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URINE RELEASE IN FREELY MOVING CATHETERISED LOBSTERS (*HOMARUS AMERICANUS*) WITH REFERENCE TO FEEDING AND SOCIAL ACTIVITIES

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Summary

Previous studies suggest that urine-borne pheromones play an important role in lobster agonistic and sexual behaviour. This paper investigates the pattern of urine release in catheterised, but otherwise freely moving, adult lobsters with respect to feeding, social and non-social activities. Lobsters on average released 4.1 ml (1 % of body mass) of urine over a 12 h period; this more than doubled to 10.6 ml over the 12 h period after feeding. Hourly monitoring revealed that most urine was released in the first hour after feeding (2.84 ml). With the exception of the first hours after feeding, urine release was intermittent, with pauses lasting up to 17 h. The probability of urine release per hour in unfed lobsters was 0.34 (median); this value increased during agonistic interactions elicited by the introduction of a conspecific (median 0.63) and during activity initiated by non-social disturbance (median 0.56). Mean urine volume during output hours in unfed lobsters amounted to 1.09 ml h^{-1}. This volume was significantly increased by the presence of a conspecific (1.88 ml h^{-1}) and decreased during activity initiated by non-social disturbances (0.56 ml h^{-1}). No sex-specific differences in urine release were found. The data demonstrate that lobsters control their urine release in a manner dependent on behavioural context. This supports recent findings suggesting the use of urine for chemical signalling in agonistic interactions.

Key words: urine output, fighting, behaviour, catheter technique, agonistic interaction, lobster, *Homarus americanus*.

Introduction

Chemical signals play an important role in the social life of many crustaceans (for critical reviews, see Dunham, 1978, 1988). Examples of the use of waterborne pheromones exist from various crustacean taxa, but most of the experimental evidence comes from research on brachyuran crabs (Bauchau, 1986; Christofferson, 1978; Eales, 1974; Gleeson, 1980, 1991; Ryan, 1966) and macruran crayfish or lobsters (Ameyaw-Akumfi and Hazlett, 1975; Atema and Cowan, 1986; Atema and Engstrom, 1971; McLeese, 1973; Tierney and Dunham, 1984). Pheromones have been shown to be released with the urine in most species tested. Urine from pre-moult females evokes the typical courtship behaviour in male brachyuran crabs (Christofferson, 1978; Eales, 1974; Gleeson, 1980). Blocking of the excretory pores of pre-moult females abolishes this response of the male crab to the female (Ryan, 1966).

In lobsters, urine signals are involved both in sexual behaviour and in the recognition of dominance (Atema, 1986; Atema and Voigt, 1995; Karavanich and Atema, 1991). Lobsters release urine through a paired set of nephropores on the ventral sides of the basal segments of the second antennae. Following release, the urine is blown forward by the powerful anteriorly projecting gill currents, which emerge on both sides just below the antennal bases (Atema, 1986). These currents appear to be perfectly suited for carrying urine-borne pheromones from one animal to another.

In contrast to most brachyuran crabs, in lobsters it is not the male but the female that actively searches for the mating partner (Atema et al., 1979; Atema, 1986). Females choose the dominant male, which usually resides in a secure shelter. Following an exchange of urine-borne signals (Atema and Voigt, 1995; Bushmann and Atema, 1994, 1997) that probably provide the female with information about the dominance of the male and the male with information about the sex and reproductive state of the visitor (Atema and Voigt, 1995; Bushmann and Atema, 1994, 1997), the female enters the shelter for a cohabitation period that can last up to 2 weeks. During this time, the female moults, the lobsters mate and thereafter the female remains in the mating shelter guarded by the male and gaining protection during her vulnerable post-moult period (Cowan and Atema, 1990; Cowan et al., 1991).

Dominant males not only gain access to the best shelters but also to mating opportunities, both limited resources for a male

lobster (Atema, 1986; Atema and Voigt, 1995). Dominance is established by fights between individual lobsters. Karavanich and Atema (1993, 1998b) allowed pairs of size-matched male lobsters to fight by placing them together in an enclosed tank for 20 min on two consecutive days. They showed that once dominance has been established between two animals subsequent agonistic interactions are very short and that the subordinate avoids the dominant individual (Karavanich and Atema, 1993, 1998b). Thus, in lobsters, dominance results from the memory of agonistic experience (Karavanich and Atema, 1993, 1998b) as in stomatopod crustaceans (Caldwell, 1979, 1985). When antennular olfaction or urine release of the lobsters was blocked, losers re-challenged the previous dominant individual in subsequent interactions (Karavanich and Atema, 1998a). This indicates that olfaction is necessary for recognition of dominant individuals and suggests that a chemical signal in the urine carries the information about individuality and dominance.

Since the significance of urine-based signals is well established for the crustacean species mentioned above, the dynamics of urine release now becomes important for the understanding of communication in these animals. If urine is used as a social signal, the animals should have control over its release and/or composition. Christofferson (1978) showed that the tank water of restrained pre-moult female *Portunus sanguinolentus* did not have the same sexually stimulating effect on males as the tank water from unrestrained females. He suggested that females stop their urine (and thus pheromone) release when restrained, so that the tank water loses biological activity. This may also explain his finding that females carried by males during precopula do not evoke behavioural responses in other mature males (Christofferson, 1978).

Few studies have investigated the dynamics of urine production in crustaceans. Cheng and Chang (1991) studied urinary rates in cannulated juvenile lobsters. Intermoult, unfed lobsters showed constant urinary rates of 2% body mass per day. Upon feeding, more than 4% of the body mass could be excreted rapidly as urine. After feeding, urine release was intermittent. Cheng and Chang (1991) did not investigate the effect of social interaction on urine release. Snyder et al. (1993) studied intra- and intersexual interactions in adult lobsters with or without antennal gland cannulation and collection bottles and found that neither sexual behaviour nor agonistic behaviour (as reported also by Karavanich and Atema, 1998a) was impaired by this restraint. However, they did not quantify urine output in their experiments. Bushmann and Atema (1994, 1997) showed that during shelter visits both visiting females and resident males release urine while facing each other.

In the present study, we use modified catheterisation techniques to monitor urine output at different temporal rates. Since urine serves as a chemical signal (Karavanich and Atema, 1998a), we expect urine release to be increased during social interactions. However, urine output should also depend on time after feeding and may be influenced by the movement activity of the lobster. The aim of this study was to determine how urine output varies over time and with respect to behavioural context. Specifically, we will address the following questions. What is the 'normal' urine release pattern of unfed, isolated lobsters? Is urine released continuously or intermittently? What is the effect of feeding and non-social activity on urine release? Is urine release increased during social interactions?

Preliminary data have been published elsewhere (Breithaupt and Atema, 1993; Breithaupt et al., 1994).

Materials and methods

We report results from two experiments with different time resolutions. In the first, urine output was recorded every 12 h for 22 days in 10 male lobsters (wet mass 374–476 g; 75–80 mm carapace length) in March 1991 at temperatures ranging between 4 and 5 °C. In the second experiment, urine output was monitored at 1 h intervals in six male and six female lobsters (383–566 g; 76–87 mm carapace length) between 20 January and 20 April 1993 at 2–8 °C.

Maintenance of animals

All animals were obtained from local fishermen. They were first kept in communal tanks of up to 10 animals. After catheterization, they were kept in separate tanks (30 l or 90 l) supplied with running ambient sea water. The animals were fed thawed/frozen squid once a week. The 10 lobsters with urine output monitored at 12 h intervals were given a 10 day acclimation/fasting period following catheterisation before their urine output was recorded.

Adhesion of catheters and urine collection technique

To monitor urine release continuously while interfering minimally with the animal, we catheterised lobsters with flexible plastic tubing (Fig. 1) using a procedure first developed successfully by Lindstrom (1991). We describe it here in some detail. The first piece of tubing, Tygon latex tubing of 3 mm i.d., was attached using cyanoacrylate glue (Zap-A-Gap) to the shell surface surrounding each nephropore (Fig. 1, inset). The bond was then reinforced by applying another coat of glue around the tube; this second coat was quickly fixed with a drop of accelerator fluid (Zip Kicker) applied to the outside of the bond. During this procedure, the lobster was strapped down on its back. When the two tubes had been adhered, they were trimmed to a length of 6 mm to create a sleeve into which smaller conduits, polyvinylchloride tubing of 1.5 mm i.d., were inserted and glued. The smaller tubes looped up and around each side of the carapace under the eyestalks. They were connected to a Y-tube using splices of latex tubing (5 mm length, 3 mm i.d.) and fixed with cyanoacrylate glue to the dorsal carapace. A single transparent polyvinylchloride tube rose from the lobster to the water surface and, depending on the required temporal resolution of urine output, was connected in one of two different ways to a vial floating at the water surface.

When monitoring at intervals of 12 h, urine was collected in

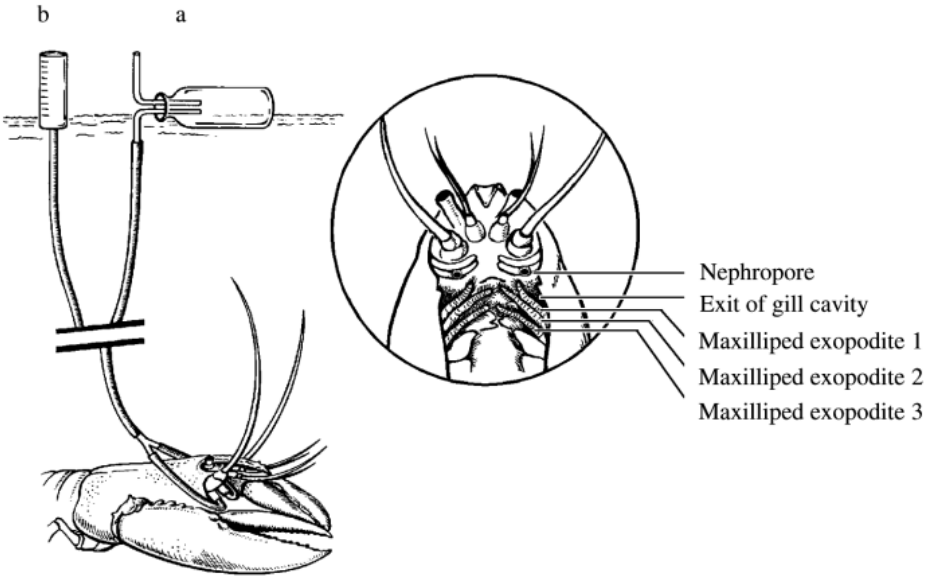

Fig. 1. Catheterization of lobsters. Urine was collected through flexible plastic tubing glued to the shell surface surrounding both nephropores. For quantification of urine output, collection devices were used that floated at the water surface (a, 50 ml glass vial; b, 20 ml syringe cylinder). The inset shows the location of nephropores above gill openings and maxilliped exopodites (see text for further details of catheterization).

a 50 ml glass vial with rubber stoppers which rested horizontally in a removable float collar (Fig. 1, a). To prevent the build-up of back pressure due to urine volume compressing the air inside the vial, a second tube connected the interior air with the external air.

To record urine output at intervals of 1 h, it was collected in a scaled transparent cylinder provided by the body of a 20 ml syringe (Fig. 1, b). This cylinder floated vertically on the water surface, supported by air-filled containers. The accuracy of reading in this arrangement was within 0.2 ml.

One concern was whether the physical variables involved in the catheter technique (hydrostatic pressure, capillary action, wall resistance) would allow the urine to flow from the lobster through the tube to a height equal to the water surface, then up the small rise into the collection vessel (e.g. Fig. 1, b). Control experiments with changing water depths revealed that negative hydrostatic pressure ('suction') is a serious concern only at water depth exceeding 30 cm when the nephropore is at least 20 cm below the water surface. At this depth, urine began to leak out of the nephropore, presumably because of the higher internal pressure in the lobster than in the air-filled tubes (e.g. 2 kPa at 20 cm depth). However, since water depth never exceeded 30 cm in our experiments, these effects were negligible. Once the urine level rises above the water surface (e.g. within the scaled cylinder shown in Fig. 1, b), hydrostatic pressure will increase in proportion to the height of the urine level above the surface (we observed up to 8 cm, corresponding to 0.8 kPa). We would expect urine output to be diminished in animals as the collection vessels filled. However, we did not find any dependence of urine output on the filling height of the collection vessels (see, for example, Fig. 2). Finally, capillary forces in the 1.5 mm diameter tubing tested in open submersed tubing caused the water level to rise above the surrounding level by almost 2 cm. This corresponds to a volume of 0.035 ml in the sampling vial, which is well below our lowest resolution of reading (0.2 ml) and thus has no detectable effect on our results. Wall resistance did not restrict the flow of urine: the flow velocity is low, and the cross-sectional area of the tubes (7 mm^2) is much greater than the size of the nephropore (approximately 1 mm^2).

Experimental procedure for collection of urine at intervals of 12 h

An acclimation/fasting period of 10 days was given concurrently to each lobster (N=10 males, see above) in its respective tank before the lobster was fitted with catheters. Each tank was provided with a shelter. Lobsters were kept under an artificial light cycle with lights on between 08:00 and 20:00 h. Animals were fed once per week at 20:00 h with a 20 g piece of squid (5 % of lobster body mass). The animals were not disturbed except when they were fed and when the collection bottles were changed. Urine collection bottles were changed every 12 h (at 08:00 h and 20:00 h) starting 12 h after fitting (08:00 h). Urine volume was determined by pouring the contents of the collection bottles into 20 ml sample vials, weighing them to the nearest of 0.1 g (assuming urine mass to be 1 g ml^{-1}) and subtracting the mass of the empty bottle.

Experimental procedure for collecting urine at intervals of 1 h and for testing the effects of the presence of conspecific and strong disturbance on urine release

Six male and six female lobsters were kept in individual 90 l tanks (70 cm×40 cm×30 cm). The lobsters were fed once per week with a 5 g piece of squid (1 % body mass), and urine production was monitored each hour throughout the day, from 08:00 h to at least 18:00 h. Each animal experienced two types of treatment (16 trials in all). In the first treatment ('social'), an unfamiliar male lobster (an individual never encountered by the study subject) was chosen from 18 different males of similar body size and introduced into one of the 12 tanks for 1 h in eight separate trials. Adding this male often resulted in fights between the lobsters. The behaviour of cohabiting lobsters was monitored at intervals of 5–10 min and classified as either aggressive (threat displays such as 'extension of claws', 'walking high on legs';

physical contacts such as 'pushing the opponent', 'clamping of claws onto opponent's claw'; and potentially damaging behaviours such as 'rapid grasp' and 'pull of opponent's appendage') or defensive ('walking away from opponent', 'tailflipping') (compare the list of agonistic behaviours described by Atema and Voigt, 1995). In the second treatment ('disturbance'), the lobsters were strongly disturbed by a 10 cm×23 cm (lobster-sized) plate pushed rapidly through the water towards the lobster once every 10 min for 1 h in eight separate trials. This plate produced a strong visual and mechanical stimulus that generally elicited activity (e.g. fast walking, tailflips) lasting for 10–60 s per disturbance (i.e. 1–6 min per treatment trial). While, on average, the duration of 'disturbance' activity was shorter than that of 'social' activity (1–60 min), 'disturbance' activity generally included faster movements (e.g. fast walking, tailflips) than 'social' activity (agonistic behaviour, e.g. threat display, pushing the opponent with claws). Thus, the physical exertion elicited by the 'disturbance' treatment was regarded as being similar in effect to the physical exertion elicited by the 'social' treatment. In addition to this physical stress, the 'disturbance' should have caused a strong psychological stress by threatening the lobster as an approaching predator would. The introduction of a conspecific in the 'social' treatment may cause a similar psychological stress as a result of the sudden dramatic change in the environment of the isolated lobster. Thus, the 'disturbance' treatment was designed to act as a control for urine release due to physical and/or psychological stress. Lobsters received treatment trials up to three times daily between 08:00 h and 18:00 h at randomly chosen times; the type of treatment and the order of presentation were also randomised.

Statistical analysis of data

To analyse changes in frequency of urine output, hourly data were dichotomised (urine release, no urine release). Changes in probability of urine release over time after feeding were analysed at intervals of 6 h using logistic regression analysis (CATMOD Procedure; SAS Institute, 1995) with maximum likelihood-estimation of parameters (Fleiss et al., 1986). The CATMOD procedure estimates the odds $[\log_e(P/1-P)]$ of urine release on the basis of the dichotomous data. From these estimates, the probability of urine release was calculated as $P = e^{(\text{intercept+estimate})}/[1+e^{(\text{intercept+estimate})}]$. Background probability of urine release of unfed untreated lobsters (used to test the effect of treatments) was calculated on the basis of the frequency of hourly urine releases of each lobster within daily periods of 5–14 h. The effect of treatments (social, disturbance) was tested using a classical randomisation test for paired comparisons within individuals (Scheffe, 1959). Here, the probability was tested that the observed distribution of data across two groups corresponded to an expected random distribution of the same data across two groups. The advantage of these statistical tests *versus* other non-parametric statistical tests (e.g. the Wilcoxon signed-rank test) is that not only the rank but also the value of the variable was taken into account. To test the effect of feeding and treatments on urine output volume, zero values were excluded from analysis. Differences in output volume were analysed using analysis of variance (ANOVA) procedures for repeated measures (JMP; SAS Institute, 1995).

Values are presented as means ± S.D., unless stated otherwise.

Results

Urine output measured at intervals of 12 h

Mean urine output of 10 unfed male lobsters measured at intervals of 12 h was 4.1±2.3 ml (mean ± S.D.) corresponding to 0.97±0.57 % of body mass, i.e. approximately 2 % of body mass per day. Mean urine output more than doubled during the 12 h following feeding to 10.6±4.7 ml (2.55±1.1 % of body mass; paired *t*-test, $P<0.01$), with individual increases of between 53 % and 460 %. During the day (08:00–20:00 h, light on), urine output was significantly greater than at night (20:00–8:00 h, light off), when lobsters tend to be more active: 1.27±0.72 % of body mass during the day *versus* 0.97±0.58 % of body mass at night (paired *t*-test, $P<0.01$).

Urine output measured every hour

Fig. 2 shows an example of the cumulative urine output of a single lobster. Following feeding, urine level first increased hourly then in steps of several hours. Pauses between measurements with urine release lasted up to 1000 min (median 105 min). Daily increases in urine output could be due either to a higher frequency of urine release bouts or to increased bout volumes or both. Since both the relative frequency and the bout volume of urine release may be of behavioural significance, they were analysed separately. The frequency of urine release was determined within daily periods of 5–14 h and standardised to release probability per hour. After feeding, the hourly probability of urine release was high ($P=0.67$ in the 6 h after feeding, Fig. 3A) and then decreased over time. After 12 h, it was no longer different from that for urine release of starved lobsters (24–144 h after feeding; logistic regression analysis, CATMOD procedure; SAS Institute, 1995). The mean hourly volume of urine output more than doubled in the

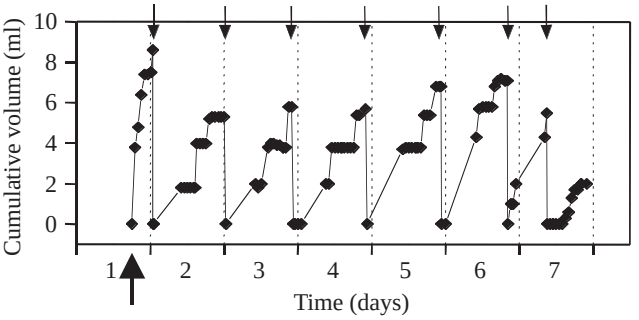

Fig. 2. Example of the cumulative urine output of a male lobster measured at intervals of 1 h after feeding (large arrow) between 08:00 h and 24:00 h. Small arrows indicate the times when collection vessels were emptied. Dotted lines indicate midnight. Stepwise increases in cumulative urine output on days 2–6 indicate pulsed urine release.

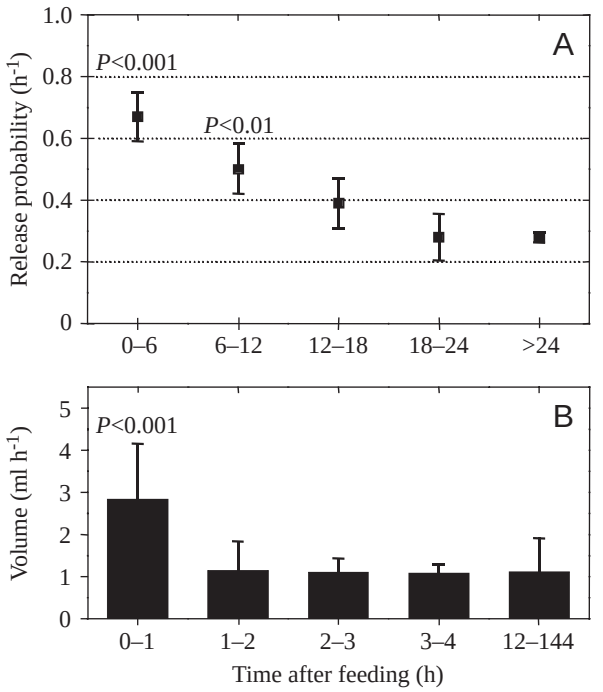

Fig. 3. Effect of feeding on urine release of six lobsters. (A) The probability of urine release per hour. Probabilities were calculated from maximum likelihood estimates of a logistic regression analysis (CATMOD procedure, SAS Institute, 1995) in six lobsters. The frequency of urine release was increased only within 12 h after feeding compared with urine release between 24 and 144 h. (B) Mean hourly urine output after feeding. Urine release was increased only at 0–1 h compared with urine output between 12 and 144 h (one-way ANOVA, N=12, six lobsters fed twice). Values are means ± S.D.

first hour after feeding (2.84±1.32 ml h^{-1}, N=12, six lobsters with two trials each) compared with the mean hourly urine output measured at least 12 h after feeding (paired t-test; P<0.001). In the second, third and fourth hour after feeding, hourly urine output volume did not differ from that of unfed lobsters (Fig. 3B). The hourly probability of urine release by starved lobsters ranged between 0.27 and 0.66 for individual lobsters (median 0.34; N=12; individuals were monitored for different periods, ranging from 35 to 237 h) (Fig. 4A, control). No difference in probability of urine release was found between animals (Friedman ANOVA). The volume released per hour by undisturbed, unfed animals could vary between 0.1 ml (lower limit of measuring resolution) and 6.3 ml (mean: 1.09±0.87 ml h^{-1}) (Fig. 4B, control).

Effect of social treatment and disturbance on urine release

Each of the six males and six females experienced two types of treatment (16 trials each). In the first treatment, an unfamiliar male lobster was introduced into each tank for 1 h in eight separate trials. Adding a second male often resulted in fights between the lobsters. This treatment caused an increase in the probability of urine release (median 0.63) compared with control time periods (i.e. days without treatments; median 0.34; P<0.05; randomisation procedure for paired comparisons within individuals) (Fig. 4A, social *versus* control). Mean urine output volumes increased from 1.09±0.90 ml h^{-1} in undisturbed animals to 1.88±1.34 ml h^{-1} during the presence of a conspecific (two-way ANOVA with replications, P<0.01) (Fig. 4B: social *versus* control). The higher urine output may not necessarily represent signal exchange in the lobsters. It could also be caused by the activity or by the disturbance due to the presence of the conspecific. To test the effect of such non-social disturbance, the lobsters experienced a second treatment: being chased by a 10 cm×23 cm (lobster-sized) plate that was moved rapidly through the water towards the lobster every 10 min for a period of 1 h. This plate produced a visual and mechanical stimulus that generally elicited escape activity including tailflips. Such disturbance caused the probability of urine release to be increased (median 0.56) compared with the controls (median 0.34; randomisation procedure for paired comparisons) (Fig. 4A). No differences were found between the effect of disturbance and social treatment on the probability of urine release. However, the volumes of urine released by the disturbed animal (mean 0.56 ml h^{-1}) were smaller than those released by fighting lobsters (mean 1.88 ml h^{-1}, P<0.01) or by undisturbed lobsters (mean 1.09 ml h^{-1}; P<0.05; two-way ANOVA for repeated measures) (Fig. 4B). Perhaps the low volumes of urine released at increased frequencies during non-social disturbance treatment were caused by the mechanics of fleeing and tailflipping, not specifically controlled by the

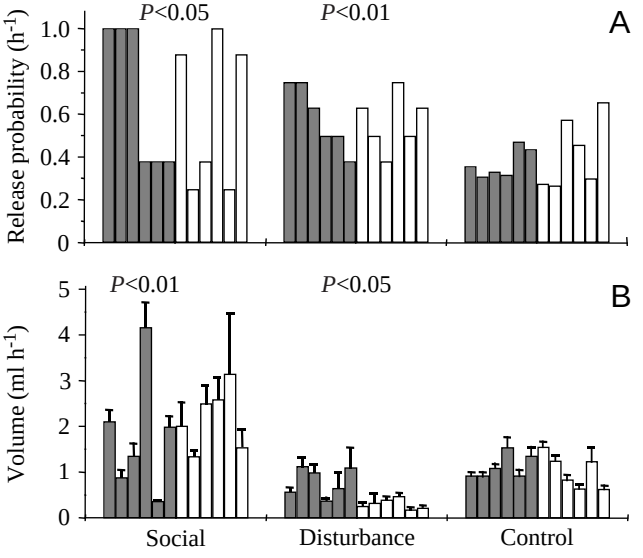

Fig. 4. Effect of treatments (social, disturbance; see text for details) on urine release of six male (filled columns) and six female (open columns) lobsters. Each individual received eight trials per treatment. Individuals are plotted in the same order for each condition. (A) The probability (relative frequency) of hourly urine release increased as a result of both social (P<0.05) and disturbance (P<0.01; randomisation test for repeated measures) treatments with respect to control time periods (hours without treatment). (B) Mean (+ S.E.M.) urine output volume increased during social treatment (P<0.01) and decreased as a result of disturbance (P<0.05; ANOVA for repeated measures).

lobster. This is in contrast to urine release during social interactions detailed below. We found no difference between the responses of male and female lobsters to the treatments (P<0.05; two-way ANOVA for repeated measures).

Urine release during agonistic interactions

The 'social' treatment elicited agonistic interactions in only 50 out of 96 trials (Fig. 5A). We analysed urine release for the trials with agonistic behaviour separately from the trials without agonistic behaviour (Fig. 5B,C). Lobsters always released urine when showing aggressive behaviour (Fig. 5B). The probability of urine release was much lower when they did not show any aggressive behaviour (11 out of 46 trials; P<0.01; Wilcoxon signed-ranks test). In addition, the volume of urine output was higher in aggressive animals (mean 2.0 ml h^{-1}) than in non-interacting or in defensive animals (mean 0.3 ml h^{-1}; P<0.01; one-way ANOVA for repeated measures). Lobsters that did not release any urine (36 of 96 trials) also did not show any aggressive behaviour. The largest amounts of urine (>5 ml) were released when an animal showed aggressive behaviour throughout the hour during which a conspecific was present. No behaviours typical of sexual interactions were observed. Both males and females showed agonistic behaviours towards the introduced male. These findings suggest that agonistic behaviour causes an increase in both the probability and the volume of urine release.

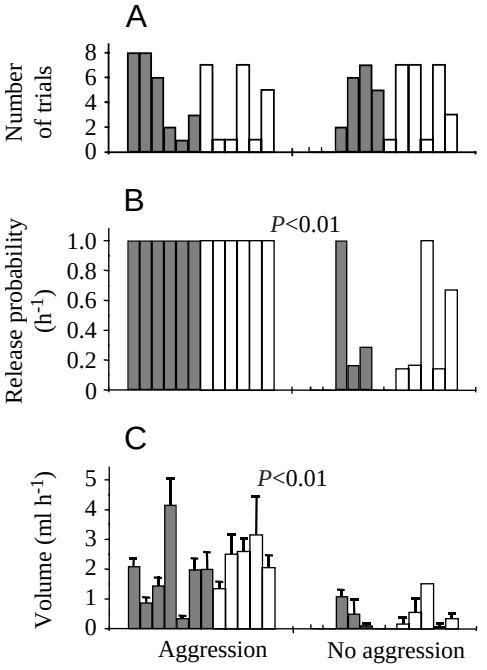

Fig. 5. Effect of aggressive behaviour during 'social' treatment on urine release by six male (filled columns) and six female (open columns) lobsters. (A) The number of trials with and without aggressive behaviour of catheterised lobsters. (B) The probability (relative frequency) of urine release per hour during trials with aggressive interactions was higher than that during trials with no aggressive interactions (P<0.01; Wilcoxon signed-ranks test). (C) Mean (+S.E.M.) urine output volume increased during trials with aggressive behaviour compared with trials without aggressive behaviour (P<0.01; ANOVA for repeated measures). Males and females are not significantly different in any of the three measures.

Discussion

Using the catheterisation technique developed for *Homarus americanus* by Lindstrom (1991) based on the technique of Holliday (1977) for crabs (*Cancer pagurus*), we studied the urine release patterns of early adult lobsters and the effects of feeding, non-social disturbance and aggressive interactions. A similar technique has been used on lobsters by Snyder and Chang (1991), Cheng and Chang (1991) and Snyder et al. (1993). However, in those studies, either the animals were not allowed to move freely (Snyder and Chang, 1991; Cheng and Chang, 1991) or urine release was not quantified (Snyder et al., 1993).

Unfed animals at low ambient temperature produced approximately 8 ml of urine per day, or 2 % of body mass. This production rate pertains to a low metabolic rate during the cold (4–5 °C) winter months. Urine output may be greater during the warmer (18–22 °C) summer months when lobsters are more active. Snyder and Cheng (1991), without specifying temperature, measured a daily urine output of 2.7 % of body mass in male and 3 % in female lobsters of the same size as in our study. Cheng and Chang (1991) reported a daily urine output of 2 % of body mass in juvenile lobsters at 20 °C. Curiously, in our study, urine output was greater during the day when lobsters tend to rest in their shelters and are less active than during the nocturnal activity period.

The results from hourly monitoring were consistent with data from juvenile lobsters (Cheng and Chang, 1991) in showing more than a doubling of urine output in the first hour after feeding (Figs 2, 3B). The probability of any urine being released during any 1 h period is greatest in the 6 h after feeding (67 %), declining over the next 6 h period to 50 %, and levelling off to 30 % subsequently until the next feeding (Fig. 3A). Thus, on average, urine is released in bursts every third hour. Similarly, in juvenile lobsters, urine flow was reported to return to basal rates only a few hours after feeding and then to be released in bursts at intervals of 1–3 h (Cheng and Chang, 1991).

The probability of release is interesting in a behavioural context where urine is used as a communication signal (e.g. Karavanich and Atema, 1998a,b; Bushmann and Atema, 1997). The probability of urine release varied between 0.27 and 0.66 for the individual lobsters (median 0.34). Pauses between urine releases could last up to 1000 min (median 105 min). The temporal resolution of 1 h in the present study was not sufficient to resolve the shortest bursts of urine release. When monitoring urine release at intervals of 5 s, bursts of urine release as short as 30 s were measured (T. Breithaupt and J. Atema, in preparation). Thus, pauses between urine release may be underestimated by up to 2 h. The longer the breaks between urine release bouts, the more the pulses of urine release stand out in the environment of the lobster. Thus, it appears that, even without changing composition, urine can stand out as a signal, because its release is pulsatile and not continuous. There is circumstantial evidence that the

composition of urine can also be changed, given the existence of a potential pheromone gland located behind the sphincter muscle in the nephropore (Bushmann and Atema, 1996). We provide further evidence for a signal function for urine below.

Upon introduction of a conspecific, the probability of urine release increased in four males (three of them up to 100 %, see Fig. 4A, social *versus* control) and three females (100 %, 88 %, 88 %). If only those treatments in which agonistic interactions were observed were counted, then the probability of urine release was 100 % in all animals (Fig. 5B), i.e. lobsters always released urine when they showed agonistic behaviours. Because of the limited temporal resolution in this study, urine output could not be correlated with individual behaviours of the catheterised lobster. A thorough analysis of urine release during agonistic interactions using higher temporal resolution is given elsewhere (T. Breithaupt and J. Atema, in preparation). The introduction of an unfamiliar male not only increased the probability of urine release but also increased the volume of urine released from an average over all animals of approximately $1\,ml\,h^{-1}$ at rest to $2\,ml\,h^{-1}$ during social interaction. As during the hour after feeding, urine release volumes also increased during agonistic interactions. After feeding, urine release ranged from 1.1 to 4.8 ml (2.8 ml on average); during fights, urine release was 0.3–5.8 ml (1.8 ml on average). This is interesting, since it means that, even without uptake of food, large amounts of urine (up to 5.8 ml) can be released during a fight. Cheng and Chang (1991) concluded from their finding of a rapid discharge of urine upon feeding that the bladder is usually filled. Burger and Smythe (1953) also reported that the bilateral bladders were usually distended, together containing up to 6 ml of urine in a 450 g lobster.

The control treatment clearly showed that urine release during social interaction was not a result of physical and/or psychological stress. When chased around their tank by a lobster-sized plate, the volume of urine released during that hour decreased significantly. The mean volumes in all 12 animals dropped from $1.09\,ml\,h^{-1}$ when undisturbed to $0.56\,ml\,h^{-1}$ during and following this disturbance. The volumes were reduced in all but one (male) animal (Fig. 4B). Interestingly, while the volume decreased, the probability of urine release during the eight replicate treatments increased significantly (Fig. 4A). We speculate that the escape behaviour caused an initial brief and reflexive release of urine, after which the nephropore sphincter muscle prevented further release.

These results suggest that urine release in the presence of a conspecific resulted from the social context (agonistic interactions) but not from the increased activity or fear during the interactions. Bushmann and Atema (1997) recorded, using the same technique described here, urine release in males and females visiting a male in his shelter. Both males and females increased their urine release when interacting with the sheltered male. Male urine output on average increased from $10\,\mu l\,min^{-1}$ to approximately $50\,\mu l\,min^{-1}$, while female urine output increased from 10 to $40\,\mu l\,min^{-1}$. This would correspond to a maximum of $3\,ml\,h^{-1}$ (in the males), an amount similar to that recorded here in some fighting animals.

What is the social function of urine release?

At least three functions of urine release are theoretically possible. (1) Urine has a direct destructive effect on the opponent. It may be used in agonistic interactions as a weapon by destroying tissue (e.g. the frontal sensory epithelium of the eyes, chemo- or mechanoreceptors) of the opponent, thus causing the opponent to withdraw. Terrestrial arthropods are known to use chemical weapons for defence against predators (Eisner, 1970). There is no evidence for this function in lobsters. (2) Urine is used as a communication signal and contains components that release specific behaviour patterns in the receiving lobster. These components could specify sex or dominance status. Male and female lobsters respond strongly with investigatory and aggressive behaviour patterns when exposed to male and female urine (Atema and Cowan, 1986). Evidence for a sex-specific releaser effect is also given by Bushmann and Atema (1997). In their study, but not in an earlier study by Snyder et al. (1993), only females with unblocked urine output were mated by the male. Bushmann and Atema (1997) explain the differences between these studies by the more natural conditions used in their own study and conclude from their finding that female chemical signals in the urine reduced resident male aggression (also noted by Atema and Engstrom, 1971) and increased the likelihood of male mating attempts. Huber et al. (1997) found metabolites of biogenic amines (specifically serotonin sulphate) in the urine of lobsters. This and other amines have been linked to aggressive behaviour. They hypothesise that dominance status may be communicated between lobsters *via* these metabolites. The expected effect of dominance signalling would be that one of the fighting lobsters would give up fighting and retreat when detecting a higher level of such amine metabolites in the opponent's rather than its own urine. So far, however, behaviourally relevant differences in urine composition between dominant and submissive animals have neither been found nor experimentally tested. (3) Urine is used as a communication signal and contains components that do not release immediate responses but have a priming effect on the receiving lobster. Results in support for this hypothesis were recorded by Karavanich and Atema (1998a) when investigating agonistic behaviour of lobsters while urine release was manipulated *via* open and closed catheters. In first fights between unfamiliar lobsters, the fight duration was longer and the aggression between the animals was always greater than in subsequent fights between the same animals. In second fights between the same pair, the losers from the first fights generally backed off immediately, avoiding a second fight. However, closing the catheters during the second fight prevented this learning effect, suggesting that components in the urine are received by the loser and are used for recognition of the previous winner. Karavanich and Atema (1998b) demonstrated that losers recognise the previous winner individually and that this memory can last between 1 and 2 weeks if pairs are kept separate between the first and second fight.

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