that change in roaming with time was dependent on treatment. As for shelter dwelling, this was mainly caused by a presumably spurious increase in roaming at day 16. Time spent roaming differed markedly within each treatment, but again the differences between treatments were small and inconsistent. However, roaming showed an overall increase with size within each treatment (lme: F= 4.64, P= 0.033).

Digging activity (cheliped shuffling):

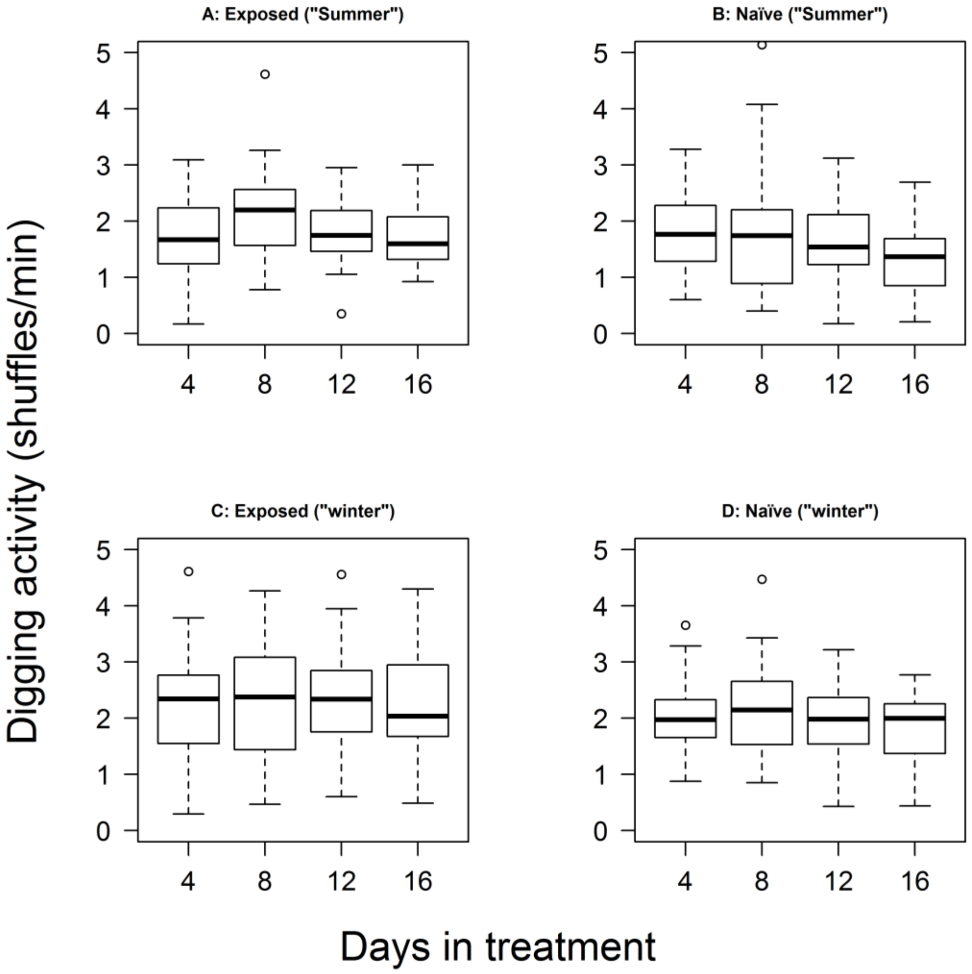

Figure 10: Digging activity as a proxy for shelter manipulation. Recorded as number of observed "cheliped shuffles" per minute inside shelter. Plotted are the raw data. For the values depicted see Fig. 8.

There was a significant interaction between treatment and NDT when analyzing digging activity (lme: F= 5.16, p= 0.0017, Fig. 10). Exposed lobsters upheld their digging activity with time irrespective of photoperiod, as opposed to Naïve lobsters that tended to reduce the digging activity with time (Naïve Summer, B: t= -4.27, p< 0.0001; Naïve Winter, D: t= -1.69, p= 0.092). There were only small differences between the treatments.

Climbing:

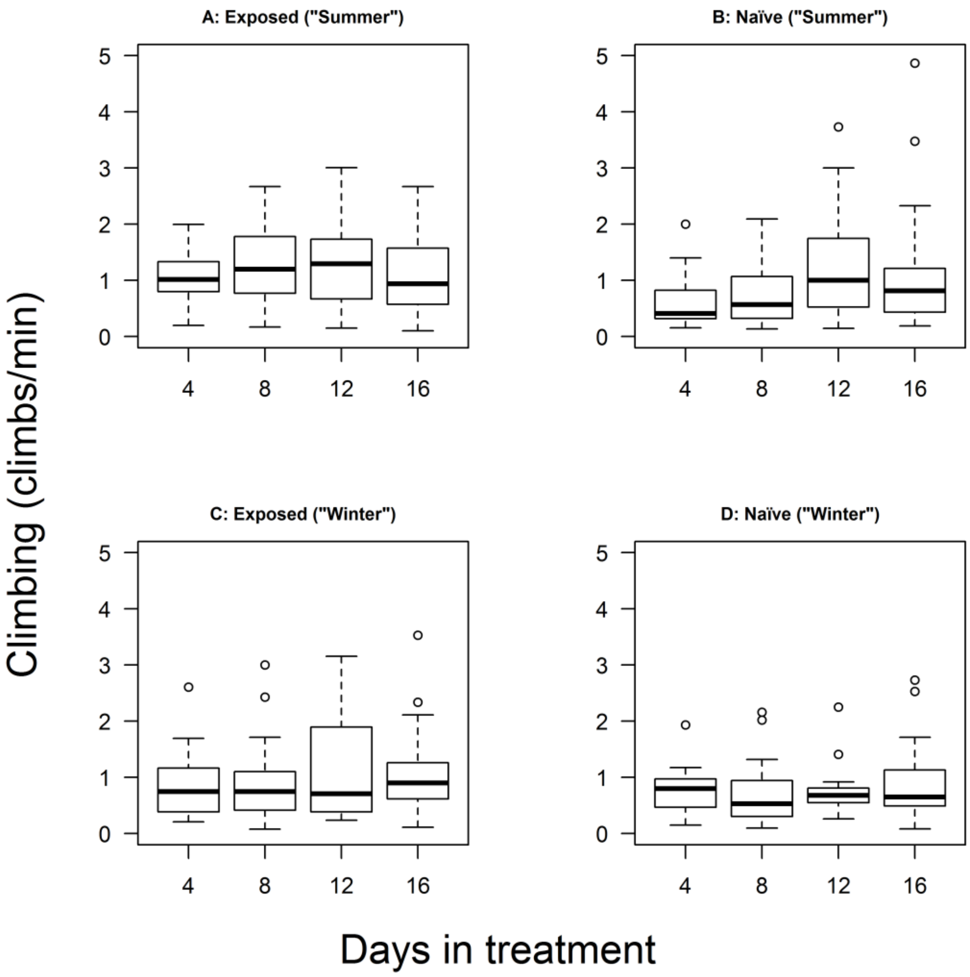

Figure 11: Climbing activity. Recorded as number of observed climb attempts or completed climbs per minute spent roaming. Plotted are the raw data. For the values depicted see Fig. 8.

There was a general increase in climbing activity with time (lme: F= 16.20, p< 0.0001), and also an increase in climbing activity with size within each treatment (lme: F= 17.60, p< 0.0001, Fig. 11).

In summary, there were no consistent effects of exposure to predator odors. Although behavior in some treatments may show statistically significant changes with time, the differences between each treatment were small (Appendix 3). The behavior of Exposed lobsters remained essentially constant with time irrespective of light regime. The biological significance of these data is restricted, and individual observation of lobsters in this type of test-environment did not seem appropriate to assess the effect of odor exposure on behavior.

3.2 Experiment 2 - Interactions

The paired interaction experiments were carried out after 20 days in treatment. Lobsters were sized matched for each pair. Exposed lobsters were on average 1.24% larger so size (CL) was included as a covariate in the statistical model to control for this size difference. The winner in each trial was determined based on the aggression score and individual score (Table 5).

Dominance was first analyzed focusing only on the aggression score (Fig. 12A). There was no significant interaction between odor exposure and photoperiod (glmm: df= 47, F= 1.607, p= 0.211, Fig. 12A), establishing that the effect of exposure to predator odors did not depend on photoperiod. There was a strong effect of odor exposure alone on the lobsters ability to win agonistic Interactions, and Exposed lobsters established dominance in 35 of 50 trials, which was highly significant compared to the Naïve lobsters (glmm: df= 48, F= 14.32, p= 0.0004, Fig. 12A). Photoperiod alone (Summer versus Winter simulations) did not have any effect on the outcome of the Interactions (df= 48, F= 0.018, p= 0.89), nor did the size (CL) (df= 48, F= 0.191, p= 0.66), in the closely size-matched lobsters.

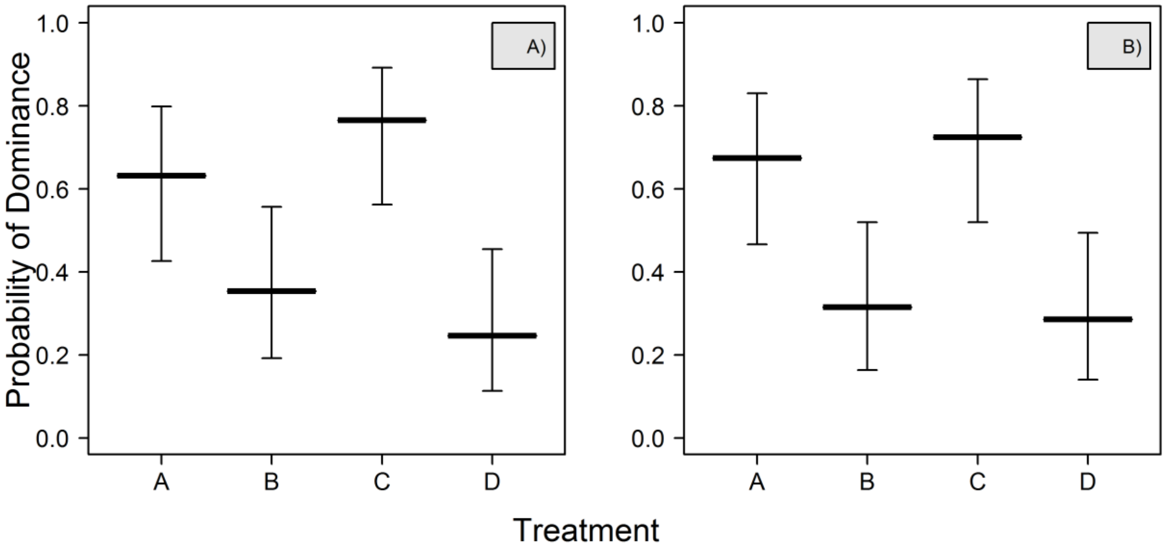

Figure 12: Probability of gaining dominance for each treatment. The error bars show 95% confidence intervals for the probabilities. **A:** Dominance determined by Interactions, i.e. the aggression score alone, which included all aggressive displays or physical contact (see also Table 5). **B:** Dominance determined by both Interactions and Individual behaviors, i.e. aggression score and individual score combined. Groups: A: Exposed (Summer), B: Naïve (Summer), C: Exposed (Winter), D: Naïve (Winter).

Concerning which group that initiated the first encounter, there was no significant interaction between odor and photoperiod (glmm: df= 47, F= 1.44, p= 0.236), meaning that the effect of odor exposure on which individual that initiated encounters did not depend on photoperiod, i.e. summer or winter simulations did not differ. However, there was a significant effect of odor exposure alone (glmm: df= 48, F= 5.38, p= 0.025), where the Naïve lobsters initiated the first encounter more often than Exposed lobsters (31 and 19 first encounters, respectively).

The dominant lobster, i.e. the winner of agonistic Interactions, displayed the most appropriate Individual behavior (Fig. 13). The winners spent significantly longer time occupying shelter (t= 6.43, p< 0.0001), less time on roaming (t= -5.26, p< 0.0001) and less time freezing (t= -2.88, p= 0.035) than the losers. The winners rarely displayed freezing behavior (n=9) as opposed to the losers (n=30). Consequently, when summarizing all Individual behaviors into an “individual score” the winners had significantly higher scores (t= 7.24, p< 0.0001) than the losers. There was no effects of size on Individual behavior (t= -0.95, p= 0.345).

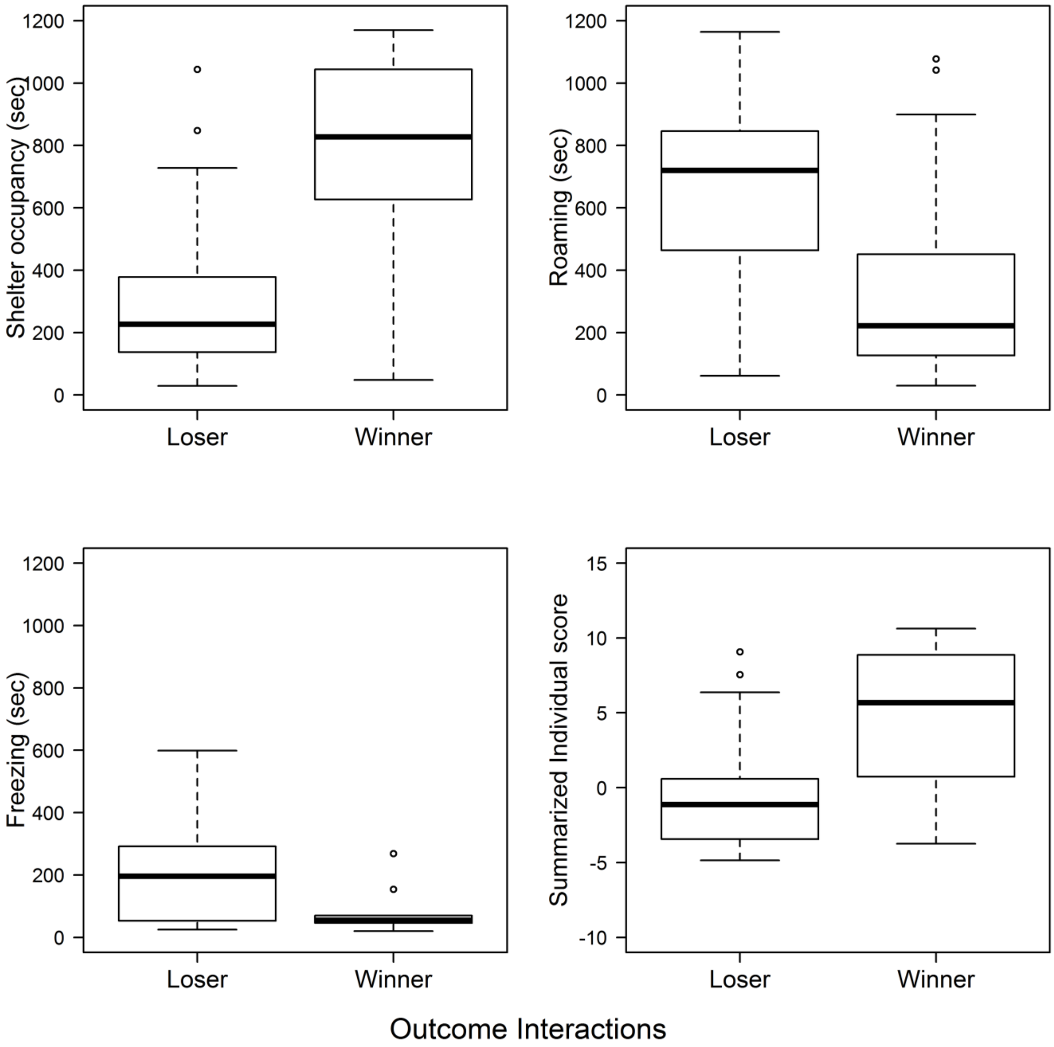

Figure 13: Individual behaviors for winners and losers of agonistic Interactions. Sample size: Shelter occupancy; n=29 and n=43 for losers and winners, respectively. Roaming; n=50 for both groups. Freezing; n=30 and n=9 for losers and winners, respectively. Summarized Individual score based on values for Individual behaviors in Table 5 (n=50 in both groups). For explanation of the values depicted see Fig.8.

When combining the aggression- and indirect scores to attain a new dominance score, there was still no significant interaction between odor exposure and photoperiod (glmm: df= 47, F= 0.180, p= 0.674, Fig. 12B). As was the case for Interactions alone, odor exposure had a strong effect on the ability to gain dominance (df= 48, F= 14.34, p= 0.0004, Fig. 12B), and Exposed lobsters similarly established dominance in 35 of 50 trials. Again, there were no effects of photoperiod or size (CL) on dominance (F= 0.011, p= 0.918 and F= 0.151, p= 0.699, respectively). Thus, exposure to predator odor was a key determinant for pair dominance.

3.3 Experiment 3 – Simulated release

After a four hour simulated transport a total of 120 lobsters were released in two 4m^2 tanks, with 30 Exposed and 30 Naïve lobsters dispersed in each tank (15 lobsters/m^2). Agonistic encounters were observed for more than 30 min post-release, but after three hours less crowding and no physical interactions were observed for 15 minutes indicating that lobsters had begun to settle into shelters and that hierarchies had started to establish. Sampling was performed on day 6, 21 and 35 post-release. Tank effects, i.e. the effect of prior light regime (Summer or Winter simulations), were considered negligible due to low inter-tank variation (Standard deviation of mean between tanks was $2.2*10^{-6}$). For simplicity, shelter occupancy will not be referred to as probabilities in the following text, but keep in mind that a change in shelter occupancy with time means a *change in the probability* of occupancy with time.

There was a significant interaction between odor exposure and number of days post-release (i.e. days in treatment) (glmm: F= 28.26, p= 0.002, Fig. 14), which means that shelter occupancy differed between Exposed and Naïve lobsters with time. At the first sampling (day 6) the Exposed lobsters had significantly lower shelter occupancy than Naïve lobsters (t= -6.91, p= 0.001, Fig. 14), but increased occupancy with time. The overall improvement from day 6 to day 35 was highly significant (t= 7.20, p< 0.001, Fig. 14). In contrast, Naïve lobsters significantly reduced shelter occupancy from day 6 to day 35 (t= -3.95, p= 0.011). At the final sampling after 35 days the Exposed lobsters had significantly higher shelter occupancy than Naïve lobsters (t= 4.16, p= 0.009, Fig. 14). Thus, Exposed lobsters initially were at a disadvantage but improved over time, and after 35 days they were better than Naïve lobsters.

The probability of being injured did not differ between Exposed and Naïve lobsters (F= 0.83, p= 0.49), but it significantly increase with time (F= 9.40, p= 0.020). Similarly, probability of survival did not differ between the groups (F= 1.06, p= 0.41), but significantly decreased with time post-release (F= 10.63, p= 0.016). This indicated that some level of competition was present over prolonged periods. At day 35, Exposed and Naïve lobsters had suffered a mortality of 25% (8/30 in tank 1, 7/30 in tank 2), and 26.6% (9/30 in tank 1, 7/30 in tank 2), respectively. The density was reduced to 10.75/m^2 and 11.5/m^2 for tank 1 and 2, respectively.

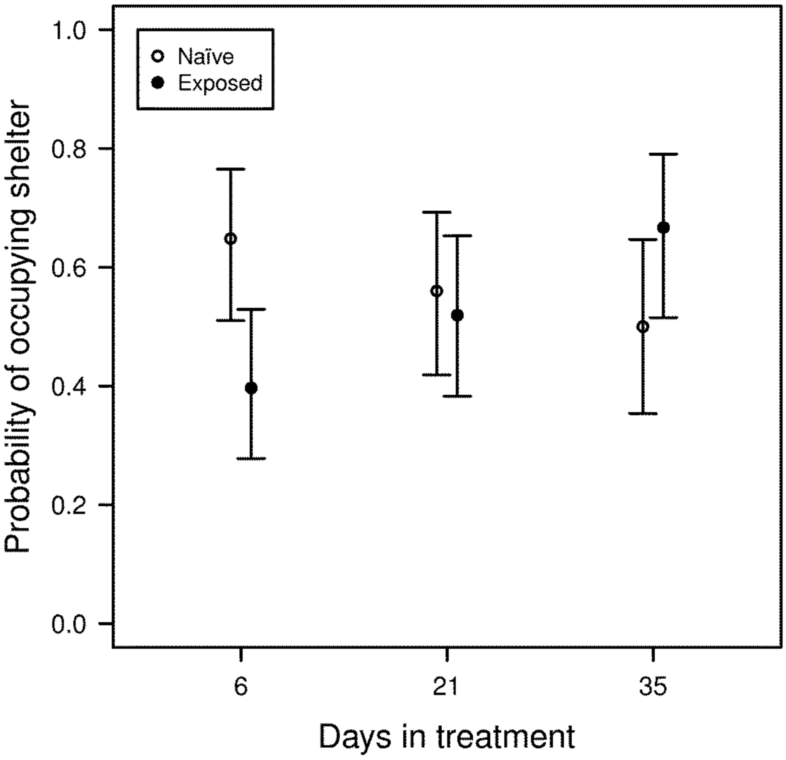

Figure 14: The figure shows the probability of occupying shelter for Exposed and Naïve lobsters at the three sampling days. The data are pooled for both tanks due to low inter-tank variation ($2.2*10^{-6}$). Error bars show 95% confidence intervals for the probabilities.

As seen in Fig. 14 the sum of the probabilities does not equal 1 for any of the days, and it may require some clarification for the reasons behind this feature. Overall mortality was equal in both tanks and did not differ between Exposed and Naïve lobsters. However, mortality was not necessarily identical in both groups at each sampling. To illustrate with an arbitrary example: If sampling at day 6 showed that group A had experienced higher mortality than group B, lobsters were not removed from group B to give the groups equal number of lobsters when re-released. This was done to resemble a realistic setting as closely as possible. Thus, although the initial number of released lobsters was equal in both groups (n=30), they did not always compete under equal conditions. For example, group A has 25 survivors and group B has 15 survivors. They are competing for 20 shelters and manage to hold 10 shelters each. Shelter occupancy in group A is then 0.4, while for group B it is 0.66. The sum of the proportions is 1.06. If one group has fewer survivors but simultaneously holds equal or higher amount of shelters they will by default have higher probability of shelter occupancy.

4. Discussion

No consistent effects of exposure to predator odors were found in the individual observations. Shelter-seeking behavior seemed to vary randomly for each individual lobster between each test-day, while there were only minor differences in shelter dwelling, roaming and climbing activity. Exposed lobsters continued to dig at constant levels, while Naïve lobsters showed decreasing digging activity with time, but the mean differences were marginal. This considerable variation *within* each treatment, combined with the small differences in mean response *between* treatments restricts the biological significance of these data when assessing the effect of odor exposure on behavior.

In the two subsequent experiments there was a clear effect of exposure to predator odors. In the paired interaction trials Exposed lobsters established dominance significantly more often, and winners of agonistic interactions also spent more time in shelter, roamed less and displayed less freezing behavior. Interestingly, the Naïve lobsters initiated significantly more first interactions than Exposed lobsters. In the simulated release experiment, although Exposed lobsters performed worse on the first sampling after six days, they significantly increased shelter occupancy with number of days post-release. In contrast, Naïve lobsters reduced shelter occupancy with number of days post-release, and at day 35 the Exposed lobsters had significantly higher probability of occupying shelter. Both groups experienced increased risk of injury and increased mortality with time.

4.1 Experimental design

The rearing environment of hatchery produced organisms is generally characterized by a high level of stability with respects to abiotic factors and food supply combined with limited space and an absence of predators. This is a simple existence compared to the vast aquatic environment experienced by their wild counterparts that must continuously cope with spatiotemporal fluctuations in temperature, predation risk and available resources. The hatchery-induced stability and lack of relevant environmental stimulation will prevent natural selection (Salvanes & Braithwaite 2006), may lead to animals with less developed sensory capabilities (Sandemann & Sandemann 2000) and result in appearance of morphological and behavioral deficits (Svåsand et al. 1998; Bell et al. 2005; Salvanes & Braithwaite 2006). Thus, extrapolating data from trials with hatchery reared juveniles to explain the behavior of wild lobsters should be made with utmost caution. However, since juveniles of European lobster cannot be found in the wild (Mercer et al. 2001), one have to make certain assumptions on their behavior based on experimental studies and from work on related species. Along with these assumptions follow different sources of bias which need to be identified for results to be transferable to a post-release situation.

Size, molt stage, sex and prior experience are important determinants for the outcome of agonistic interactions (Atema & Voigt 1995). Size is recognized as the most important variable so lobsters were size matched in Experiments 2 and 3. In Experiment 2 lobsters were matched pair-wise as closely as possible. Exposed lobsters were on average 1.24% larger, which is well below the 5% criterion used by Vye et al. (1997, see also Scrivener 1971, cited in Atema & Voigt 1995) and should not bias the results. Size (CL) was also incorporated in statistical models, and there was no significant size effect on dominance. In addition, asymmetries in claw size may affect dominance in several crustacean species (Scrivener 1971, cited in Atema & Voigt; Stein 1976; Sneddon et al. 1997). Claws were not measured here, but the visual impression was that size matched individuals also had similar claw dimensions.

Neither the sex nor the exact molt stage was determined in this study, both of which can affect agonistic behavior (Atema & Voigt 1995). Lobsters were molt-staged based on exoskeletal flexibility, a method also used by Rutishauser et al. (2004). Only intermolt juveniles with rigid exoskeletons that had not molted in the last 8 days were used in the experiments. Moreover, external sex characteristics are not visible until the juveniles reach ~25-33 mm CL (Agnalt IMR, pers. comm.). Thus, euthanization would have been necessary for both molt staging and sex determination. This could not be done as lobsters were reused. Even though adult males of American lobster are more aggressive than females (Scrivener 1971, cited in Atema & Voigt 1995; Karnofsky et al. 1989), this does not seem to be the case for European lobster (Skog 2009). Regardless, in juvenile lobster a direct sex-dependent advantage in shelter competition and establishment of dominance is likely not present (Jacobson 1977, cited in Atema & Voigt 1995; Peeke et al. 1998).

In Experiment 2, lobsters paired against each other were both naïve to intraspecific interactions. This was not the case in the simulated release experiment, where all lobsters had experienced one 20 minute interaction trial, and prior experience and recognition of social status could have affected the results. However, neither adult nor juvenile lobsters recognize a former opponent after seven days in isolation (Karavanich & Atema 1998; Rutishauser et al. 2004). Lobsters had been isolated for 11 days prior to the release, so any effect of previous experience or individual recognition is unlikely. Consequently, it is reasonable to assume that size, molt stage, sex or prior experience did not have any significant effects, and the observed differences in behavior should be attributable to the rearing conditions or inherent individual variation in behavior.

Animals can be highly responsive towards environmental stimuli within sensitive periods in ontogeny (Bateson & Gluckman 2011), such as the transition from pelagic larvae to benthic postlarvae in homarid lobsters. This may be a logical starting point for evaluating effects of long term exposure to predator odors, as shelter-related behaviors seem inherently present past this stage (van der Meeren 1993; James-Pirri & Cobb 1999; Castro & Cobb 2005). Hatchery reared postlarvae respond selectively to olfactory stimuli (Wahle 1992; Boudreau et al. 1993) and may also detect suitable substrates chemically (Alberts-Hubatsch et al. 2011). Further, from stage IV to V they undergo behavioral changes related to shelter-seeking and reduce activity level (Castro & Cobb 2005). However, mass-releases of early stage postlarvae have been attempted on numerous occasions (Nicosia & Lavalli 1999) but with ambivalent stock enhancement effects. The focus was therefore directed towards older juveniles. The lobsters used in the present study were on average 13.5 ± 1.5 mm CL (mean ± SD), which corresponds well to the size used in large scale release programs (~15 mm CL; Agnalt et al. 1999; van der Meeren 2000; Schmalenbach et al. 2011). Between settling and the emergent phase (~18-25 mm CL, Lawton & Lavalli 1995) juveniles are presumably tightly associated with shelter as a trade-off against predation risk (Wahle 1992), and within this period there are likely no major behavioral shifts. Consequently, it may not be the optimal window in ontogeny to influence predator-avoiding behavior through exposure to environmental stimuli, but olfaction will presumably have a primary role considering the potential lack of other biologically relevant stimuli within their burrows.

The choice of predator species could have affected the lobsters' response to odor exposure. Few studies have dealt with European lobster in this respect, although Nilsen (2007) found that Atlantic cod (*Gadhus morhua*) odor affected shelter-seeking behavior in EBP juveniles. Studies on American lobster juveniles have demonstrated an innate response toward predators naturally present in well-known lobster habitats (Wahle 1992; Boudreau et al. 1993; Castro & Cobb 2005). This supports the choice of predators in the present study as Atlantic cod, ballan wrasse (*Labrus berggylta*) and green crabs (*Carcinus maenas*) are all naturally co-occurring in lobster habitats along the Norwegian coast (Agnalt IMR, pers. comm.). Ballan wrasse and green crabs have also been observed to predate on newly released juveniles in large scale releases (van der Meeren 2000) and field tethering studies (Ball et al. 2001; Mercer et al.

2001), and lobsters have been found in cod stomach samples shortly after release (van der Meeren 2000; Ball et al. 2001; Mercer et al. 2001). It is reasonable to assume that co-evolution with these species have equipped lobsters with the ability to recognize their odors.

Based on promising results from preliminary studies (Appendix 5) it was decided to expose the lobsters to a mix of the three predator odors in pulses of long duration. Since habituation, or response waning, is more rapid with weak stimuli delivered in high frequencies (Hinde 1966), pulses were introduced in a high-flow regime with no pulse intermittency to produce a strong stimulus. Preliminary trials also indicated that odor exposure during the light phase would result in different behavior compared to exposure in the dark phase. Since it has been suggested that releases should be performed in early winter when predators are less active (van der Meeren 2000), this gave an interesting opportunity to assess whether lobsters acclimated to a winter photoperiod would respond differently to predator odors than those from a summer photoperiod. However, photoperiod was not found to affect behavior in any particular direction, and lobsters generally behaved in the same way irrespective of the light regime. In retrospect, this additional variable complicated the experimental design and may have been excluded.

There are essentially no studies on the effect of prolonged exposure to predator odors on any lobster species (although see Nilsen 2007), and to my knowledge there have been no releases of lobsters after long-term exposures. Most of the information on odor exposure and behavior is thus based on studies with fish, where workers that attempt to enhance predator avoidance generally apply learning paradigms (Suboski & Templeton 1989; Fernø et al. 2011), i.e. juveniles are trained to avoid predators by aversive conditioning. Pairing of inherently recognizable predator cues with an unconditioned fright stimulus like conspesific alarm odor may increase predator avoidance upon reintroduction of the alarm odor (Olla & Davis 1989; Brown & Smith 1997; Berejikian et al 1998). Even though avoidance learning can be effective in laboratory experiments, few field releases of anti-predator trained juveniles have been performed, and results have generally been inconclusive concerning the survival benefit of these training programs (Olla & Davis 1989; Otterå et al. 1999; Berejikian et al. 1998; Hawkins et al. 2007). In other words, we do not know whether this learning paradigm will improve behavior or survival post-release. The lack of positive results from field studies may indicate that the conditioned stimulus is not present above threshold levels post-release. Consequently, in the present study it was decided not to employ a classical Pavlovian learning paradigm, but explore whether the juveniles could be stimulated to enhance predisposed predator-avoiding behavior (Wahle 1992; Boudreau et al. 1993; Castro & Cobb 2005) exclusively through prolonged odor exposure. It was hypothesized that exposure to a presumably fright-inducing stimulus could alter the lobsters' motivation (Hinde 1966) to hide and compete for shelter when introduced to novel environments.

4.2 Experiment 1: Individual observations

In the individual trials lobsters were observed in a small and relatively low complex test-environment. In contrast to what was predicted exposure to predator odors did not improve shelter-seeking or influence other behaviors irrespective of the photoperiod in which lobsters had been reared. Smaller lobsters generally found shelter more rapidly and tended to show lower activity levels.

The objective of this experiment was to assess shelter-related behavior in presumably fright motivated animals versus naïve unmotivated controls. Naïve EBP lobsters exposed to predator-related cues may increase shelter use (Wahle 1992; Castro & Cobb 2005; Nilsen 2007), and lobsters consistently avoid leaving shelter during daytime as a trade-off against predation risk (Lawton 1987; Mehrtens et al. 2005). Consequently, one would expect Exposed lobsters to show enhanced predator-avoidance behavior if prior exposure had influenced their behavioral state (i.e. more frightened). This would be evident as a more intimate connection with shelter and restricted activity in the open area (Spanier et al. 1998). However, this was not the case as Exposed lobsters showed no consistent changes in behavior after 4-16 rounds of odor exposure. The duration of the individual trials could have been too short to reveal differences in shelter-related behavior, but since predation on hatchery reared juveniles is most intense during the first 15 minutes post-release (van der Meeren 2000; Ball et al. 2001) a prolonged time frame would give results biased towards conditions in an experimental setting.

The most probable explanation for the lack of differences between Exposed and Naïve lobsters is that shelter-seeking and substrate manipulation are "hard-wired" behaviors to which the lobsters are predisposed (van der Meeren 1993; Wickins & Barry 1996; James-Pirri & Cobb 1999), and that exposure to predator odors prior to the observations was insufficient to trigger a strong behavioral response when no real threats were present. The first observations made after four days in treatment resulted in 88.3% of the animals finding shelter within 15 minutes, but there was no improvement with experience as 85% found shelter in the final observations. Exposed lobsters showed close to constant digging activity over time while Naïve lobsters tended to reduce digging activity, although the differences were very small. This was in concurrence with what Pottle & Elner (1982) found in American lobster juveniles where 90.6% found shelter within 15 minutes. Wickins & Barry (1996) have also shown that experience does not enhance motivation to burrow in EBP juveniles. However, van der Meeren (2001) reported that shelter-seeking behavior was improved by previous experience. Based on the large individual variation reported from various studies there seems to be a need for more standardized test-protocols for observations on shelter-seeking behavior and shelter manipulation and whether these behaviors can be improved before release.

The substantial variation in the individual observations could also be ascribed to inherent behavioral plasticity. Individuals vary consistently in their readiness to take risks which is reflected in their behavior in different contexts (Huntingford 2004). In the present study, some behavioral syndromes were stable over time (Gherardi et al. 2012), and lobsters would generally either; i) rapidly find shelter, dig entrances and show a minimum of activity in the open, ii) not enter shelter, "pace" around in open areas and climb along the walls, or iii) walk very slowly, enter shelter after several minutes and not show any digging activity. However, individual lobsters were highly plastic in their behavior, and one lobster could display very different behaviors between observations only four days apart. A similar situation was observed in brown rockfish (*Sebastes auriculatus*), where behavioral syndromes were stable over time, but the same fish could exhibit two completely different behaviors in trials 10 days apart (Lee & Bereijikian 2008). It was suggested that this behavioral plasticity could reduce susceptibility to unintentional selection. The observed behavioral syndromes may thus be adaptations to spatiotemporal variations in predation pressure and food supply in wild EBP lobsters and misinterpreted as maladaptive behaviors in an experimental setting.

Habituation towards the predator odors may have occurred after prolonged odor exposure in the single celled compartments. There are few studies on habituation in lobsters, but spiny lobsters (*Panulirus argus*) rapidly habituate to food-related odors (Daniel & Derby 1988), whereas the escape responses in postlarval lobsters did not show habituation to physical stimuli (Jackson & MacMillan 2000). Hemmi & Merkle (2009) concluded that habituation in fiddler crab (*Uca vomeris*), at least under natural conditions, is highly specific towards predator-related cues with only minor changes reintroducing the response. This is supported by studies on Arctic char (*Salvelinus alpinus*), where repeated exposures to pike odor did not result in habituation (Vilhunen 2005). In the present study, performance did not change with increasing duration of exposure, so habituation seemed unlikely.

4.3 Experiment 2: Interactions

To assess the effect of odor exposure on agonistic behavior, two lobsters were placed together and a dominance score determined the winner and loser for each pair. Naïve lobsters initiated more first interactions, but the Exposed lobsters were as predicted more adept in their competitive behavior and established dominance significantly more often than Naïve lobsters. Dominant individuals also spent more time occupying shelter and less time on roaming, while submissive lobsters frequently displayed freezing behavior. There were no differences in agonistic behavior related to the photoperiod the animals had experienced.

Dominant lobsters had possession of shelter for longer time than submissive ones, indicating that shelter is a valuable resource that lobsters will compete for when in shortage (van der Meeren 2005; Peeke et al. 1998; Spanier et al. 1998). Possession and defense of shelter can be a valid measure of social dominance (Peeke et al. 2000). In juvenile American lobster the dominant individual in a similar situation remains aggressive until its opponent consistently retreats (Huber & Kravitz 1995). However, in the present study dominant European lobsters spent relatively more time occupying shelter and guarding the entrances, and less time exposed showing aggression towards the subordinate, whereas submissive lobsters continued to roam around the tank and make approach-retreat bouts towards the shelter. This frequently resulted in further aggressive encounters and subsequent escape or freezing behavior in the subdominant. Release of these “bold losers” would presumably reduce survival. Conversely, if exposure to predator odors can both reduce unwarranted boldness and improve competitive behavior as indicated in the present study, it holds potential to improve post-release survival.

The ritualistic nature of agonistic interactions observed in American lobster (Huber & Kravitz 1995; Karavanich & Atema 1998) were less evident in the present study, and encounters rapidly escalated to physical interactions of short to moderate duration ($\leq$ 3 min). This was also observed in larger European lobster (Skog 2009). Size matched American lobsters evaluate the strength of an opponent through sequential assessment strategies with successive steps of increasing intensity, providing them a possibility to retreat without escalation (Huber & Kravitz 1995). Consequently, by a less ritualized sequence of aggression weak opponents may more frequently initiate interactions with stronger ones, rather than retreating to avoid physical confrontation.

Socially naïve juveniles readily engage in agonistic interactions in staged fights or high density rearing environments (Huber & Kravitz 1995; Rutishauser et al. 2004; Skog 2009, Aspaas 2012). In the present study, Naïve lobsters engaged significantly more interactions, evident as either frontal or “ambush” approach or attempts to displace a resident lobster from shelter. This could indicate that Naïve lobsters were initially bolder and more aggressive, which is supported by Aspaas (2012) who observed that socially naïve lobsters more frequently initiated aggressive encounters. However, the optimal animal should be bold only when it pays off to be bold (Gherardi 2012), and lobsters that readily engage unknown opponents may experience an increased risk of injury. Exposed lobsters won significantly more fights but initiated less of them, and one could argue that these animals had assumed a more appropriate strategy by adjusting their behavior when the situation changed instead of being consistently bold (see Gherardi et al. 2012).

4.4 Experiment 3: Simulated release

In the simulated release experiment Exposed and Naïve lobsters were released together in 4m^2 tanks with substrate and a shelter-to-lobster ratio of 0.5. It was hypothesized that Exposed lobsters, having experienced a more complex and potentially fright-inducing chemical background, would be more adept at shelter-seeking in competition with Naïve lobsters that had been held in an impoverished rearing environment. Interestingly, the Naïve lobsters displayed higher initial shelter occupancy, but Exposed lobsters significantly increased shelter occupancy as the experiment progressed.

Lobsters fight to establish dominance and form stable hierarchies both in the wild (Karnofsky et al. 1989) and in experimental settings (Paille et al. 2002; Rutishauser et al. 2004). Dominance and territoriality has been confirmed as early as stage V in the American lobster (James-Pirri & Cobb 1999; Paille et al. 2002). In the present study, behavior was not observed over long periods of time, but in the period immediately after release lobsters were readily engaging in agonistic behavior and the same individuals were observed to frequently be involved in consecutive encounters presumably caused by the high stocking densities. As observed in the interaction trials, they did not seem to follow the strict “rules of conduct” observed for American lobster (Huber & Kravitz 1995). Full-contact fights were observed without ritualistic threat displays typically involving two to five lobsters. The fights were observed for more than 30 minutes post-release. Prolonged fights are reported to attract several other lobsters (van der Meeren 1993), and aggression is observed to be density-dependent (van der Meeren 1993; Paille et al. 2002). The initial density of 15 lobsters/m^2 was much higher than what van der Meeren and co-workers (2000) used in a large scale release, where they mainly deployed one lobster per meter shoreline. Thus, lobsters were expected to interact both due to high density (frequent encounters) and as a consequence of competition for shelters. Fights often ended in mass-retreats, usually by one or more lobsters escaping by tail-flipping.

When the tanks were observed three hours post-release the level of aggression had decreased markedly, and no full-contact fights were observed for 15 minutes. Lobsters were still roaming around the tank, but less crowding indicated that some lobsters had settled into shelter and several lobsters were observed in shelter manipulation. Threat displays frequently resulted in retreats without physical contact indicating that hierarchies maintained by aggressive displays had been established (Atema & Voigt 1995). After six days Naïve lobsters occupied a significantly higher proportion of the available shelters than Exposed lobsters. Lobsters outside of shelter now tended to reside along the tank walls and some were in shallow burrows presumably representing attempts to make alternative shelter (Howard & Bennett 1979; Botero & Atema 1982). Results from the first sampling thus did not concur with the prediction that exposure to predator odors would enhance competitive behavior.

Even though being at an initial disadvantage the Exposed lobsters significantly increased shelter occupancy between days 6 and 35. To accomplish this it is reasonable to assume that they had overcome both a residence advantage and established hierarchies. Lobsters also show higher fidelity to their shelter when shelters are in shortage (Paille et al. 2002). The prior residence effect (Peeke et al. 1998; Deverill et al. 1999; Figler et al. 1999; Metcalfe et al. 2003), where the animal that first takes possession of a valuable resource gains an advantage over subsequent intruders especially if the intruder is of equal size, would presumably give an advantage to lobsters occupying shelter shortly after release. This is supported by Aspaas (2012) who in a similar study found that the group of lobsters initially possessing shelter continued to do so over the next month. This was not the case in the present study. Yet, the Exposed lobsters managed to displace resident lobsters from shelter. This indicates a long-term effect of odor exposure on competitive behavior. The main challenge remains to explain *why* and *how* the Exposed lobsters were initially outperformed post-release, and how they outcompeted the Naïve lobsters over time.

Out-of-water transportation of lobsters should ideally be followed by a period of in-water acclimation (van der Meeren 1991; 1993) that promotes less conspicuous post-release behavior (van der Meeren 2000). One could argue that since none of the groups in the present study were acclimated this would not affect the results. However, based on the number of shelters occupied by each group (Peeke et al. 2000) the dominance pattern was altered compared to the interaction trials. Both the transport procedure and lack of acclimation may have affected the lobsters' ability to cope with a novel situation. Emersion for 4 hours cause hypoxia and a rapid stress response (Chang 2005), and stress related to out-of-water transportation has been observed to affect behavior as seen by more pelagic rushes and increased conspicuousness (van der Meeren 1991). In the present study the pelagic rushes were less frequent, but seemingly unprovoked tail-flip escapes were observed when lobsters encountered either conspesifics, the central standpipe or when roaming along the tank walls. Thus, it appeared as the transportation made lobsters more prone to "startling" which may be interpreted as an elevated stress-level. If the Exposed lobsters were released with an elevated baseline stress-level the novel environment could have been experienced as too severe, compromising their ability to cope with the many novel challenges, in particular if there was a cumulative effect of all stressors present up until the time of release; prolonged odor exposure, the transport procedure, lack of acclimation and the release itself. This could allow the initially more bold Naïve lobsters, presumably with lower baseline stress-levels prior to transportation, to settle and gain a resident advantage (Peeke et al. 1998). Subsequently, when the Exposed lobsters had acclimated to the new environment they would have a superior competitive ability as observed in the interaction experiment, and increase shelter occupancy with time.

One could argue that handling in connection with sampling at days 6 and 21 reset the entire system and new hierarchies were established each time. Assuming that shelter-seeking has a strong innate component (van der Meeren 1993; James-Pirri & Cobb 1999), lobsters initially without shelter could thus have been more motivated to gain occupancy after re-release. Lobsters without shelter were observed to reside in depressions in the substrate illustrating their predisposition to either find existing or create new shelters (Howard & Bennett 1979; Botero & Atema 1982). Hence, if the hierarchies were disrupted when lobsters were sampled and shelter entrances destroyed, lobsters without shelter could have outcompeted the presumably less motivated residents. The observations that lobsters sampled from within shelter seemed more strongly affected than those sampled elsewhere could have further reinforced competition. When shelters were opened the resident lobster often made a tail-flip escape. This was also observed in the individual observations, where lobsters taken from within shelter frequently attempted to escape. Removal or "destruction" of shelter may have been experienced as a predator attack (Barshaw & Lavalli 1988) on a familiar home shelter (Karnofsky et al. 1989; Mehrtens et al. 2005). Upon re-release the former shelter may be experienced as unsafe prompting a search for alternative shelter. If Exposed lobsters in addition had a higher level of motivation to gain possession of shelter this could further reinforce competition. The proportion of injured and number of dead individuals increased at each sampling indicating that there were persistent competition and agonistic interactions (Linnane et al. 2000). The aggression level could have been further escalated by the 5°C temperature increase during the experiment (Appendix 1) as lobsters display more agonistic behavior with increasing temperatures (van der Meeren 1993). Since the tanks were not continuously monitored none of these intervening explanations can be ruled out.

4.5 Long term effect of odor exposure

Behavioral plasticity

A possible cause for the long term effect of odor exposure on competitive behavior may be development of a higher level of behavioral plasticity in animals living in chemically enriched environments. Neuronal plasticity underlies the behavioral plasticity that can be seen throughout an animals' life (Bateson & Gluckman 2011). When Sandemann & Sandemann (2000) exposed freshwater crayfish (*Cherax destructor*) to enriched (communal rearing) and impoverished (isolated in featureless single units) rearing conditions a difference was evident after two weeks. Animals reared in the impoverished environment had then lower rates of cell proliferation- and survival in the olfactory and accessory lobes receiving higher order input from the main sensory systems. This indicates a high degree of neuronal plasticity in the decapod brain. Chemical stimulation of hatchery reared lobsters may produce similar effects. Juveniles in the present study had only experienced the chemical environment of recirculated seawater since hatching, and were now exposed to high concentrations of predator odors generating a completely different olfactory Umwelt.

The possibility of enhanced behavioral plasticity is supported by findings of Aspaas (2012), who observed that lobsters reared communally for 45 days displayed higher ability to occupy shelters after a simulated release. This may indicate that the enriched environment stimulated brain development (Sandemann & Sandemann 2000) promoting increased behavioral plasticity and ability to adjust to a new situation (competition). A high level of behavioral plasticity has also been observed in communally held spiny lobster (*Jasus edwardsii*) reared one year in captivity, where high daytime activity was reduced with 50% by rearing lobsters with predators and feeding them at night (Oliver et al. 2006). Thus, an enriched rearing environment promotes an ability to adjust behavior according to the situation.

In the interaction trials Naïve lobsters seemed initially bolder and more readily engaged interactions. Exposed lobsters initiated fewer interactions, but established dominance more frequently. It may be argued that the chemical enhancement resulted in a higher level of behavioral plasticity in the Exposed lobsters, i.e. higher ability to adjust behavior to the specific situation. Whether this was connected to predator odors in particular, or could have been achieved with other forms of olfactory or visual stimuli represents an interesting question for further studies. In the simulated release experiment, a lower level of behavioral plasticity in Naïve lobsters could give them an advantage in readily approaching novel objects (new shelters). However, lobsters with conspicuous behavior attract predators in release settings (van der Meeren 2000). In a realistic situation with release of lobsters in lower densities and in the presence of predators, it may be appropriate to assume a more cryptic and careful behavior while having a well-developed competitive behavior when challenged. The exposed lobsters performed better with time, indicating a positive long-term effect of chemical enrichment.

Long term effect on motivation

The last but perhaps most interesting explanation for the observed differences between Exposed and Naïve lobsters would be a long-term effect of prolonged odor exposure on the lobsters' motivational systems. A stimulus that acts continuously to determine the animals' specific state or responsiveness can be distinguished as a motivating stimulus, and if the external situation is constant (i.e. the rearing environment) altered behavior is caused by changes in the animals' internal state (Hinde 1966). Thus, although not constantly present, predator odor may act to modulate the lobsters' motivation, where the following response towards environmental disturbances depends on which motivational system that is most strongly activated, e.g. fright or aggression. Exposure to odors from three predators was assumed to represent a fright-inducing stimulus. The effect of odor exposure seemed to be connected to the level of environmental disturbance and the lobsters' ability to cope with different novel situations.

In the individual observations neither handling nor introduction to a novel environment elicited a pronounced behavioral response, i.e. the fright-related motivational system was not active above threshold levels. In the paired interaction trials, Exposed lobsters showed superior competitive behavior indicating an effect of prolonged odor exposure, where a real threat (i.e. an environmental trigger) was required to promote activation of fright-related motivation above threshold levels, prompting them to compete more actively for possession of shelter (Fig. 15). However, when lobsters were released after 30 days of odor exposure their competitive behavior seemed initially compromised. This indicated an inability to cope in the new environment, which may have resulted from the lack of acclimation prior to release. Yet, they increased shelter occupancy over the next month when detrimental effects from the transport and release procedure presumably had been reduced. This suggests that prolonged exposure to predator odor had an underlying long term effect, possibly related to an *extended activation* of fright-related motivation.

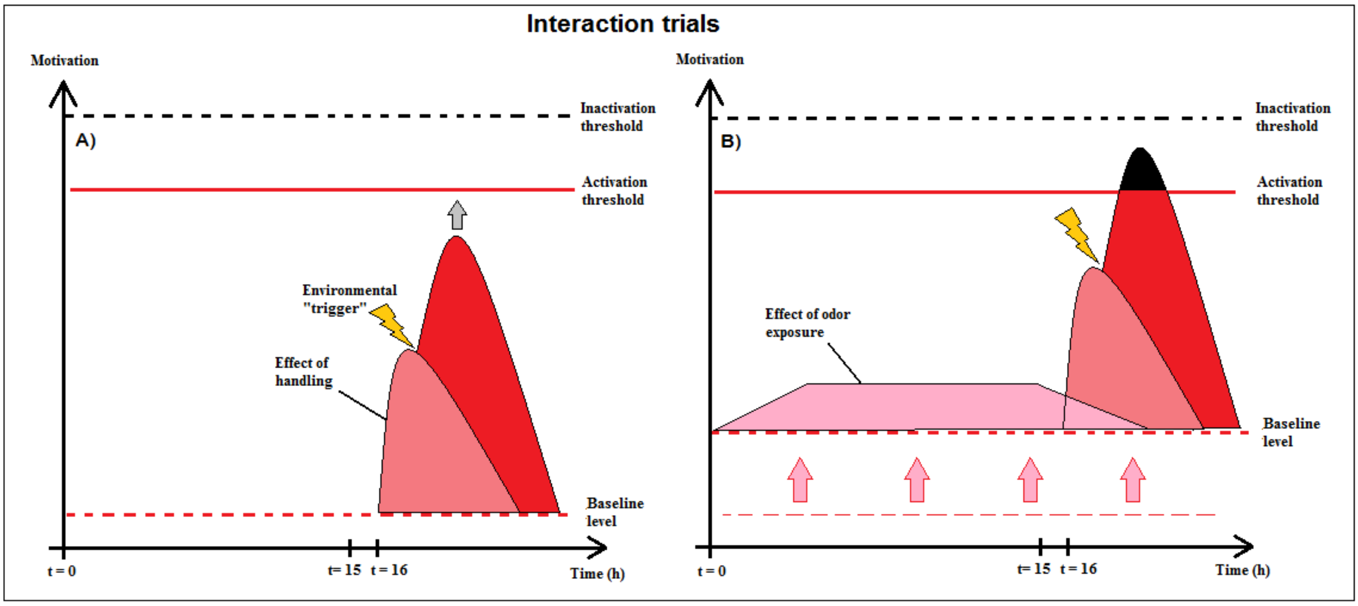

Figure 15 – Interaction trials. t=0 and t=15 is the beginning and end of odor exposure, while t=16 corresponds to the start of the observations. Stimulation above the activation threshold promotes a fright-fight response, while stimulation above the inactivation threshold represents acute stress compromising the lobsters coping ability and results in fright-flight (i.e. escapes, avoidance or freezing). **A:** Naïve lobsters had never experienced fright inducing predator odors, and thus had a low baseline fright-related motivation. Handling (environmental disturbance) prior to the observations did not promote motivation above the activation threshold, but the presence of an environmental trigger in the form of interactions/competition increased competitive motivation, although still at sub-threshold levels. **B:** Exposed lobsters had an elevated baseline fright-related motivation, and thus react to a weaker stimulus than the Naïve lobsters, or react more strongly to a stimulus of equal magnitude. Handling did not represent a strong enough stimulus by itself, but the presence of an environmental trigger add to the stress caused by odor exposure and handling, promoting motivation to reach activation threshold levels. Thus, when the Exposed lobsters were engaged in fights they had an activated fright-related motivational system, which resulted in fight behavior and establishment of dominance (i.e. they were more motivated to gain possession of shelter).

Frightful stimuli can induce both changes in behavioral motivation and a physiological stress response, where the behavioral response can persist for several days (Pickering et al. 1982; Utne & Bacchi 1997; Folkedal et al. 2010). Yet, prolonged activation of e.g. the fright-related motivational system is to my knowledge rarely observed. However, moving to a different motivational system; the aggressive motivation in male cichlids (*Haplochromis burtoni*) declines after stimulation to low levels with a half-life of seven days (Heiligenberg & Kramer 1972). Consequently, after one month only 6.25% of the initial increase in aggressive motivation remains, but in a competitive situation even this becomes relatively high if other individuals lack aggressive motivation. Similarly, if fright-related motivation was long-lived with prolonged halflife in the simulated release, this could explain why Exposed lobsters managed to increase their shelter occupancy with time after initially being at a disadvantage. Should this be the case it could represent a highly relevant method for training of juveniles prior to release, given that acclimation prior to release will prevent the initial disadvantage observed in the present study. See Appendix 6 for further elaboration on this explanation.

4.6 Concluding remarks

This study indicates a potential for manipulating lobster behavior through exposure with predator odors. Individual observations in a small test-environment with substrate and shelter did not reveal any differences between lobsters exposed and naïve to predator odors. Time in shelter could vary from 20 seconds to 14 minutes within each treatment, thus indicating variable fidelity to shelter although 87% of the lobsters did find shelter and showed some shelter manipulation. Considering the large individual variation in combination with presumably innate shelter-seeking behavior (van der Meeren 1993; James-Pirri & Cobb 1999) this percentage may not be improvable in a low complex experimental setting.

The paired interaction tests showed a positive effect of exposure to predator odor, and it seemed like competition was needed to reveal the differences between Exposed and Naïve lobsters. The odor exposure could have elevated the underlying fright-related motivation to gain shelter, which was only triggered when the lobster encountered a real threat. Alternatively, the chemical enrichment of the rearing environment and not the predator odors *per se*, may have led to development of more behaviorally plastic individuals better suited to cope with novel situations. Whichever may have caused the effect; this could increase post-release survival. Further studies should be performed to clarify if the effect comes from predator odors specifically or chemical enhancement in general.

The results from the simulated release were both the most unexpected and most interesting. The Exposed lobsters recovered from an initial disadvantage and increased shelter occupancy significantly during a 35 day period, implying that they had gained some advantage that became evident in the course of the experiment. This may represent the most interesting aspect to elaborate on in further studies; can prolonged exposure to predator odors affect lobsters for long periods, e.g. through a prolonged increase in motivation to compete for shelters? If the initial results are improved with acclimation prior to release and Exposed lobsters consistently perform better than Naïve lobsters, this would represent a potential means of low-cost space efficient predator training easily applicable in a hatchery setting. A more realistic approach with potential predators present in release experiments could bridge the experimental and full-scale release situations. A small scale release with use of divers or cameras to obtain information about their actual behavior in the wild could clarify if the effect of odor exposure was only artifacts of the rearing environment. The results so far do not yet merit a costly experimental release, and more small-scale studies should be performed to assess the consistency of these findings.

When training juveniles for increased predator avoidance, the most commonly applied paradigm is short-term aversive conditioning. Releases have not been carried out with conditioned lobsters. Some releases have been done with fish based on clear effects from laboratory experiments, but conditioning has not resulted in increased survival. The reasons for this are unknown. More research is needed to elucidate the mechanisms related to chemical stimuli and predator avoidance in lobster. Such knowledge is important both to further develop the lobster farming industry and to increase the understanding of the behavioral complexity in lobster juveniles. Indeed, if simply exposing lobsters to relevant predator odors for a prolonged time without using a conditioning paradigm have some potential of enhancing competitive behavior this should be investigated further.

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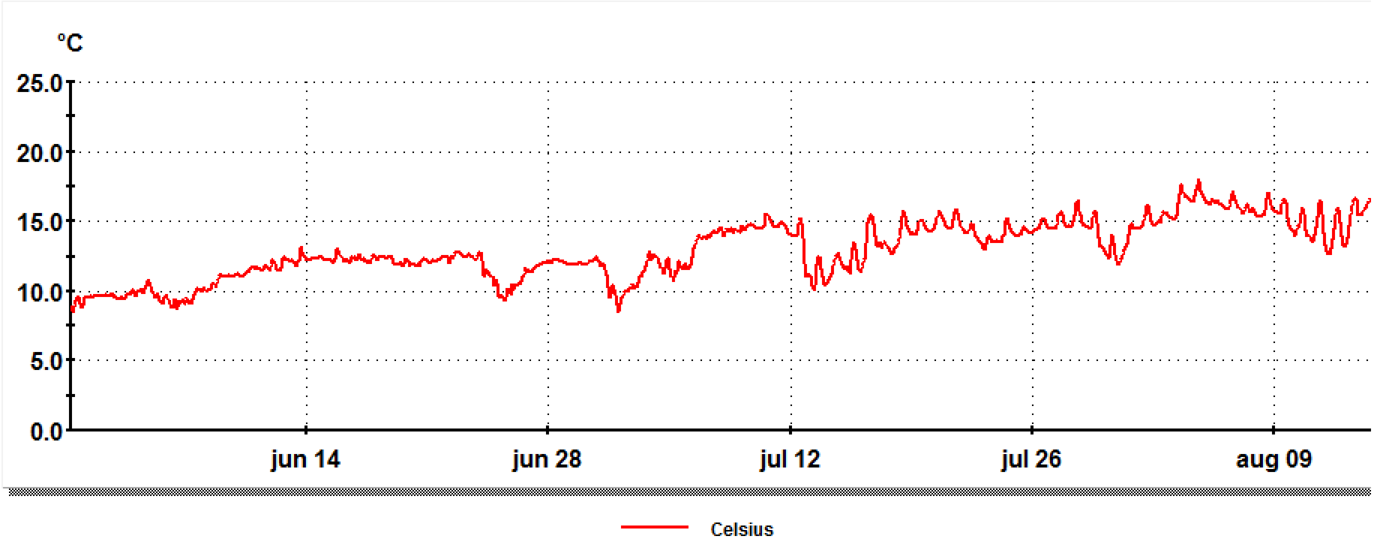
6. Appendices

Appendix 1 – Temperature:

Mean temperature from the start of Experiment 1 (8th of June) to the end of Experiment 3 (14th of August).



From:- 31 May 2011 08:03:39 To:- 14 August 2011 22:03:39

Appendix 2 - R-syntax:

2a) Analysis of size differences by Tukey HSD multiple comparisons test to see which treatments differed.

```
fit1.lm <- lm (CL ~ Treatment)

library (multcomp)

mc <- glht (fit1.lm, linfct= mcp (Treatment='Tukey'))

summary (mc)
```

2b) Experiment 1: Individual observations

i) Survival analysis on mean time to find shelter depending on NDT and Treatment. NDT= number of days in treatment, Treatment= A, B, C or D.

```
library (survival)

fit1.surv <- survreg (Surv (Time.find, Shelter.found) ~ CL + NDT*Treatment,
dist='weibull')

anova (fit1.surv)

summary (fit1.surv)
```

Default syntax for contrast analysis; *relevel* for each Treatment. This is the method used for contrast analyses on subsequent models as well.

```
Days <- NDT-4

Days <- NDT-8

Days <- NDT-12

Days <- NDT-16

Treatment1 <- relevel (Treatment, ref='X')  → X= treatment A, B, C or D

fit1a.surv <- survreg (Surv (Time.find, Shelter.found) ~ CL + Days*Treatment1,
dist='weibull')

summary (fit1a.surv)
```

ii) General behavior: Response variables were Box-Cox transformed and analyzed with a linear mixed effects model by the lme function in R. Predictor variables were NDT and Treatment.

```
library (nlme)

library (car)

p1 <- powerTransform (response)

Response <- bcPower (response, p1$lambda)

fit1.lme <- lme (Response ~ CL + NDT*Treatment, random=~+1|lobster, corr=corAR1(),
na.action='na.omit')

anova (fit1.lme)

summary (fit1.lme)
```

2c) Experiment 2: Interactions

i) Probability of Dominance was analyzed with a glmm by the glmmPQL function in R.

Full model including interaction between Odor and Photoperiod:

```
library (MASS)

fit1.glmm <- glmmPQL (Dominance ~ CL + Odor*Photoperiod, random=~+1|nr,
family=binomial)

anova.lme (fit1.glmm)

summary (fit1.glmm)
```

ii) Probability of initiating the first encounter was analyzed with a glmm. Odor and Photoperiod were used as predictor variables.

```
library(MASS)

fit3.glmm <- glmmPQL (Initiator ~ CL + Odor*Photoperiod, random=~+1|nr, binomial)

anova.lme (fit3.glmm)

summary (fit3.glmm)
```

iii) Individual behaviors. Values were Box-Cox transformed and analyzed with a linear mixed effects model by the lme function in R. Dominance as predictor and CL was included as a covariate.

```
library (nlme)

library (car)

p1 <- powerTransform (individual behavior)

Individual <- bcPower (individual behavior, p1$lambda)

individual.lme <- lme (Individual ~ CL + Dominance, random=~+1|nr)

anova.lme (indirect.lme)

summary (indirect.lme)
```

2d) Experiment 3 - Simulated release:

i) Probability of shelter occupancy was analyzed with a glmm by the glmmPQL function in R. Predictor variables were NDT (as a categorical predictor) and Odor exposure. Photoperiod was included as a random effects factor.

```
library (MASS)

Days<- as.factor (NDT)

fit1.glmm <- glmmPQL (cbind (success, failure) ~ Days*Odor,
random=~+1|photoperiod, cor=corAR1(), binomial)

anova.lme (fit1.glmm)

summary (fit1.glmm)
```

ii) Probability of injury and mortality was analyzed in the same way as probability of shelter occupancy:

```
library (MASS)

Days<- as.factor (NDT)

fit2.glmm <- glmmPQL (cbind (Injured/Dead, Healthy/Alive) ~ Days*Odor,
random=~+1|photoperiod, cor=corAR1(), binomial)

anova.lme(fit2.glmm)
```

Appendix 3 – Statistics: model output from Experiment 1

Table showing the model output from Experiment 1 (General behavior): mean response - differences between each treatment at each test-day. The model used is a linear mixed effects model on Bow-Cox transformed data.

Shelter dwelling	**4 days**	**8 days**	**12 days**	**16 days**
A vs B	t= -0.55 p= 0.58	t= 0.07 p= 0.95	t= 0.81 p= 0.42	t= 1.21 p= 0.23
A vs C	t= 0.6 p= 0.49	t= 0.55 p= 0.59	t= 0.25 p= 0.81	t= -0.036 p= 0.97
A vs D	t= 1.94 p= 0.054	t= 1.43 p= 0.15	t= 0.51 p= 0.61	t= -0.32 p= 0.75
B vs C	t= 1.20 p= 0.21	t= 0.50 p= 0.62	t= -0.56 p= 0.57	t= -1.29 p= 0.20
B vs D	t= 2.54 p= 0.012	t= 1.43 p= 0.15	t= -0.25 p= 0.80	t= -1.54 p= 0.13
C vs D	t= 1.36 p= 0.18	t= 1.00 p= 0.32	t= 0.30 p= 0.76	t= -0.31 p= 0.76
Roaming	**4 days**	**8 days**	**12 days**	**16 days**
A vs B	t= 0.87 p= 0.39	t= 0.39 p= 0.70	t= -0.28 p= 0.78	t= -0.75 p= 0.46
A vs C	t= -0.78 p= 0.44	t= -0.40 p= 0.69	t= 0.13 p= 0.90	t= 0.55 p= 0.58
A vs D	t= -1.48 p= 0.14	t= -1.02 p= 0.31	t= -0.28 p= 0.78	t= 0.39 p= 0.70
B vs C	t= -1.67 p= 0.097	t= -0.81 p= 0.42	t= 0.42 p= 0.68	t= 1.32 p= 0.19
B vs D	t= -2.39 p= 0.018	t= -1.45 p= 0.15	t= -0.018 p= 0.98	t= 1.13 p= 0.26
C vs D	t= -0.76 p= 0.45	t= -0.69 p= 0.49	t= -0.45 p= 0.66	t= -0.16 p= 0.87
Cheliped shuffles	**4 days**	**8 days**	**12 days**	**16 days**
A vs B	t= 0.24 p= 0.81	t= -1.12 p= 0.26	t= -2.47 p= 0.014	t= -3.24 p= 0.002
A vs C	t= 2.78 p= 0.006	t= 3.19 p= 0.002	t= 3.16 p= 0.002	t= 2.75 p= 0.007
A vs D	t= 2.70 p= 0.008	t= 2.45 p= 0.015	t= 1.84 p= 0.068	t= 1.11 p= 0.27
B vs C	t= 2.65 p= 0.009	t= 4.44 p= 0.000	t= 5.80 p= 0.000	t= 6.22 p= 0.000
B vs D	t= 2.58 p= 0.011	t= 3.64 p= 0.0004	t= 4.29 p= 0.000	t= 4.34 p= 0.000
C vs D	t= 0.005 p= 1.00	t= -0.65 p= 0.52	t= -1.29 p= 0.20	t= -1.67 p= 0.10
Climbing activity	**4 days**	**8 days**	**12 days**	**16 days**
A vs B	t= -1.68 p= 0.10	t= -1.32 p= 0.18	t= -0.62 p= 0.54	t= 0.062 p= 0.95

A vs C	t= 0.077 p= 0.94	t= 0.057 p= 0.95	t= 0.023 p= 0.98	t= -0.011 p= 0.99
A vs D	t= 0.032 p= 0.97	t= -0.41 p= 0.68	t= -0.88 p= 0.38	t= -1.12 p= 0.27
B vs C	t= 1.80 p= 0.075	t= 1.40 p= 0.16	t= 0.64 p= 0.52	t= -0.073 p= 0.94
B vs D	t= 1.62 p= 0.11	t= 0.80 p= 0.43	t= -0.35 p= 0.73	t= -1.20 p= 0.23
C vs D	t= -0.042 p= 0.97	t= -0.51 p= 0.61	t= -0.97 p= 0.33	t= -1.17 p= 0.24

Appendix 4 – Raw data:

Experiment 1: Individual observations

Category			
id	identifies the lobster	**inside**	dwelling
CL	size	**shuffle**	digging frequency
gr.	treatment	**climb**	climbing frequency
test	which observation	**roam**	time spent roaming
days	days in treatment	**dig.out**	digging outside shelter
find	time-to-shelter	**freeze**	time spent freezing
status.find	whether shelter is found		

id	CL	gr.	test	days	find	status	inside	shuffle	climb	roam	dig.out	freeze
D2	16,4	A	1	4	63	1	360	1,667	0,889	540	NA	NA
T3	12,6	A	1	4	338	1	562	2,883	NA	338	NA	NA
T2	14,8	A	1	4	497	1	403	2,233	1,569	497	NA	NA
T9	14	A	1	4	295	1	97	3,093	0,299	803	NA	NA
U1	15,4	A	1	4	52	1	848	1,840	NA	52	NA	NA
T6	16,8	A	1	4	84	1	283	0,848	NA	617	NA	NA
T5	14,9	A	1	4	65	1	81	1,481	0,952	819	NA	NA
J5	12,7	A	1	4	730	1	170	2,471	NA	332	398	NA
L8	15,1	A	1	4	72	1	828	1,667	NA	72	NA	NA
M5	10,8	A	1	4	77	1	823	0,437	NA	63	14	NA
M6	15	A	1	4	29	1	871	1,791	NA	29	NA	NA
M7	14,4	A	1	4	236	1	664	2,530	1,017	236	NA	NA
M8	15	A	1	4	542	1	357	0,168	0,221	543	NA	NA
M9	12,4	A	1	4	67	1	290	1,034	0,197	610	NA	NA
N5	15,1	A	1	4	147	1	753	2,072	NA	147	NA	NA
N6	14,9	A	1	4	446	1	454	1,322	1,995	421	25	NA
N8	14,9	A	1	4	116	1	784	1,301	1,552	116	NA	NA
N9	14,4	A	1	4	900	0	NA	NA	1,333	900	NA	NA
O5	11	A	1	4	201	1	699	0,944	1,194	201	NA	NA
O6	13,5	A	1	4	308	1	344	1,570	0,887	541	15	NA
O7	15,1	A	1	4	94	1	806	2,382	NA	94	NA	NA
O9	14,1	A	1	4	900	0	NA	NA	NA	44	856	NA
P5	12,7	A	1	4	287	1	613	1,272	NA	287	NA	NA
P6	16,2	A	1	4	900	0	NA	NA	1,200	900	NA	NA
P7	16,1	A	1	4	278	1	622	0,579	NA	278	NA	NA
P8	15,8	A	1	4	146	1	715	2,014	1,297	185	NA	NA
P9	13,9	A	1	4	189	1	451	1,064	0,267	449	NA	NA
Q5	12,6	A	1	4	129	1	644	1,211	NA	256	NA	NA
Q6	16,6	A	1	4	900	0	NA	NA	1,846	845	55	NA
Q7	16	A	1	4	900	0	NA	NA	1,438	584	316	NA
Q8	16,1	A	1	4	702	1	45	NA	0,797	602	193	NA
Q9	15	A	1	4	900	0	NA	NA	0,312	577	263	NA

R5	12,7	A	1	4	53	1	827	1,669	NA	73	NA	NA
R6	12,3	A	1	4	51	1	849	2,261	NA	51	NA	NA
R7	13,3	A	1	4	81	1	778	2,237	NA	122	NA	NA
R8	13,5	A	1	4	62	1	838	1,289	NA	62	NA	NA
R9	14,9	A	1	4	114	1	786	2,748	NA	114	NA	NA
S6	15	A	1	4	260	1	55	NA	1,278	845	NA	NA
S8	14,4	A	1	4	172	1	728	2,060	NA	172	NA	NA
S9	15,8	A	1	4	496	1	404	2,228	0,968	496	NA	NA
D2	16,4	A	2	8	738	1	21	2,857	1,553	541	338	NA
T3	12,6	A	2	8	94	1	806	2,680	NA	72	NA	22
T2	14,8	A	2	8	900	0	NA	NA	1,800	900	NA	NA
T9	14	A	2	8	900	0	NA	NA	0,600	900	NA	NA
U1	15,4	A	2	8	371	1	117	2,564	1,762	783	NA	NA
T6	16,8	A	2	8	76	1	524	2,405	0,169	354	NA	22
T5	14,9	A	2	8	225	1	95	1,895	1,043	805	NA	NA
J5	12,7	A	2	8	900	0	NA	NA	1,083	277	623	NA
L8	15,1	A	2	8	109	1	791	2,048	NA	109	NA	NA
M5	10,8	A	2	8	900	0	NA	NA	0,974	308	592	NA
M7	14,4	A	2	8	34	1	866	2,425	NA	34	NA	NA
M8	15	A	2	8	34	1	866	2,217	NA	34	NA	NA
M9	12,4	A	2	8	52	1	848	1,061	NA	52	NA	NA
N5	15,1	A	2	8	45	1	855	2,456	NA	45	NA	NA
N6	14,9	A	2	8	178	1	269	1,338	0,761	631	NA	NA
N8	14,9	A	2	8	900	0	NA	NA	2,067	900	NA	NA
N9	14,4	A	2	8	134	1	766	1,567	NA	114	NA	20
O5	11	A	2	8	242	1	658	1,185	0,293	205	NA	37
O9	14,1	A	2	8	97	1	803	2,466	NA	97	NA	NA
P5	12,7	A	2	8	71	1	829	1,448	NA	46	NA	25
P6	16,2	A	2	8	900	0	NA	NA	1,091	880	NA	NA
P7	16,1	A	2	8	149	1	751	1,278	NA	149	NA	NA
P8	15,8	A	2	8	255	1	645	2,698	1,882	255	NA	NA
P9	13,9	A	2	8	186	1	307	0,782	1,518	593	NA	NA
Q5	12,6	A	2	8	423	1	477	2,767	NA	423	NA	NA
Q6	16,6	A	2	8	900	0	NA	NA	2,667	900	NA	NA
Q7	16	A	2	8	276	1	441	2,721	1,307	459	NA	NA
Q8	16,1	A	2	8	900	0	NA	NA	0,781	384	516	NA
Q9	15	A	2	8	83	1	817	1,909	NA	83	NA	NA
R5	12,7	A	2	8	128	1	573	2,094	0,183	327	NA	NA
R6	12,3	A	2	8	29	1	79	2,278	1,348	623	198	NA
R7	13,3	A	2	8	108	1	792	2,197	NA	108	NA	NA
R8	13,5	A	2	8	133	1	767	0,782	NA	133	NA	NA
R9	14,9	A	2	8	33	1	773	3,260	NA	127	NA	NA
S6	15	A	2	8	19	1	117	4,615	NA	783	NA	NA
S8	14,4	A	2	8	41	1	859	2,026	NA	41	NA	NA
S9	15,8	A	2	8	110	1	737	1,791	1,840	163	NA	NA
D2	16,4	A	3	12	481	1	61	2,951	2,179	716	123	NA

T3	12,6	A	3	12	24	1	861	2,091	NA	39	NA	NA
T2	14,8	A	3	12	396	1	504	2,619	2,273	396	NA	NA
T6	16,8	A	3	12	282	1	618	1,262	NA	259	NA	23
T5	14,9	A	3	12	900	0	NA	NA	1,733	900	NA	NA
J5	12,7	A	3	12	67	1	734	1,717	NA	166	NA	NA
M5	10,8	A	3	12	109	1	791	1,290	NA	109	NA	NA
M7	14,4	A	3	12	96	1	804	2,239	0,625	96	NA	NA
M8	15	A	3	12	52	1	848	0,354	NA	52	NA	NA
M9	12,4	A	3	12	77	1	823	1,531	NA	77	NA	NA
N5	15,1	A	3	12	50	1	850	2,259	NA	50	NA	NA
N6	14,9	A	3	12	153	1	220	2,182	0,618	680	NA	NA
N8	14,9	A	3	12	900	0	NA	NA	1,295	880	20	NA
O5	11	A	3	12	66	1	820	1,244	NA	80	NA	NA
O6	13,5	A	3	12	196	1	490	1,469	1,543	350	NA	NA
O9	14,1	A	3	12	113	1	787	2,897	NA	113	NA	NA
P5	12,7	A	3	12	90	1	810	1,778	0,667	90	NA	NA
P6	16,2	A	3	12	112	1	718	2,173	NA	101	NA	21
P7	16,1	A	3	12	33	1	867	1,661	NA	33	NA	NA
P9	13,9	A	3	12	206	1	607	1,483	1,229	293	NA	NA
Q5	12,6	A	3	12	642	1	258	1,395	0,187	642	NA	NA
Q6	16,6	A	3	12	231	1	366	1,639	2,247	534	NA	NA
Q7	16	A	3	12	66	1	62	2,903	3,007	838	NA	NA
Q8	16,1	A	3	12	900	0	NA	NA	1,379	87	788	25
Q9	15	A	3	12	40	1	860	1,256	NA	40	NA	NA
R5	12,7	A	3	12	424	1	476	1,891	0,149	404	NA	20
R6	12,3	A	3	12	66	1	834	2,014	NA	66	NA	NA
R7	13,3	A	3	12	37	1	863	2,294	NA	37	NA	NA
R8	13,5	A	3	12	79	1	821	1,462	NA	79	NA	NA
R9	14,9	A	3	12	392	1	508	1,654	0,918	392	NA	NA
S6	15	A	3	12	100	1	341	1,056	0,966	559	NA	NA
S8	14,4	A	3	12	42	1	858	1,818	NA	42	NA	NA
S9	15,8	A	3	12	35	1	274	2,190	1,629	626	NA	NA
D2	16,4	A	4	16	900	0	NA	NA	2,667	900	NA	NA
T3	12,6	A	4	16	900	0	NA	NA	0,752	878	NA	22
T2	14,8	A	4	16	77	1	823	1,968	1,558	77	NA	NA
T6	16,8	A	4	16	315	1	585	1,641	0,571	315	NA	NA
T5	14,9	A	4	16	900	0	NA	NA	2,333	900	NA	NA
J5	12,7	A	4	16	20	1	130	0,923	0,619	485	285	NA
M5	10,8	A	4	16	122	1	778	1,542	NA	122	NA	NA
M7	14,4	A	4	16	106	1	794	2,343	0,566	106	NA	NA
M8	15	A	4	16	108	1	792	1,061	NA	108	NA	NA
M9	12,4	A	4	16	161	1	629	1,717	NA	271	NA	NA
N5	15,1	A	4	16	623	1	188	2,553	0,843	712	NA	NA
N6	14,9	A	4	16	445	1	187	1,604	2,020	713	NA	NA
N8	14,9	A	4	16	900	0	NA	NA	1,667	900	NA	NA
O5	11	A	4	16	61	1	688	1,221	0,283	212	NA	NA

O6	13,5	A	4	16	105	1	516	1,279	0,938	384	NA	NA
O7	15,1	A	4	16	130	1	770	2,104	0,462	130	NA	NA
O9	14,1	A	4	16	235	1	665	2,707	0,678	177	58	NA
P5	12,7	A	4	16	188	1	712	1,011	NA	188	NA	NA
P6	16,2	A	4	16	900	0	NA	NA	1,571	840	60	NA
P7	16,1	A	4	16	64	1	836	1,579	NA	64	NA	NA
P9	13,9	A	4	16	265	1	40	3,000	2,163	860	NA	NA
Q5	12,6	A	4	16	98	1	270	1,556	0,102	590	40	NA
Q6	16,6	A	4	16	900	0	NA	NA	1,533	900	NA	NA
Q7	16	A	4	16	252	1	528	1,591	2,419	372	NA	NA
Q9	15	A	4	16	36	1	864	1,319	NA	36	NA	NA
R5	12,7	A	4	16	209	1	8	NA	0,348	862	NA	30
R6	12,3	A	4	16	87	1	813	2,214	NA	87	NA	NA
R7	13,3	A	4	16	246	1	527	2,049	0,804	373	NA	NA
R9	14,9	A	4	16	70	1	209	1,435	1,216	691	NA	NA
S6	15	A	4	16	900	0	NA	NA	1,467	900	NA	NA
S8	14,4	A	4	16	254	1	582	1,959	0,377	318	NA	NA
S9	15,8	A	4	16	211	1	137	1,314	1,573	763	NA	NA
A6	12,1	B	1	4	900	0	NA	NA	0,317	567	333	NA
B6	10,5	B	1	4	620	1	280	1,286	0,401	598	NA	22
B7	12,4	B	1	4	417	1	483	1,366	0,752	319	67	31
B8	12,4	B	1	4	900	0	NA	NA	0,198	607	293	NA
B9	11,6	B	1	4	633	1	267	2,921	0,190	633	NA	NA
C6	14,2	B	1	4	110	1	754	2,069	0,822	146	NA	NA
C7	12,2	B	1	4	32	1	868	1,797	NA	32	NA	NA
C8	12,4	B	1	4	198	1	702	1,111	NA	186	12	NA
C9	13,3	B	1	4	108	1	792	1,439	NA	108	NA	NA
D6	11,6	B	1	4	361	1	14	NA	0,407	589	297	NA
D7	12	B	1	4	48	1	852	0,915	NA	48	NA	NA
D8	10,8	B	1	4	382	1	518	1,737	NA	382	NA	NA
D9	13	B	1	4	61	1	839	0,715	NA	61	NA	NA
E6	15	B	1	4	194	1	706	1,870	0,619	194	NA	NA
E7	13,3	B	1	4	354	1	17	NA	0,316	759	124	NA
E8	15,1	B	1	4	127	1	773	2,406	NA	95	NA	32
F6	13	B	1	4	53	1	847	1,063	1,132	53	NA	NA
F7	13,6	B	1	4	93	1	614	1,661	0,210	286	NA	NA
F9	11,8	B	1	4	900	0	NA	NA	0,200	900	NA	NA
G6	14	B	1	4	229	1	671	1,610	1,048	229	NA	NA
G7	15,1	B	1	4	900	0	NA	NA	2,000	900	NA	NA
G8	15,6	B	1	4	174	1	726	1,901	NA	174	NA	NA
G9	14,6	B	1	4	308	1	592	1,419	0,390	308	NA	NA
T1	15,5	B	1	4	566	1	334	3,054	1,060	566	NA	NA
T10	15,3	B	1	4	58	1	842	2,280	NA	58	NA	NA
U2	14,1	B	1	4	623	1	205	2,049	0,259	695	NA	NA
T4	15	B	1	4	165	1	706	3,059	NA	194	NA	NA
H6	14,8	B	1	4	900	0	NA	NA	0,820	366	534	NA

H9	15	B	1	4	215	1	685	1,489	0,279	215	NA	NA
I6	14,7	B	1	4	900	0	NA	NA	1,400	900	NA	NA
I7	15,7	B	1	4	900	0	NA	NA	0,602	598	302	NA
I8	15	B	1	4	62	1	839	1,073	NA	61	NA	NA
I9	15,2	B	1	4	445	1	110	2,182	0,155	776	14	NA
J6	14,6	B	1	4	214	1	686	1,574	0,561	214	NA	NA
J7	14,4	B	1	4	191	1	709	2,285	0,373	161	NA	30
J8	15,9	B	1	4	334	1	311	3,280	1,222	589	NA	NA
J9	14,4	B	1	4	151	1	749	2,163	NA	151	NA	NA
K6	14	B	1	4	336	1	564	1,064	0,759	316	NA	20
K7	15,1	B	1	4	722	1	178	5,730	0,332	722	NA	NA
K8	14,7	B	1	4	48	1	852	1,127	NA	48	NA	NA
K9	14,3	B	1	4	203	1	697	0,603	1,182	203	NA	NA
L7	16,6	B	1	4	167	1	662	1,813	NA	238	NA	NA
L9	15	B	1	4	853	1	47	2,553	0,318	755	NA	98
A6	12,1	B	2	8	213	1	687	2,271	0,282	213	NA	NA
B7	12,4	B	2	8	93	1	807	0,669	NA	93	NA	NA
B8	12,4	B	2	8	900	0	NA	NA	1,159	466	434	NA
B9	11,6	B	2	8	797	1	103	4,078	0,320	749	NA	48
C6	14,2	B	2	8	79	1	821	1,827	NA	79	NA	NA
C7	12,2	B	2	8	900	0	NA	NA	0,430	838	62	NA
C8	12,4	B	2	8	900	0	NA	NA	1,067	900	NA	NA
C9	13,3	B	2	8	159	1	721	1,248	1,006	179	NA	NA
D6	11,6	B	2	8	37	1	863	1,390	NA	37	NA	NA
D7	12	B	2	8	63	1	837	0,717	NA	63	NA	NA
D8	10,8	B	2	8	900	0	NA	NA	0,333	900	NA	NA
D9	13	B	2	8	98	1	802	0,599	NA	98	NA	NA
E6	15	B	2	8	194	1	706	1,955	0,619	194	NA	NA
E7	13,3	B	2	8	454	1	394	0,914	0,259	464	42	NA
E8	15,1	B	2	8	145	1	755	2,066	NA	125	NA	20
F6	13	B	2	8	165	1	735	0,898	0,364	165	NA	NA
F7	13,6	B	2	8	330	1	570	1,895	0,608	296	34	NA
F9	11,8	B	2	8	260	1	610	0,590	0,207	290	NA	NA
G6	14	B	2	8	287	1	613	0,881	2,091	287	NA	NA
G7	15,1	B	2	8	720	1	150	0,400	2,000	750	NA	NA
G8	15,6	B	2	8	186	1	714	2,773	0,968	186	NA	NA
G9	14,6	B	2	8	96	1	804	2,537	NA	96	NA	NA
T1	15,5	B	2	8	900	0	NA	NA	0,667	900	NA	NA
T10	15,3	B	2	8	215	1	685	1,664	0,279	215	NA	NA
U2	14,1	B	2	8	475	1	312	3,462	0,408	588	NA	NA
T4	15	B	2	8	104	1	796	1,583	NA	104	NA	NA
H6	14,8	B	2	8	479	1	421	2,138	0,570	421	58	NA
H9	15	B	2	8	53	1	847	0,779	NA	53	NA	NA
I6	14,7	B	2	8	900	0	NA	NA	1,533	900	NA	NA
I7	15,7	B	2	8	370	1	450	2,267	0,133	450	NA	NA
I8	15	B	2	8	263	1	157	1,911	0,323	743	NA	NA

I9	15,2	B	2	8	244	1	656	2,287	NA	218	NA	26
J6	14,6	B	2	8	485	1	415	1,880	0,654	459	NA	26
J7	14,4	B	2	8	142	1	758	1,741	0,423	142	NA	NA
J8	15,9	B	2	8	39	1	336	2,679	1,069	449	NA	115
K6	14	B	2	8	844	1	56	1,071	0,427	844	NA	NA
K7	15,1	B	2	8	830	1	70	5,143	1,736	795	35	NA
K8	14,7	B	2	8	129	1	771	0,700	NA	129	NA	NA
K9	14,3	B	2	8	38	1	862	0,626	1,579	38	NA	NA
L7	16,6	B	2	8	51	1	828	1,159	NA	72	NA	NA
L9	15	B	2	8	400	1	500	1,800	0,300	400	NA	NA
A6	12,1	B	3	12	221	0	245	2,449	0,824	655	NA	NA
B7	12,4	B	3	12	82	1	818	1,247	NA	82	NA	NA
B9	11,6	B	3	12	124	1	776	1,237	NA	124	NA	NA
C6	14,2	B	3	12	134	1	766	1,645	1,791	134	NA	NA
C7	12,2	B	3	12	194	1	706	0,765	NA	194	NA	NA
C8	12,4	B	3	12	48	1	685	1,051	1,395	215	NA	NA
C9	13,3	B	3	12	180	1	720	1,250	1,000	180	NA	NA
D6	11,6	B	3	12	496	1	404	3,119	0,395	456	40	NA
D7	12	B	3	12	900	0	NA	NA	2,118	878	22	NA
D8	10,8	B	3	12	141	1	759	1,660	NA	141	NA	NA
D9	13	B	3	12	77	1	823	0,656	NA	77	NA	NA
E6	15	B	3	12	148	1	752	1,436	NA	148	NA	NA
E7	13,3	B	3	12	411	1	489	0,736	0,146	411	NA	NA
E8	15,1	B	3	12	153	1	747	1,847	0,392	153	NA	NA
F6	13	B	3	12	64	1	836	0,646	NA	64	NA	NA
F7	13,6	B	3	12	188	1	712	1,264	0,385	156	32	NA
F9	11,8	B	3	12	679	1	221	1,900	1,149	679	NA	NA
G6	14	B	3	12	221	1	679	NA	NA	221	NA	NA
G7	15,1	B	3	12	900	0	NA	NA	3,733	900	NA	NA
G8	15,6	B	3	12	54	1	823	2,552	0,779	77	NA	NA
G9	14,6	B	3	12	183	1	717	1,841	NA	183	NA	NA
T1	15,5	B	3	12	605	1	295	3,051	0,496	605	NA	NA
T10	15,3	B	3	12	107	1	733	1,228	0,561	107	NA	NA
U2	14,1	B	3	12	336	1	177	2,034	0,664	723	NA	NA
T4	15	B	3	12	213	1	687	2,271	NA	213	NA	NA
H6	14,8	B	3	12	469	1	431	2,367	1,244	434	35	NA
H9	15	B	3	12	900	0	NA	NA	3,000	900	NA	NA
I6	14,7	B	3	12	588	1	340	0,176	1,500	560	NA	NA
I7	15,7	B	3	12	78	1	743	1,777	0,382	157	NA	NA
I9	15,2	B	3	12	53	1	847	0,921	NA	53	NA	NA
J6	14,6	B	3	12	262	1	567	2,116	1,261	333	NA	NA
J7	14,4	B	3	12	412	1	488	1,352	1,748	412	NA	NA
J8	15,9	B	3	12	111	1	729	2,716	0,541	111	NA	NA
K6	14	B	3	12	59	1	685	NA	NA	215	NA	NA
K7	15,1	B	3	12	722	1	178	2,360	2,327	722	NA	NA
K8	14,7	B	3	12	67	1	833	1,297	NA	45	NA	22

K9	14,3	B	3	12	134	1	766	0,705	1,791	134	NA	NA
L7	16,6	B	3	12	114	1	786	1,298	0,526	114	NA	NA
L9	15	B	3	12	156	1	744	1,935	NA	136	NA	20
A6	12,1	B	4	16	226	1	566	2,120	0,188	320	14	NA
B7	12,4	B	4	16	42	1	858	0,839	NA	42	NA	NA
B9	11,6	B	4	16	117	1	783	1,533	NA	117	NA	NA
C6	14,2	B	4	16	70	1	830	1,446	NA	70	NA	NA
C7	12,2	B	4	16	258	1	642	1,776	NA	230	NA	28
C8	12,4	B	4	16	900	0	NA	NA	0,733	900	NA	NA
C9	13,3	B	4	16	88	1	812	0,739	NA	88	NA	NA
D6	11,6	B	4	16	245	1	655	2,107	0,837	215	NA	30
D8	10,8	B	4	16	672	1	198	1,515	0,533	675	27	NA
D9	13	B	4	16	80	1	820	0,512	NA	80	NA	NA
E7	13,3	B	4	16	538	1	362	0,829	0,223	538	NA	NA
E8	15,1	B	4	16	403	1	497	1,690	1,042	403	NA	NA
F6	13	B	4	16	177	1	723	1,245	1,017	177	NA	NA
F7	13,6	B	4	16	138	1	762	0,866	0,435	138	NA	NA
F9	11,8	B	4	16	516	1	384	0,625	0,814	516	NA	NA
G7	15,1	B	4	16	900	0	NA	NA	4,867	900	NA	NA
G8	15,6	B	4	16	388	1	512	2,695	0,619	388	NA	NA
G9	14,6	B	4	16	262	1	638	1,411	0,458	262	NA	NA
T1	15,5	B	4	16	172	1	728	1,319	NA	172	NA	NA
T10	15,3	B	4	16	215	1	685	1,401	NA	215	NA	NA
T4	15	B	4	16	145	1	755	2,623	NA	145	NA	NA
H6	14,8	B	4	16	178	1	722	2,078	0,337	178	NA	NA
H9	15	B	4	16	777	1	123	0,488	3,475	777	NA	NA
I6	14,7	B	4	16	900	0	NA	NA	2,000	900	NA	NA
I7	15,7	B	4	16	146	1	663	1,357	NA	237	NA	NA
I9	15,2	B	4	16	388	1	512	1,172	0,330	364	24	NA
J6	14,6	B	4	16	71	1	829	1,158	1,690	71	NA	NA
J7	14,4	B	4	16	248	1	652	1,564	1,210	248	NA	NA
K6	14	B	4	16	285	1	575	0,209	NA	325	NA	NA
K7	15,1	B	4	16	472	1	428	2,243	1,017	472	NA	NA
K8	14,7	B	4	16	34	1	866	0,762	NA	34	NA	NA
K9	14,3	B	4	16	184	1	307	1,368	2,327	593	NA	NA
L7	16,6	B	4	16	319	1	581	1,343	NA	319	NA	NA
L9	15	B	4	16	294	1	606	1,683	0,408	294	NA	NA
U1	13,7	C	1	4	28	1	872	3,784	NA	28	NA	NA
T7	15,2	C	1	4	305	1	595	2,420	0,984	305	NA	NA
I2	12	C	1	4	157	1	743	2,342	0,382	157	NA	NA
I3	13	C	1	4	89	1	811	0,296	0,674	89	NA	NA
I4	12,4	C	1	4	24	1	876	2,055	NA	24	NA	NA
J1	13,5	C	1	4	172	1	728	2,143	0,698	172	NA	NA
J2	13,8	C	1	4	115	1	635	1,984	NA	265	NA	NA
J3	13,7	C	1	4	49	1	819	2,418	NA	81	NA	NA
J4	11,9	C	1	4	270	1	630	2,381	NA	225	15	30

K1	14	C	1	4	40	1	860	1,674	NA	40	NA	NA
K2	12,4	C	1	4	266	1	634	1,420	NA	95	171	NA
K3	13,1	C	1	4	498	1	402	2,687	0,391	460	38	NA
L1	13,9	C	1	4	127	1	773	1,552	NA	127	NA	NA
L2	12,3	C	1	4	77	1	823	1,021	NA	77	NA	NA
L3	11,9	C	1	4	71	1	829	0,507	NA	71	NA	NA
L4	14,7	C	1	4	133	1	752	3,271	NA	148	NA	NA
M1	10,8	C	1	4	154	1	746	2,413	NA	154	NA	NA
M4	12,5	C	1	4	20	1	880	2,795	NA	20	NA	NA
N1	12,2	C	1	4	47	1	853	2,110	NA	47	NA	NA
N2	12	C	1	4	314	1	586	3,276	0,573	314	NA	NA
N4	11,8	C	1	4	531	1	369	2,764	1,695	531	NA	NA
N7	14	C	1	4	75	1	825	1,309	0,800	75	NA	NA
O1	11,8	C	1	4	82	1	610	3,049	0,207	290	NA	NA
O2	14,4	C	1	4	366	1	452	2,920	1,339	448	NA	NA
O4	11,1	C	1	4	355	1	545	1,321	0,990	303	32	20
P1	13,7	C	1	4	103	1	797	1,129	NA	103	NA	NA
P3	12	C	1	4	502	1	398	3,317	0,598	502	NA	NA
P4	12,1	C	1	4	163	1	737	1,791	NA	163	NA	NA
Q2	12,2	C	1	4	709	1	191	0,942	2,609	115	566	28
Q3	13,6	C	1	4	900	0	NA	NA	NA	424	476	NA
Q4	12,7	C	1	4	900	0	NA	NA	1,217	493	356	NA
R1	16,1	C	1	4	214	1	234	4,615	0,991	666	NA	NA
R2	12,5	C	1	4	852	1	48	3,750	0,327	733	97	22
R3	13	C	1	4	84	1	816	2,426	NA	84	NA	NA
R4	15,2	C	1	4	550	1	245	2,694	0,275	655	NA	NA
S1	15,3	C	1	4	58	1	842	1,995	NA	58	NA	NA
S2	15,3	C	1	4	900	0	NA	NA	1,133	900	NA	NA
S3	12,2	C	1	4	144	1	756	2,540	NA	144	NA	NA
S4	13	C	1	4	270	1	630	1,810	0,222	270	NA	NA
S5	12,1	C	1	4	50	1	850	0,776	1,200	50	NA	NA
U1	13,7	C	2	8	101	1	799	3,379	NA	101	NA	NA
T7	15,2	C	2	8	535	1	230	2,348	0,716	670	NA	NA
I1	12,8	C	2	8	900	0	NA	NA	0,078	765	40	95
I2	12	C	2	8	900	0	NA	NA	0,375	480	420	NA
I3	13	C	2	8	480	1	420	1,857	0,271	442	38	NA
I4	12,4	C	2	8	24	1	806	0,596	NA	94	NA	NA
J1	13,5	C	2	8	681	1	219	2,466	1,101	654	NA	27
J2	13,8	C	2	8	883	1	17	3,529	0,747	883	NA	NA
J3	13,7	C	2	8	38	1	740	2,351	NA	160	NA	NA
J4	11,9	C	2	8	370	1	530	3,057	NA	282	NA	88
K1	14	C	2	8	900	0	NA	NA	1,000	900	NA	NA
K2	12,4	C	2	8	64	1	766	0,705	0,896	134	NA	NA
K3	13,1	C	2	8	110	1	790	1,063	0,545	110	NA	NA
L1	13,9	C	2	8	900	0	NA	NA	1,068	337	563	NA
L2	12,3	C	2	8	43	1	742	1,051	NA	158	NA	NA

L3	11,9	C	2	8	127	1	773	0,466	NA	127	NA	NA
L4	14,7	C	2	8	76	1	540	2,889	0,500	360	NA	NA
M4	12,5	C	2	8	177	1	723	1,660	NA	177	NA	NA
N1	12,2	C	2	8	390	1	510	2,941	1,290	279	111	NA
N2	12	C	2	8	60	1	822	3,285	NA	78	NA	NA
N4	11,8	C	2	8	382	1	518	2,780	0,785	382	NA	NA
N7	14	C	2	8	77	1	823	1,239	0,779	77	NA	NA
O1	11,8	C	2	8	176	1	31	3,871	0,138	869	NA	NA
O2	14,4	C	2	8	900	0	NA	NA	3,000	900	NA	NA
O4	11,1	C	2	8	251	1	649	1,294	1,283	187	64	NA
P1	13,7	C	2	8	66	1	834	1,583	0,909	66	NA	NA
P3	12	C	2	8	117	1	180	3,667	0,750	720	NA	NA
P4	12,1	C	2	8	161	1	576	2,396	0,235	255	69	NA
Q2	12,2	C	2	8	566	1	334	2,156	0,742	566	NA	NA
Q3	13,6	C	2	8	392	1	508	3,071	0,153	392	NA	NA
Q4	12,7	C	2	8	900	0	NA	NA	2,432	814	64	22
R1	16,1	C	2	8	47	1	165	4,000	1,714	735	NA	NA
R2	12,5	C	2	8	129	1	771	1,712	0,465	129	NA	NA
R3	13	C	2	8	49	1	733	2,374	NA	167	NA	NA
R4	15,2	C	2	8	900	0	NA	NA	0,510	824	NA	76
S1	15,3	C	2	8	267	1	633	4,265	1,124	267	NA	NA
S2	15,3	C	2	8	900	0	NA	NA	1,247	866	NA	34
S3	12,2	C	2	8	144	1	756	3,095	0,417	144	NA	NA
S4	13	C	2	8	269	1	631	2,377	0,223	269	NA	NA
S5	12,1	C	2	8	20	1	811	1,110	NA	89	NA	NA
U2	13,7	C	3	12	51	1	761	3,075	NA	139	NA	NA
T7	15,2	C	3	12	224	1	676	2,130	0,804	224	NA	NA
I1	12,8	C	3	12	285	1	615	1,756	NA	285	NA	NA
I2	12	C	3	12	73	1	827	1,959	NA	73	NA	NA
I3	13	C	3	12	295	1	605	1,983	NA	295	NA	NA
I4	12,4	C	3	12	25	1	875	1,303	NA	25	NA	NA
J1	13,5	C	3	12	242	1	658	2,827	2,479	242	NA	NA
J2	13,8	C	3	12	900	0	NA	NA	0,267	900	NA	NA
J3	13,7	C	3	12	86	1	740	2,432	NA	160	NA	NA
J4	11,9	C	3	12	99	1	801	2,322	NA	99	NA	NA
K1	14	C	3	12	95	1	745	1,772	NA	155	NA	NA
K2	12,4	C	3	12	51	1	849	1,696	1,176	51	NA	NA
K3	13,1	C	3	12	31	1	869	2,140	NA	31	NA	NA
L1	13,9	C	3	12	900	0	NA	NA	1,292	743	157	NA
L2	12,3	C	3	12	28	1	754	1,273	3,158	38	108	NA
L3	11,9	C	3	12	102	1	798	0,602	NA	102	NA	NA
L4	14,7	C	3	12	181	1	719	3,255	NA	181	NA	NA
M1	10,8	C	3	12	338	1	562	1,281	0,612	294	44	NA
M4	12,5	C	3	12	334	1	566	2,756	0,436	275	25	34
N1	12,2	C	3	12	153	1	747	3,213	0,392	153	NA	NA
N2	12	C	3	12	26	1	839	3,647	NA	61	NA	NA

N4	11,8	C	3	12	605	1	51	2,353	1,625	849	NA	NA
N7	14	C	3	12	34	1	866	0,624	NA	34	NA	NA
O1	11,8	C	3	12	189	1	44	2,727	0,370	811	NA	45
O2	14,4	C	3	12	581	1	319	3,950	2,262	557	24	NA
O4	11,1	C	3	12	155	1	745	1,530	0,387	155	NA	NA
P1	13,7	C	3	12	209	1	691	1,476	0,574	209	NA	NA
P3	12	C	3	12	25	1	773	3,648	NA	127	NA	NA
P4	12,1	C	3	12	252	1	648	2,593	0,238	252	NA	NA
Q2	12,2	C	3	12	661	1	239	2,510	0,343	525	50	86
Q3	13,6	C	3	12	247	1	653	2,848	NA	247	NA	NA
Q4	12,7	C	3	12	900	0	NA	NA	2,400	900	22	NA
R1	16,1	C	3	12	108	1	298	3,020	1,894	602	NA	NA
R2	12,5	C	3	12	462	1	438	1,918	0,548	438	NA	24
R3	13	C	3	12	95	1	583	2,573	NA	317	NA	NA
R4	15,2	C	3	12	267	1	129	2,791	NA	771	NA	NA
S1	15,3	C	3	12	203	1	697	4,562	0,296	203	NA	NA
S2	15,3	C	3	12	679	1	221	1,900	1,944	679	NA	NA
S3	12,2	C	3	12	217	1	683	2,899	0,829	217	NA	NA
S4	13	C	3	12	134	1	766	2,115	NA	134	NA	NA
S5	12,1	C	3	12	93	1	807	1,115	NA	93	NA	NA
U2	13,7	C	4	16	54	1	846	3,121	NA	54	NA	NA
T7	15,2	C	4	16	780	1	120	3,500	1,308	780	NA	NA
I1	12,8	C	4	16	365	1	535	0,897	NA	343	NA	22
I2	12	C	4	16	402	1	5	NA	0,737	895	NA	NA
I3	13	C	4	16	105	1	795	1,208	NA	105	NA	NA
I4	12,4	C	4	16	56	1	759	0,870	0,851	141	NA	NA
J1	13,5	C	4	16	237	1	302	1,589	1,045	574	NA	24
J2	13,8	C	4	16	900	0	NA	NA	0,414	869	NA	31
J3	13,7	C	4	16	51	1	733	2,374	NA	167	NA	NA
J4	11,9	C	4	16	357	1	305	1,770	NA	501	NA	94
K1	14	C	4	16	80	1	541	1,664	2,340	359	NA	NA
K2	12,4	C	4	16	200	1	700	1,800	NA	200	NA	NA
K3	13,1	C	4	16	133	1	767	2,034	0,902	133	NA	NA
L1	13,9	C	4	16	117	1	783	1,686	NA	85	NA	32
L2	12,3	C	4	16	832	1	68	1,765	0,721	832	NA	NA
L3	11,9	C	4	16	38	1	862	0,487	NA	38	NA	NA
L4	14,7	C	4	16	110	1	790	2,962	NA	110	NA	NA
M1	10,8	C	4	16	73	1	827	2,031	NA	73	NA	NA
M4	12,5	C	4	16	377	1	523	3,442	0,183	327	NA	50
N1	12,2	C	4	16	900	0	NA	NA	1,263	665	235	NA
N2	12	C	4	16	69	1	684	3,860	0,556	216	NA	NA
N7	14	C	4	16	84	1	816	1,029	NA	84	NA	NA
O2	14,4	C	4	16	465	1	49	2,449	1,763	851	NA	NA
O4	11,1	C	4	16	900	0	NA	NA	1,061	735	145	20
P1	13,7	C	4	16	218	1	682	1,496	0,550	218	NA	NA
P3	12	C	4	16	75	1	794	2,267	NA	106	NA	NA

P4	12,1	C	4	16	102	1	307	2,932	0,788	533	60	NA
Q2	12,2	C	4	16	598	1	302	3,179	0,111	542	NA	56
Q3	13,6	C	4	16	33	1	608	1,875	0,616	292	NA	NA
Q4	12,7	C	4	16	300	1	NA	NA	3,533	900	NA	NA
R1	16,1	C	4	16	740	1	20	3,000	2,114	880	NA	NA
R2	12,5	C	4	16	243	1	657	2,648	0,861	209	NA	34
R3	13	C	4	16	68	1	832	2,091	NA	68	NA	NA
R4	15,2	C	4	16	291	1	93	2,581	NA	760	NA	47
S1	15,3	C	4	16	356	1	544	4,301	0,557	323	NA	33
S2	15,3	C	4	16	420	1	25	NA	1,029	875	NA	NA
S3	12,2	C	4	16	900	0	NA	NA	1,375	480	420	NA
S4	13	C	4	16	127	1	773	2,018	0,945	127	NA	NA
S5	12,1	C	4	16	900	0	NA	NA	0,966	497	403	NA
A1	13	D	1	4	217	1	683	0,878	NA	217	NA	NA
A2	11,8	D	1	4	195	1	705	1,362	0,308	195	NA	NA
A3	12,2	D	1	4	257	1	643	2,706	NA	257	NA	NA
A4	10,8	D	1	4	608	1	292	1,849	0,888	608	NA	NA
A5	13	D	1	4	55	1	845	2,201	NA	55	NA	NA
A7	13,6	D	1	4	307	1	593	2,327	NA	307	NA	NA
A9	12,2	D	1	4	165	1	735	2,694	1,091	165	NA	NA
B1	12,7	D	1	4	900	0	NA	NA	0,233	258	533	109
B2	13,6	D	1	4	102	1	798	2,481	1,176	102	NA	NA
B3	12	D	1	4	174	1	726	1,653	0,345	174	NA	NA
B4	11,8	D	1	4	216	1	684	1,053	NA	216	NA	NA
B5	12,5	D	1	4	900	0	NA	NA	0,660	636	264	NA
C1	14,2	D	1	4	116	1	784	2,985	NA	116	NA	NA
C2	14	D	1	4	99	1	766	1,645	NA	134	NA	NA
C3	12,7	D	1	4	49	1	851	1,974	NA	49	NA	NA
C4	11	D	1	4	432	1	468	2,692	NA	307	125	NA
C5	12,2	D	1	4	199	1	701	2,140	0,905	199	NA	NA
D3	11,8	D	1	4	204	1	696	1,897	0,588	204	NA	NA
D4	12,5	D	1	4	143	1	757	1,823	NA	143	NA	NA
D5	13	D	1	4	468	1	432	2,083	0,769	468	NA	NA
E1	13,2	D	1	4	139	1	761	2,050	NA	139	NA	NA
E2	12,5	D	1	4	50	1	850	1,906	NA	50	NA	NA
E3	11,6	D	1	4	121	1	197	3,655	0,153	392	311	NA
E4	13,3	D	1	4	199	1	701	2,225	NA	199	NA	NA
E5	12,7	D	1	4	96	1	804	1,642	NA	96	NA	NA
F1	12,6	D	1	4	204	1	696	2,414	0,978	184	20	NA
F2	10,8	D	1	4	216	1	684	1,228	NA	216	NA	NA
F3	11,6	D	1	4	170	1	730	3,288	NA	170	NA	NA
F4	15,8	D	1	4	900	0	NA	NA	1,933	900	NA	NA
F5	13,6	D	1	4	62	1	838	1,933	0,968	62	NA	NA
G1	12,8	D	1	4	44	1	856	1,752	NA	44	NA	NA
G3	11,8	D	1	4	134	1	766	1,645	NA	134	NA	NA
G4	12,9	D	1	4	72	1	828	2,101	0,833	72	NA	NA

G5	15	D	1	4	70	1	830	1,807	NA	70	NA	NA
T8	14	D	1	4	70	1	830	2,964	NA	42	NA	28
H1	12,5	D	1	4	194	1	706	2,125	NA	194	NA	NA
H2	13	D	1	4	44	1	856	1,612	NA	44	NA	NA
H3	12,6	D	1	4	208	1	692	1,734	0,759	158	50	NA
H4	11,9	D	1	4	103	1	797	1,656	NA	103	NA	NA
H5	13,6	D	1	4	123	1	777	2,085	NA	123	NA	NA
A1	13	D	2	8	129	1	771	0,856	NA	129	NA	NA
A2	11,8	D	2	8	97	1	503	2,147	NA	97	NA	NA
A3	12,2	D	2	8	900	0	NA	NA	1,065	676	178	46
A4	10,8	D	2	8	522	1	41	2,927	0,838	859	NA	NA
A5	13	D	2	8	119	1	781	2,766	1,008	119	NA	NA
A7	13,6	D	2	8	900	0	NA	NA	NA	611	232	57
A9	12,2	D	2	8	525	1	375	3,200	0,343	525	NA	NA
B1	12,7	D	2	8	900	0	NA	NA	0,508	708	164	28
B2	13,6	D	2	8	80	1	820	2,415	NA	80	NA	NA
B3	12	D	2	8	89	1	811	2,219	NA	89	NA	NA
B4	11,8	D	2	8	37	1	709	1,269	0,628	191	NA	NA
B5	12,5	D	2	8	672	1	228	4,474	1,319	546	126	NA
C1	14,2	D	2	8	64	1	836	2,656	NA	64	NA	NA
C2	14	D	2	8	122	1	778	2,082	NA	122	NA	NA
C3	12,7	D	2	8	57	1	843	0,996	NA	57	NA	NA
C4	11	D	2	8	253	1	647	2,318	NA	198	NA	55
C5	12,2	D	2	8	81	1	819	1,612	NA	81	NA	NA
D3	11,8	D	2	8	154	1	746	0,965	0,432	139	15	NA
D4	12,5	D	2	8	281	1	619	1,551	0,269	223	58	NA
D5	13	D	2	8	264	1	636	2,075	0,517	232	NA	32
E1	13,2	D	2	8	97	1	803	2,092	NA	72	NA	25
E2	12,5	D	2	8	89	1	431	1,531	0,128	469	NA	NA
E3	11,6	D	2	8	900	0	NA	NA	0,116	516	364	20
E4	13,3	D	2	8	68	1	832	2,308	0,882	68	NA	NA
E5	12,7	D	2	8	405	1	282	1,064	0,097	618	NA	NA
F3	11,6	D	2	8	111	1	789	2,738	2,162	111	NA	NA
F4	15,8	D	2	8	900	0	NA	NA	2,023	860	NA	40
F5	13,6	D	2	8	22	1	624	2,692	0,652	276	NA	NA
G1	12,8	D	2	8	68	1	832	1,947	NA	68	NA	NA
G3	11,8	D	2	8	58	1	842	0,855	NA	58	NA	NA
G4	12,9	D	2	8	157	1	508	2,244	NA	392	NA	NA
G5	15	D	2	8	30	1	870	2,276	NA	30	NA	NA
T8	14	D	2	8	110	1	490	3,429	0,545	110	NA	NA
H1	12,5	D	2	8	66	1	834	1,439	NA	66	NA	NA
H2	13	D	2	8	237	1	663	1,719	NA	200	NA	37
H3	12,6	D	2	8	657	1	243	2,469	0,440	546	111	NA
H4	11,9	D	2	8	72	1	818	1,027	NA	82	NA	NA
H5	13,6	D	2	8	694	1	206	3,204	0,110	544	150	NA
A1	13	D	3	12	370	1	530	1,019	0,811	370	NA	NA

A2	11,8	D	3	12	43	1	857	1,400	NA	43	NA	NA
A3	12,2	D	3	12	609	1	291	1,649	0,296	609	NA	NA
A4	10,8	D	3	12	114	1	460	1,435	0,682	440	NA	NA
A5	13	D	3	12	54	1	846	2,199	NA	54	NA	NA
A7	13,6	D	3	12	169	1	731	2,134	NA	169	NA	NA
B1	12,7	D	3	12	900	0	NA	NA	0,545	880	NA	20
B2	13,6	D	3	12	92	1	808	2,970	NA	92	NA	NA
B3	12	D	3	12	112	1	788	1,523	NA	112	NA	NA
B4	11,8	D	3	12	82	1	818	1,834	NA	82	NA	NA
B5	12,5	D	3	12	85	1	815	2,356	1,412	85	NA	NA
C1	14,2	D	3	12	434	1	466	3,219	0,305	394	NA	40
C2	14	D	3	12	321	1	579	2,487	0,748	321	NA	NA
C3	12,7	D	3	12	35	1	865	1,595	NA	35	NA	NA
C4	11	D	3	12	450	1	450	2,533	NA	411	NA	39
C5	12,2	D	3	12	33	1	631	1,807	NA	269	NA	NA
D3	11,8	D	3	12	60	1	840	0,429	NA	60	NA	NA
D4	12,5	D	3	12	90	1	810	2,148	NA	90	NA	NA
D5	13	D	3	12	64	1	682	1,848	0,550	218	NA	NA
E1	13,2	D	3	12	142	1	758	2,454	NA	142	NA	NA
E2	12,5	D	3	12	97	1	803	2,017	NA	97	NA	NA
E3	11,6	D	3	12	900	0	NA	NA	0,259	694	181	25
E4	13,3	D	3	12	29	1	871	2,204	NA	29	NA	NA
E5	12,7	D	3	12	205	1	695	1,554	NA	205	NA	NA
F3	11,6	D	3	12	100	1	353	2,380	0,658	547	NA	NA
F4	15,8	D	3	12	900	0	NA	NA	2,250	880	20	NA
F5	13,6	D	3	12	188	1	712	2,275	NA	188	NA	NA
G1	12,8	D	3	12	45	1	855	2,316	NA	45	NA	NA
G3	11,8	D	3	12	64	1	836	1,292	NA	64	NA	NA
G5	15	D	3	12	261	1	639	2,817	0,920	261	NA	NA
T8	14	D	3	12	74	1	826	2,615	0,811	74	NA	NA
H1	12,5	D	3	12	79	1	821	1,023	NA	79	NA	NA
H2	13	D	3	12	316	1	584	1,952	0,570	316	NA	NA
H3	12,6	D	3	12	900	0	NA	NA	0,694	865	35	NA
H4	11,9	D	3	12	77	1	823	0,875	NA	77	NA	NA
H5	13,6	D	3	12	248	1	652	1,748	NA	155	93	NA
A1	13	D	4	16	136	1	764	0,707	0,882	136	NA	NA
A2	11,8	D	4	16	646	1	46	NA	0,583	824	NA	30
A3	12,2	D	4	16	218	1	422	1,991	1,010	416	NA	62
A4	10,8	D	4	16	900	0	NA	NA	0,568	845	23	32
A5	13	D	4	16	131	1	769	1,717	NA	131	NA	NA
A7	13,6	D	4	16	846	1	54	2,222	NA	846	NA	NA
B1	12,7	D	4	16	900	0	NA	NA	1,029	875	NA	25
B2	13,6	D	4	16	99	1	509	2,475	0,614	391	NA	NA
B3	12	D	4	16	222	1	678	1,239	NA	222	NA	NA
B4	11,8	D	4	16	270	1	149	2,013	0,649	555	196	NA
B5	12,5	D	4	16	900	0	NA	NA	0,507	829	22	49

C1	14,2	D	4	16	469	1	345	1,913	NA	555	NA	NA
C2	14	D	4	16	44	1	856	1,893	NA	44	NA	NA
C4	11	D	4	16	402	1	498	2,771	NA	339	NA	63
C5	12,2	D	4	16	54	1	730	2,712	NA	170	NA	NA
D3	11,8	D	4	16	215	1	685	0,438	NA	215	NA	NA
D4	12,5	D	4	16	317	1	583	2,264	0,729	247	43	27
D5	13	D	4	16	395	1	260	1,154	NA	504	88	48
E1	13,2	D	4	16	341	1	559	1,503	1,232	341	NA	NA
E2	12,5	D	4	16	236	1	664	2,078	NA	236	NA	NA
E3	11,6	D	4	16	900	0	NA	NA	0,298	403	497	NA
E4	13,3	D	4	16	53	1	847	1,629	NA	53	NA	NA
E5	12,7	D	4	16	689	1	24	2,500	0,083	724	130	22
F3	11,6	D	4	16	137	1	130	2,308	1,714	770	NA	NA
F4	15,8	D	4	16	900	0	NA	NA	2,533	900	NA	NA
F5	13,6	D	4	16	615	1	180	2,000	1,333	720	NA	NA
G1	12,8	D	4	16	107	1	793	2,119	NA	107	NA	NA
G3	11,8	D	4	16	98	1	802	0,748	NA	98	NA	NA
G5	15	D	4	16	900	0	NA	NA	2,733	900	NA	NA
T8	14	D	4	16	48	1	649	2,126	0,478	251	NA	NA
H1	12,5	D	4	16	51	1	849	1,201	NA	51	NA	NA
H2	13	D	4	16	660	1	240	2,250	0,182	660	NA	NA
H3	12,6	D	4	16	267	1	633	1,991	0,225	267	NA	NA
H4	11,9	D	4	16	193	1	707	0,934	NA	193	NA	NA
H5	13,6	D	4	16	220	1	680	2,471	NA	165	NA	55

Experiment 2: Interactions

		Categories	
Pair	identifies the lobster pair	**status.agg**	binary response variable based on agg.score
Id	identifies the lobster	**status.total**	binary response variable based on total score
gr.	treatment	**odor**	whether they were exposed to odors
init.	initiator of first encounter	**occupy**	time spent occypying shelter
aggression	aggression score (Table 5)	**roam**	time spent roaming
individual	individual score (Table 5)	**freeze**	time spent freezing
Total	total score: agg.score + ind.score		

pair	id	gr.	Init.	aggression	individual	total	status. agg	status. total	odor	occupy	roam	freeze
1	D5	D	1	-11,00	3,34	-7,66	0	0	no	557	553	40
1	J2	C	0	11,00	1,65	12,65	1	1	yes	422	688	NA
2	C5	D	1	20,50	9,69	30,19	1	1	no	1090	95	NA
2	K3	C	0	1,50	-1,94	-0,44	0	0	yes	29	763	NA
3	C4	D	1	4,00	-0,85	3,15	0	0	no	NA	205	52

3	L3	C	0	12,00	10,63	22,63	1	1	yes	1170	30	NA
4	C2	D	1	8,50	7,55	16,05	0	1	no	848	124	NA
4	T7	C	0	11,50	2,54	14,04	1	0	yes	283	196	NA
5	C1	D	1	-6,00	-4,20	-10,20	0	0	no	NA	1009	115
5	K1	C	0	13,00	8,84	21,84	1	1	yes	1027	173	NA
6	B4	D	1	-0,50	-0,91	-1,41	0	0	no	NA	219	NA
6	N1	C	0	1,00	10,63	11,63	1	1	yes	1170	30	NA
7	E5	D	0	-22,00	-3,98	-25,98	0	0	no	NA	956	599
7	J1	C	1	8,00	-1,46	6,54	1	1	yes	125	841	NA
8	F3	D	0	-0,50	0,53	0,03	0	0	no	339	792	NA
8	I2	C	1	10,50	4,54	15,04	1	1	yes	641	433	NA
9	B1	D	0	11,50	-3,29	8,21	0	0	no	NA	790	85
9	I1	C	1	13,50	0,73	14,23	1	1	yes	282	508	NA
10	A5	D	1	-1,00	3,68	2,68	0	0	no	378	112	NA
10	L1	C	0	1,50	5,73	7,23	1	1	yes	763	390	NA
11	A4	D	1	1,50	7,01	8,51	1	1	no	862	282	20
11	M1	C	0	-5,00	-1,39	-6,39	0	0	yes	165	904	75
12	A3	D	1	-2,00	-4,85	-6,85	0	0	no	NA	1164	35
12	K2	C	0	2,00	9,09	11,09	1	1	yes	1047	153	NA
13	T8	D	1	-4,50	-4,70	-9,20	0	0	no	NA	1127	NA
13	I3	C	0	2,00	10,38	12,38	1	1	yes	1149	47	NA
14	F6	B	0	8,00	8,87	16,87	1	1	no	1041	73	NA
14	T3	A	1	-2,00	-4,61	-6,61	0	0	yes	NA	1106	NA
15	D6	B	0	10,00	9,70	19,70	1	1	no	1124	41	NA
15	M5	A	1	-8,00	-1,93	-9,93	0	0	yes	NA	464	NA
16	F7	B	1	-5,00	-3,71	-8,71	0	0	no	NA	891	237
16	S8	A	0	13,00	6,25	19,25	1	1	yes	723	187	NA
17	E7	B	1	5,00	-1,15	3,85	1	0	no	NA	276	55
17	T9	A	0	2,00	6,37	8,37	0	1	yes	728	168	25
18	T1	B	1	4,50	-0,25	4,25	0	0	no	NA	61	NA
18	T5	A	0	10,00	-0,25	9,75	1	1	yes	NA	61	NA
19	D8	B	0	-7,50	-1,29	-8,79	0	0	no	42	633	510
19	O5	A	1	3,50	7,26	10,76	1	1	yes	980	217	NA
20	F9	B	0	-9,50	-1,22	-10,72	0	0	no	95	722	307
20	Q5	A	1	5,50	3,99	9,49	1	1	yes	697	436	NA
21	G9	B	1	-8,50	-0,32	-8,82	0	0	no	227	770	NA
21	S9	A	0	4,50	7,04	11,54	1	1	yes	828	207	NA
22	D9	B	1	-10,00	-0,69	-10,69	0	0	no	149	704	222
22	M9	A	0	10,50	3,81	14,31	1	1	yes	569	464	66
23	L9	B	0	25,00	9,23	34,23	1	1	no	1051	127	NA
23	Q7	A	1	0,00	-3,47	-3,47	0	0	yes	NA	833	NA
24	C7	B	1	-2,50	3,14	0,64	0	0	no	493	472	NA
24	R5	A	0	1,50	4,30	5,80	1	1	yes	612	433	NA
25	B9	B	0	-9,50	0,59	-8,91	0	0	no	92	282	NA
25	S5	A	1	16,00	8,95	24,95	1	1	yes	1028	149	NA
26	H4	D	0	-2,50	-0,05	-2,55	0	0	no	253	639	230

26	S3	C	1	21,00	5,56	26,56	1	1	yes	739	383	NA
27	E1	D	0	-8,50	0,25	-8,25	0	0	no	149	478	NA
27	Q4	C	1	2,00	-1,91	0,09	1	1	yes	NA	459	NA
28	H2	D	0	-5,50	-1,50	-7,00	0	0	no	140	880	83
28	P1	C	1	9,00	5,48	14,48	1	1	yes	698	320	NA
29	G5	D	1	12,00	5,97	17,97	1	1	no	828	223	NA
29	R4	C	0	2,00	-3,85	-1,85	0	0	yes	NA	923	53
30	B5	D	0	-4,50	-1,14	-5,64	0	0	no	NA	274	NA
30	A1	C	1	14,00	5,91	19,91	1	1	yes	780	141	NA
31	G4	D	1	1,00	1,17	2,17	0	0	no	380	719	NA
31	Q2	C	0	3,00	2,16	5,16	1	1	yes	365	451	54
32	G2	D	1	-9,50	0,05	-9,45	0	0	no	249	725	193
32	O1	C	0	6,50	3,44	9,94	1	1	yes	642	458	70
33	F4	D	1	4,00	-3,09	0,91	1	1	no	48	1078	NA
33	S2	C	0	-12,00	1,56	-10,44	0	0	yes	293	452	381
34	B2	D	1	9,50	10,08	19,58	1	1	no	1121	63	NA
34	R3	C	0	-7,50	-4,39	-11,89	0	0	yes	NA	1053	NA
35	B3	D	1	41,50	-1,68	38,32	1	1	no	NA	763	154
35	R2	C	0	-11,00	-2,60	-13,60	0	0	yes	NA	623	454
36	E2	D	1	-8,00	-3,75	-11,75	0	0	no	NA	899	248
36	S4	C	0	5,50	7,81	13,31	1	1	yes	979	204	NA
37	H3	D	0	-5,50	-4,26	-9,76	0	0	no	NA	1022	40
37	N2	C	1	7,00	7,54	14,54	1	1	yes	949	209	NA
38	A6	B	1	-7,50	-1,18	-8,68	0	0	no	40	602	439
38	M9	A	0	9,00	8,98	17,98	1	1	yes	1070	104	NA
39	J7	B	1	11,50	8,82	20,32	1	1	no	980	84	NA
39	O6	A	0	-2,00	-0,98	-2,98	0	0	yes	140	756	30
40	J6	B	1	6,00	1,36	7,36	0	0	no	340	473	292
40	N8	A	0	22,50	-3,75	18,75	1	1	yes	NA	899	46
41	K9	B	1	54,50	1,42	55,92	0	0	no	156	91	NA
41	T2	A	0	97,00	-0,98	96,02	1	1	yes	NA	235	NA
42	H6	B	0	-29,50	-0,50	-30,00	0	0	no	73	507	546
42	P9	A	1	11,00	-3,19	7,81	1	1	yes	78	1042	NA
43	I9	B	1	30,00	-1,35	28,65	1	1	no	NA	325	34
43	D2	A	0	18,00	-3,03	14,97	0	0	yes	NA	727	265
44	I7	B	1	2,00	-3,43	-1,43	0	0	no	NA	824	196
44	P6	A	0	27,00	9,35	36,35	1	1	yes	1055	105	NA
45	I6	B	1	10,00	8,17	18,17	1	1	no	1010	180	NA
45	M7	A	0	-17,00	-1,10	-18,10	0	0	yes	137	777	NA
46	T4	B	0	-10,50	4,93	-5,58	0	0	no	502	62	NA
46	M8	A	1	9,50	5,63	15,13	1	1	yes	666	221	269
47	G8	B	0	5,00	0,82	5,82	0	0	no	252	547	41
47	Q6	A	1	16,50	3,61	20,11	1	1	yes	664	461	NA
48	G7	B	1	41,00	-0,02	40,98	1	1	no	313	752	NA
48	N6	A	0	-10,50	-2,59	-13,09	0	0	yes	35	812	202
49	H9	B	0	7,00	9,39	16,39	1	1	no	1055	97	NA

49	N9	A	1	0,00	-3,53	-3,53	0	0	yes	NA	846	190
50	T10	B	0	-7,50	9,07	1,57	0	0	no	1044	152	NA
50	O7	A	1	2,50	-0,08	2,43	1	1	yes	95	448	NA

Experiment 3: Simulated release

tank	day	odor	success	shelters	failure	injured	healthy	dead	alive	Initial #	group
1	6	no	17	30	9	10	16	4	26	30	D
1	21	no	12	27	12	12	12	6	24	30	D
1	35	no	10	25	11	14	7	9	21	30	D
2	6	no	18	30	10	10	18	2	28	30	B
2	21	no	16	29	10	14	12	4	26	30	B
2	35	no	12	27	11	13	10	7	23	30	B
1	6	yes	12	30	18	13	17	0	30	30	C
1	21	yes	14	27	12	12	14	4	26	30	C
1	35	yes	15	25	7	9	13	8	22	30	C
2	6	yes	11	30	17	10	18	2	28	30	A
2	21	yes	13	29	13	15	11	4	26	30	A
2	35	yes	15	27	8	14	9	7	23	30	A

Appendix 5 - Preliminary observations:

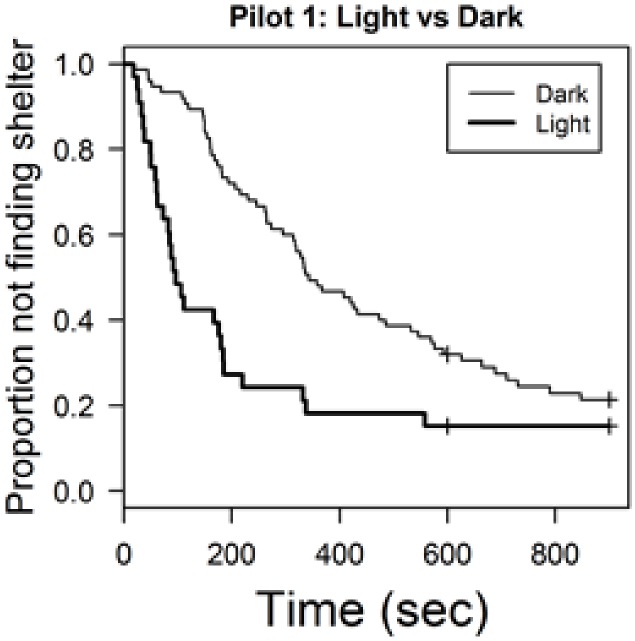
Pilot 1: Text written in 2011

From the 15th to 22nd of March (2011) individual observations were done on EBP lobster juveniles to assess the behaviour after exposure to predator odors. Lobsters were exposed for several different durations of exposure and in two different light regimes. This pilot was also done to familiarize with the behaviour that could be expected in an experimental setting. In some separate trials interactions between Exposed and Naive lobsters were observed.

We used juvenile Atlantic cod and juvenile Ballan wrasse, both of which had been reared in hatcheries. Both predators and lobsters had a temperature regime in the region of 13.5°C. Predators were transferred from their holding tanks to smaller plastic tanks with no water flow for approximately two hours to make a concentrated odor solution (oxygen added continuously). For the exposure event lobsters were taken out of their single celled compartments and placed singly in small featureless plastic trays, along with approximately one liter of water containing the predator odor in each tray. The trays were then left for the desired amount of time (1, 4 or 18 hours), after which the predator water was replaced with fresh seawater. This was done to ensure that all lobsters received exposure for the same amount of time, since they could not be observed simultaneously.

All tests were carried out in two rectangular 50 liter tanks, which were filled with around 30 l of seawater. Shell sand was used as substrate, and a shelter made out of three cobble stones. The walls were cover with sand glued on with silicone. At the start of each trial, one lobster was released in each tank simultaneously and the trial continued for 15 minutes. The method of deployment was not standardized, and the lobsters were not acclimated before start of each trial. This may have been an important source of error, and will be done differently in the main experiment. There were no significant differences between the three durations of exposure to predator odor, but the sample size was low for each group. Nevertheless, there was clear effect of light regime on shelter-seeking, and in light treatments the lobsters found shelter significantly faster than in the dark treatments.

There were many factors that could have affected behavior in this pilot. Lobsters were of unequal size and could not be exposed to odors while in the single celled compartments, which is the plan for the main experiment. Lobsters of different size may react to predator odors in a different way, which was evident in some cases (a visual impression that shelter-seeking was stronger in smaller lobsters). The fact that each lobsters was handled several times before a trial since they could not be exposed in the single cells may have caused an elevated stress level, which in turn could have masked a possible response toward the predator odor. Predators were juvenile and hatchery reared, which could have affected the results since predators in the wild have a larger size spectrum and may discharge stronger chemical stimuli. Predators caught from the coastal waters will have a different odor profile, and that these can stimulate a stronger response in lobster behavior. Therefore, wild caught and larger predators will be used in the main experiment.

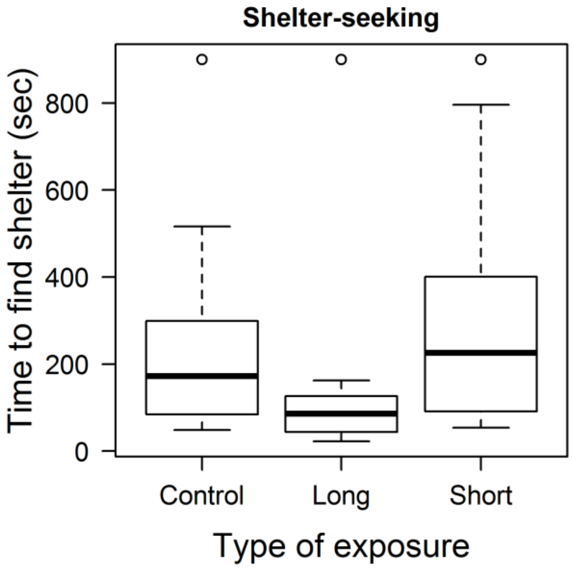


Pilot 2:

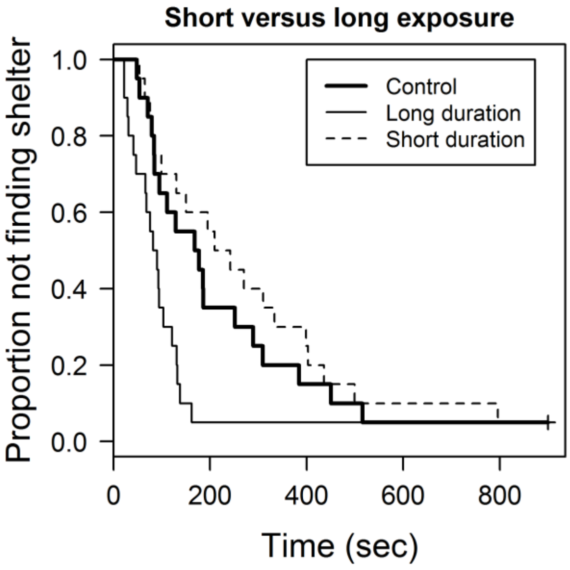
From the 4th to 6th of June before starting of the main experiment, a second pilot was performed on a separate batch of juveniles to decide on which duration of predator exposure that should be used. Lobsters were divided into three groups;

1) Control (n=20)
2) Short and repeated exposure (n=20)
3) Long duration without pulse intermittency (n=20)

Group 1 remained naïve to predator odors, group 2 received one hour long pulses four times per day (1 hour between each pulse) for three days, and group 3 received 14 hour long pulses for three days. On the 6th of June they were observed individually in 15 minute trials. Shelter-seeking did not differ between Group 1 and 2 (survival analysis: z= 0.75, p= 0.45), but group 3 showed better shelter-seeking than both group 1 and 2 (group 1 vs 3, survival analysis: z= -2.01, p= 0.045, group 2 vs 3, survival analysis: z= -2.75, p= 0.006). Based on these results long duration was used in the main experiment.



Shelter-seeking



Short versus long exposure

R syntax:

```
fit1.surv <- survreg(Surv(find.shelter,status.find)~exposure, dist='weibull')
anova(fit1.surv)
summary(fit1.surv)
exposure2 <- relevel(exposure, ref='Short')
fit2.surv <- survreg(Surv(find.shelter,status.find)~exposure2, dist='weibull')
summary(fit2.surv)
```

Appendix 6: Synthesis - Long term effect on motivation

There are four major causal systems, or *motivational systems*, of behavior; hunger, sex, fright and aggression (Colgan 1986), where motivation can be classified as goal directed behavior reflecting the animals internal state and how it is affected by external conditions (Folkedal 2010 and references therein). A stimulus that acts continuously to determine the animals' specific internal state or responsiveness can be distinguished as a motivating stimulus, and if the external situation is constant, altered behavior is caused by changes in the animals' internal state (Hinde 1966).

In the present study, where juvenile lobsters were subjected to odors from three predator species, odor exposure was assumed to represent a fright-inducing stimulus. Since the external situation was more or less constant, i.e. lobsters were maintained in small single celled compartments, predator odors may have acted to modulate the lobsters' internal (motivational) state, and their response towards environmental disturbances would depend on which motivational system that was most strongly activated, e.g. fright or aggression.

According to Spruijt et al. (2001), the activation of a certain motivational system, at least in more complex animals, is dependent on the reward gained by performing a certain behavior. The reward value, or *incentive*, of the specific behavior depends on the animals' current internal state, e.g. the hungrier animals are, the higher the rewarding value of food, or as in the present study considering that EBP lobsters presumably are shelter-bound as trade-off against predation risk (Wahle 1992); the more frightened lobsters are, the higher the incentive value of possessing shelter. If two or more motivational states are in conflict, e.g. hunger and thirst, or aggression and fright, the animal must evaluate benefits and costs of each behavior. The best motivational system for a specific situation is then the one where the reduction between actual and expected (ontogenetically determined reference) state gives the highest reward (Spruijt et al. 2001).

In the present study, the effect of odor exposure seemed to be connected to the level of environmental disturbance and also the lobsters' ability to cope with the different novel situations:

- **Individual observations:** Low environmental disturbance: Swift handling procedure, emersion for ≤ 1 min, introduction to relatively low complexity test-environment.

- **Interaction trials:** Moderate environmental disturbance: Swift handling procedure, emersion for ≤ 1 min, introduction to relatively low complexity test-environment, but also interactions and competition for a limited resource.
- **Simulated release:** High environmental disturbance: Handling related to packing, emersion and ice-cooling for ~4 hours, rapid transition from light to dark and increased light intensity, multiple agonistic encounters, lack of acclimation before release to a novel environment.

According to Hinde (1966), a given stimulus does not always evoke the same response in all animals. In the present study, the effect of e.g. handling could depend on the lobsters' internal state, where Exposed lobsters were presumed to be in a state of elevated fright, i.e. their motivation was affected by odor exposure. Subsequently, when introduced to the test-environment they could respond differently to the same stimuli than Naïve lobsters.

In the individual observations neither handling nor introduction to a novel environment resulted in consistent behavioral responses in any of the groups (Fig. 16), i.e. the low level of environmental disturbance presumably resulted in weak or no activation of the fright-related motivational system, and their behavior did not indicate a frightful situation (few or no flight reactions like escape or freezing). The reward value (i.e. *the incentive*) of gaining shelter may depend on their internal state, and thus without increased motivation (i.e. altered internal state) shelter-seeking behavior was not enhanced.

It is suggested that hatchery reared animals always have a baseline level of stress due to the rearing environment or various experimental procedures (Rehnberg & Schreck 1987; Vilhunen et al. 2005) (Fig. 16A), but this level may be elevated for lobsters exposed to frightful stimuli (Fig. 16B). Even though there was no apparent response to odor exposure, the lack of a behavioral reaction is not equivalent to an inability to perceive the stimulus (Rehnberg & Schreck 1987). For example, coho salmon (*Oncorhynchus kisutch*) can respond to a chemical stimulus either behaviorally, physiologically (through a stress response) or in a combination of these, but the responses need not be co-occurring (Rehnberg & Schreck 1987). Although handling may elicit a stress response, it did not seem to be a strong enough fright-inducing stimulus to promote motivation above activation threshold levels. Likewise, handled chinook salmon (*Oncorhynchus tshawytscha)* and Arctic char displayed the same anti-predator behavior as non-handled fish (Berejikian et al. 2003; Vilhunen 2006). In the present study, handling before individual observations was short, and lobsters were never emersed for more than one minute thus avoiding hypoxia (Chang 2005).

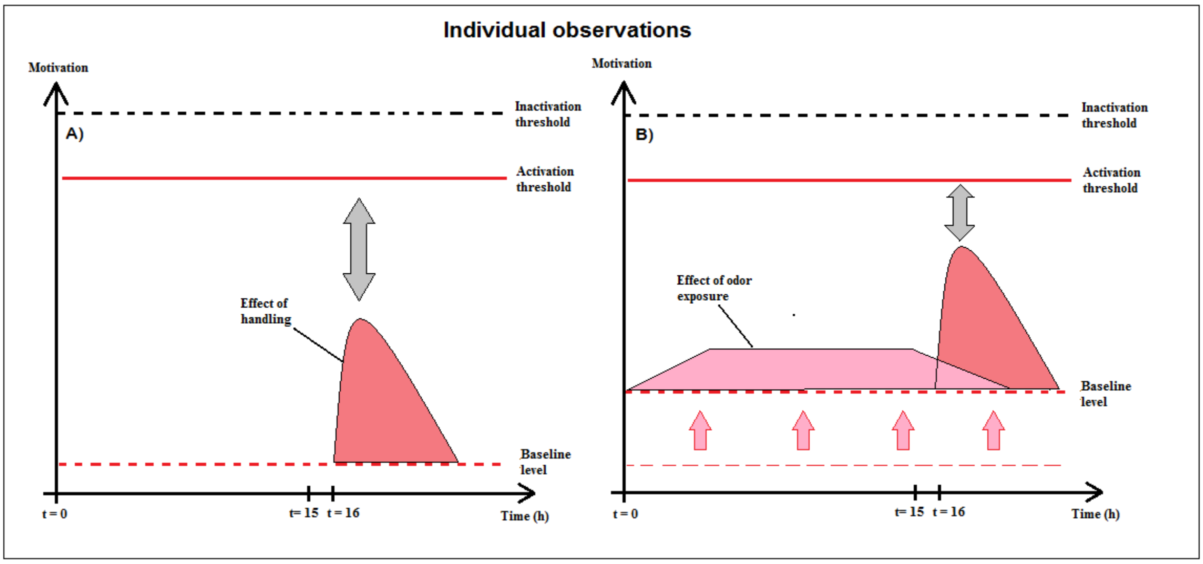


Figure 16 - Individual observations: A possible effect of exposure to predator odors on the underlying fright-driven motivation in juvenile lobsters. t=0 and t=15 is the beginning and end of odor exposure, while t=16 corresponds to the start of observation. Stimulation above the activation threshold promotes a fright response and presumably enhances shelter-seeking and reinforce association with shelter, while stimulation above the inactivation threshold represents acute stress compromising the lobsters coping abilities (e.g. resulting in escape, avoidance or freezing). **A:** Naïve lobsters have never experienced fright inducing chemical stimuli, and thus have a low baseline fright-related motivation. Some baseline stress will presumably be ever-present in a rearing environment. Handling or introduction to a new environment (environmental disturbance) was not a strong enough stimulus to promote motivation above the activation threshold level. **B:** Exposed lobsters have an elevated baseline fright-related motivation (i.e. stress caused by fright inducing stimuli), which implies that they will react to a relatively weaker stimulus than the Naïve lobsters, or react more strongly to a stimulus of equal magnitude. However, handling did not represent a strong enough stimulus and no fright-motivated behavioral response was observed.

Rearing animals in isolation can promote aggression compared with socially experienced individuals (Dunham 1972), but can also enhance their fright-level (Gallagher et al. 1972). In the present study, all animals had been reared in solitary confinement but based on the interaction trials they were not all equally aggressive or frightened. This implies that exposure to predator odor has a potential to affect behavior, but a real threat (i.e. interactions) had to be introduced in the environment to promote fright-related motivation above an activation threshold (Fig. 17).

In the interaction trials there was genuine competition where shelter represented a limited resource. Based on the notion of incentive value (Spruijt et al. 2001); the more frightened the animal, the higher the incentive value of shelter. In this case, the behavior elicited to gain access to shelter presumably reduced the difference between the lobsters' actual internal state (fright: faced with a threat) and the ontogenetically based reference state (shelter-bound). Thus, activation of the fright-related motivational system was stimulated by the specific award gained by performing certain behaviors, in this case to win fights against an opponent and subsequently gain possession of shelter. The benefit of gaining shelter (protection from a threat) must have surpassed the cost of gaining access (agonistic behavior).

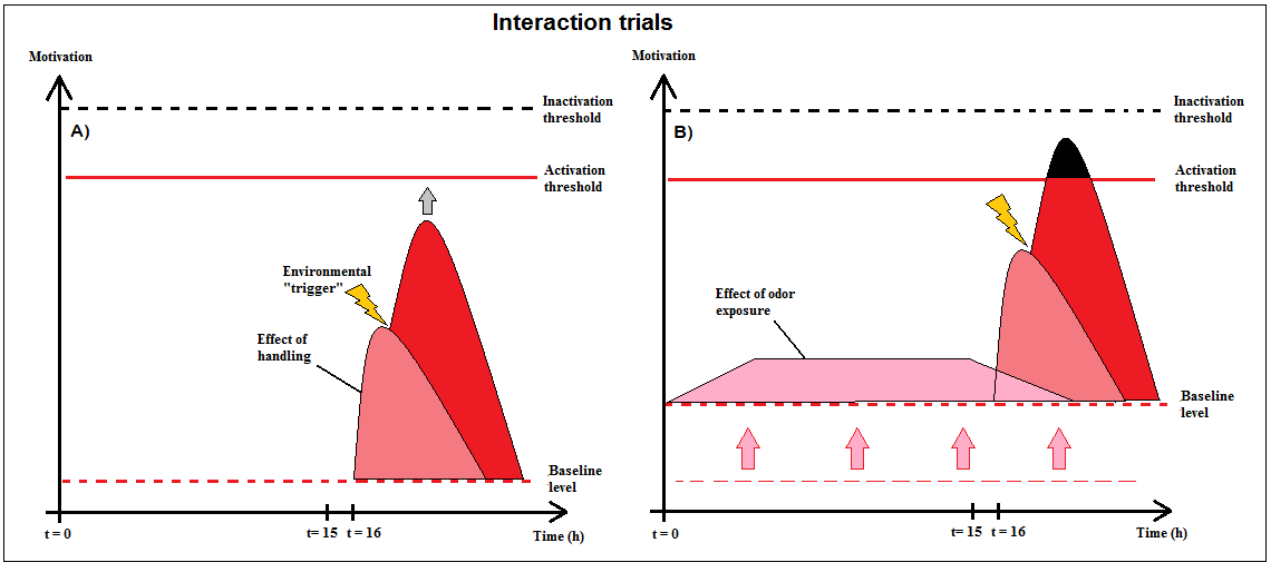


Figure 17 (Fig. 15 revisited) – Interaction trials. t=0 and t=15 is the beginning and end of odor exposure, while t=16 corresponds to the start of the observations. Stimulation above the activation threshold promotes a fright-fight response, while stimulation above the inactivation threshold represents acute stress compromising the lobsters coping ability and results in fright-flight (i.e. escapes, avoidance or freezing). **A:** Naïve lobsters had never experienced fright inducing predator odors, and thus had a low baseline fright-related motivation. Handling (environmental disturbance) prior to the observations did not promote motivation above the activation threshold, but the presence of an environmental trigger in the form of interactions/competition increased competitive motivation, although still at sub-threshold levels. **B:** Exposed lobsters had an elevated baseline fright-related motivation, and thus react to a weaker stimulus than the Naïve lobsters, or react more strongly to a stimulus of equal magnitude. Handling did not represent a strong enough stimulus by itself, but the presence of an environmental trigger add to the stress caused by odor exposure and handling, promoting motivation to reach activation threshold levels. Thus, when the Exposed lobsters were engaged in fights they had an activated fright-related motivational system, which resulted in fight behavior and establishment of dominance (i.e. they were more motivated to gain possession of shelter).

Active motivational systems are deactivated by various forms of feedback, either through physiological feedback mechanisms (e.g. satiation) or behavioral mechanisms (Folkedal et al. 2010 and references therein). In the present study, dominant lobsters generally receded to shelter and reduced other activities after they had won agonistic encounters, while subordinate individuals could continue to make several approach-retreat bouts. The dominant lobster tended to only engage in further aggression if the subdominant came in close proximity to the shelter. This was not in concurrence with Huber & Kravitz (1995), who observed that the dominant lobster would initiate further aggression until the loser consistently retreats. This indicates that, in the present study, the fighting itself had no incentive value and was rather a necessary step to gain access to shelter. Thus, motivation may have been deactivated when the dominant had already taken possession of shelter (i.e. the actual state was now equal to the reference state; shelter-bound), but could be re-activated if the subdominant approached or indeed attempted to take the shelter.

In the simulated release, complexity of the test-environment was further enhanced with a new set of environmental impulses, i.e. larger area, different light intensity, different type of shelters and multiple possible agonistic encounters, and the Exposed lobsters were presumably unable to cope by the same behavioral response as in the paired interaction trials, and it was the Naïve lobsters that initially had the highest shelter occupancy at the first sampling. Shelter occupancy is a valid measure of social dominance (Peeke et al. 2000), which implied that Exposed lobsters were not as dominant as observed earlier. However, they managed to significantly increase shelter occupancy over the next month, and at the final sampling held more shelters than Naïve lobsters. Thus, some advantage that was not evident at the day of release or after 6 days, may have been become evident in the following 29 days of the experiment which included a sampling procedure also at day 21. There was no exposure to predator odor in the test-environment, so this mechanism must presumably have been present at the time of release.

Before release all lobsters were included in a simulated transport lasting 4 hours, which included emersion for a prolonged period in low temperatures (see section 4.4). Lobsters were not acclimated before release. Since the only difference between the Exposed and Naïve lobsters was the odor exposure itself, it was assumed that the Exposed lobsters' initial inability to cope may have resulted from prolonged odor exposure. If the exposure contributed to increase the underlying fright-related motivation as indicated in the interaction experiments, Exposed lobsters should have responded in a similar manner; highly motivated to win agonistic interactions in order to gain possession of shelter (i.e. protection). However, this was not the case. Added stress from the odor exposure may have resulted in Exposed lobsters displaying fright-flight responses (e.g. escape, avoidance, freezing) rather than fright-fight response (motivated aggressive behavior). The most probable explanation for this shift in behavior is that the new situation was perceived as too severe, and the animal coped through avoidance rather than fighting (Fig. 19), but as the lobsters managed to acclimate to new conditions they could again respond as observed in the interaction trials. Considering that

Naïve lobsters were presumably less motivated (Fig. 18), they may have had less fidelity to their shelters and could have been outcompeted by the highly motivated Exposed lobsters.

Support for this explanation was found in the behavior of Exposed lobsters before sampling at day 6. Many of lobsters without shelter had made burrows or were observed in prolonged digging activities. Additional stress imposed by failure to deactivate their fright-related motivation (i.e. behavioral feedback: to have possession of shelter) may lead to compensatory reactions in seeking other rewards to restore balance (Folkedal et al. 2010 and references therein). Their inability to gain shelter may have prevented feedback to down-regulate motivation, which then promoted other forms of shelter-related behavior such as burrowing. The reward value (Spruijt et al. 2001) of residing in a burrow is clearly lower than having possession of shelter, but it may still have reduced the difference between actual (severe stress) and reference state (shelter-bound) to such an extent that the benefits (somewhat more protected) exceeded the cost (digging) in this particular situation. Thus, the behavior had some reward value. However, after the Exposed lobsters presumably acclimated after some days (Fig. 19) the reward value of residing in a burrow became much lower than occupying shelter (i.e. less severe stress, higher coping abilities). This may have then have promoted the competitive ability. Considering that Naïve lobsters were likely to have settled and gained a resident advantage, the competition may have been long-lived, which can be the reason for the gradual rather than rapid improvement in shelter occupancy seen in the Exposed lobsters.

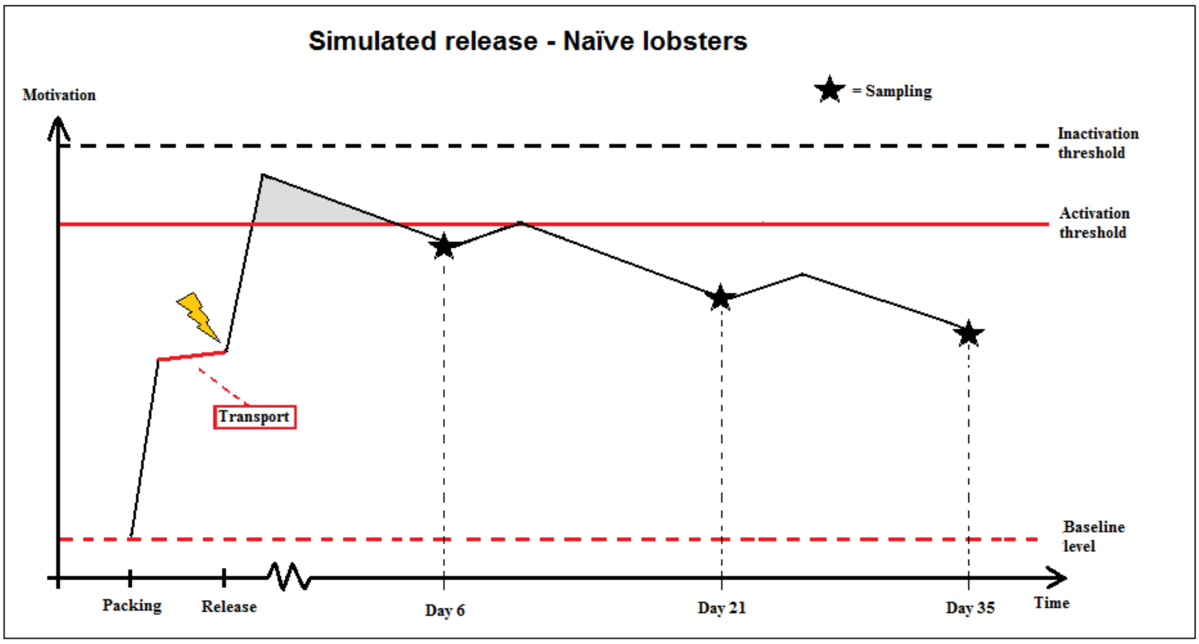


Figure 18 – Simulated release: Packing of lobsters in newspaper on ice took 15 minutes, before the transport stage was initiated and lasted for 4 hours. After transport lobsters were released at the surface (marked by yellow dart). The release represents the environmental trigger (novel environment, multiple

agonistic interactions, etc.). Sampling is marked by a black star. The first sampling was done after 6 days, and each sampling is assumed to re-elevate the fright-related motivation, but not to the same extent as the initial release since lobsters presumably had acclimated to the new conditions. The motivation has an arbitrary half-life so the fright-related motivation to gain/remain in possession of shelter decreases with time. Motivation above the activation threshold results in increased competitive behavior, while reaching the inactivation resembles acute stress temporarily resulting in inability to cope and display of fright-flight behavior (i.e. escape, freezing, avoidance).

Naïve lobsters have never experienced fright inducing chemical stimuli, and thus still have a low baseline motivation. After transport and release into a novel environment without prior acclimation the fright-related motivational system is activated. They out-competed the temporarily inactivated Exposed lobsters in the first days post-release. On subsequent samplings, their level of motivation had declined below the activation threshold along with their motivation to occupy new shelters. At each sampling their fright-related motivational system is below threshold levels which presumably resulted in lower shelter-fidelity.

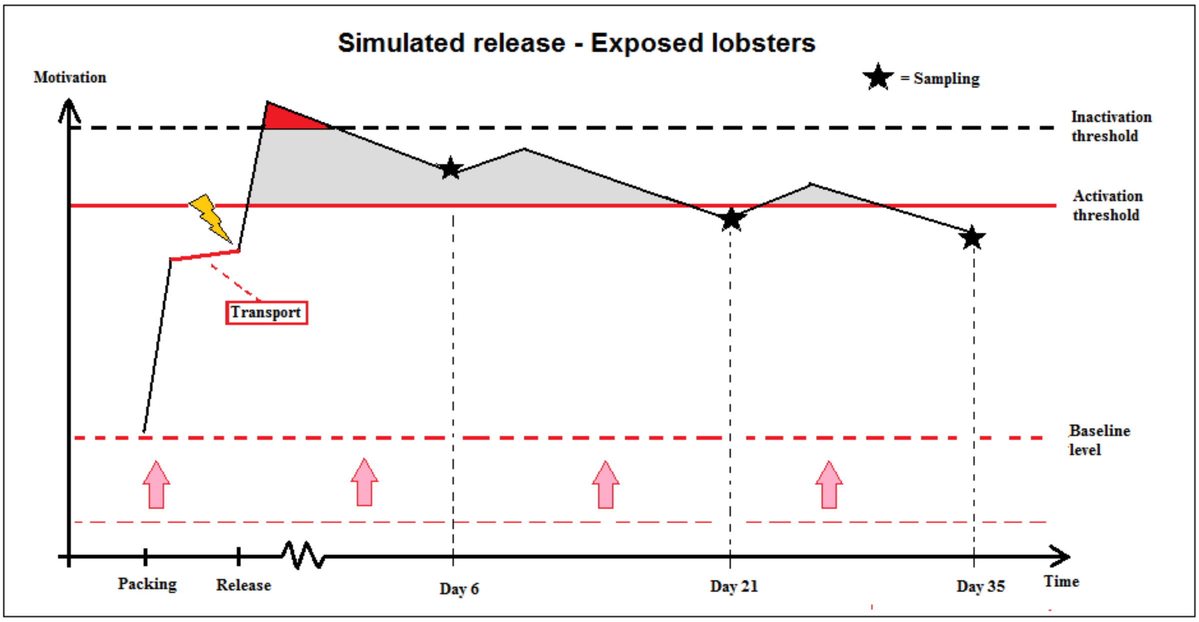


Figure 19 – Simulated release: See Fig. 18 for explanation of figure contents. Exposed lobsters have an elevated baseline motivation, and will react to a weaker stimulus than Naïve lobsters, or react more strongly to a stimulus of equal magnitude. After transport and release into a novel environment without prior acclimation their fright-related motivational system is activated but acute stress renders them unable to cope resulting in a fright-flight response (avoidance, caution, escape). They are thus out-competed before the first sampling. Subsequently, when acclimated to the new environment they are able to regain initiative. Their fright-related motivational system is active above activity threshold levels for much longer than that of Naïve lobsters, which will stimulate fright-fight behavior and competitive behavior. They outcompete Naïve lobsters over time as they will have a higher fright-related motivation to gain/remain in possession of shelters.

In behavioral studies where animals are conditioned for hours rather than days, prolonged activation of any motivational system is to my knowledge rarely observed. Still, a long lasting effect seemed to be present after lobsters had been exposed to predator odors for 30 days prior to the simulated release. Several studies have observed an effect of short-term acute stressors both on physiological and behavioral responses. The physiological stress response is usually short-lived and stress indicators return to pre-stimulatory levels after some hours (Metcalfe et al. 1987; Folkedal et al. 2010). A physiological response to predator odors has also been observed in Arctic char after 72 days of constant exposure (Laakkonen 2006), although not recognized as an acute response (change in body composition) supporting that frightful stimuli might not always trigger a stress response of the general adaptation syndrome (Rehnberg & Shcreck 1987). All the same, the behavioral response to acute fright-related stress may persist for several days (Pickering et al. 1982; Utne-Palm 2001; Folkedal et al. 2010). Thus, short term exposure to fright-related cues can both elevate the baseline stress level and have an effect on the motivational systems for some time, but what happens after prolonged exposure is largely unknown territory, also in marine decapods.

Although no long term activation have been observed concerning fright-related motivation, there are examples of long-term retention of aggressive motivation (Heiligenberg & Kramer 1972). After several weeks in isolation aggression in male cichlids decline to low levels. The attack rate can be restored by exposure to fish dummies for 10 days, and then declines to pre-stimulatory levels with a halflife of seven days. Consequently, if the exposure time is long it seems like motivation can remain activated for a longer time. After one month only 6.25% of the initial aggressive motivation remains, but in a competitive situation against unmotivated opponents, low motivation can all the same be sufficient to give an advantage. If the fright-related motivation to gain shelter in lobsters was long-lived in the simulated release, this could explain why the exposed lobsters managed to increase their shelter occupancy with time after initially being at a disadvantage (Figs. 18 and 19).

This could represent a highly relevant method for training of juveniles prior to release, granted that the increased competitive behavior displayed by the Exposed lobsters is still found when similar experiments are performed with acclimation prior to release. Both the behavioral and physiological aspect of prolonged odor exposure should be studied further. Behavioral studies should entail continuous monitoring of the tanks post-release to observe the actual events. Furthermore, predators present in the tanks would give an impression of whether odor exposure has impact on behavior under elevated risk of predation. If this was combined with a measure on the lobsters’ stress response after prolonged exposure relative to the response when they are released, one could clarify whether the odor exposure has potential to promote competitive behavior or if the stress related to transport and release procedure will mask any effects gained through exposure.