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NITROGEN METABOLISM OF TWO PORTUNID CRABS, *CARCINUS MAENAS* AND *NECORA PUBER*, DURING PROLONGED AIR EXPOSURE AND SUBSEQUENT RECOVERY: A COMPARATIVE STUDY

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Summary

***Carcinus maenas* and *Necora puber* were exposed to air for 72 h and 18 h, respectively, at 18 °C. Nitrogen excretion, blood and muscle ammonia content and blood urate and lactate content were recorded throughout the experimental emersion and following reimmersion (recovery period).**

During emersion, the rate of ammonia excretion was strongly reduced in both species, while urea and amine excretion were not enhanced. Blood and muscle ammonia content increased steadily, reaching 1.3 and 10.4 mmol l^{-1}, respectively, after an 18 h emersion in *N. puber*. In contrast, in *C. maenas*, blood ammonia levels increased slightly during the first 12 h and then remained at this level (approximately 0.12 mmol l^{-1}) until the end of emersion. Muscle ammonia content showed a non-significant increase at 12 h, after which values returned to control values (3.3 mmol l^{-1}) for the next 60 h.

Blood urate and lactate content increased in emersed *N. puber*, indicating strong internal hypoxia, but urate content did not increase in *C. maenas* until the third day of emersion.

Upon reimmersion, both species released large amounts of ammonia within a few minutes. Two different patterns of ammonia release then were observed: ammonia excretion was enhanced for a further 3 h in *N. puber*, whereas raised ammonia excretion rates were observed for a further 24 h in *C. maenas*. These patterns, the recovery of blood and muscle ammonia levels and the calculated nitrogen balance between emersed and control crabs indicated that specific processes were used to manage the nitrogen overload induced by air exposure.

Whereas *N. puber* shows little or no ability to limit ammonia accumulation in its body, *C. maenas* exhibits strong regulation of its nitrogen metabolism. The probability that amino acid synthesis is involved in this regulation and whether these species use metabolic depression as a survival strategy are discussed.

Key words: air exposure, nitrogen metabolism, nitrogen excretion, ammonia, amine, urea, urate, lactate, haemolymph, muscle, crab, *Carcinus maenas*, *Necora puber*.

Introduction

The effects of air exposure on aquatic crustaceans have received a great deal of attention regarding respiratory function and acid–base regulation (Truchot, 1975, 1979; deFur, 1988; reviewed by Burnett, 1988). Studies have also focused on the adaptative processes involved in respiration by crustaceans that have successfully made the transition to terrestrial life; a large range of respiratory patterns from facultative to obligate air-breathing are reviewed by Henry (1994). In marine decapods, air exposure induces hypoventilation, promoting an accumulation of CO_2 in the blood and a resulting respiratory acidosis. A decrease in the rate of oxygen uptake, despite the generally reduced metabolic rate in air-exposed decapods, leads to internal hypoxia and anaerobic metabolism, inducing a blood metabolic acidosis.

In marine decapods, ammonia excretion occurs mainly at the gills and, like respiratory gas exchange, is a continuous process in sea water (Regnault, 1987). Because branchial gaseous and ionic exchanges are disrupted in the absence of environmental sea water, ammonia excretion, which is the main route for the release of the end-products of nitrogen metabolism, is also expected to be impaired during air exposure.

The nitrogen metabolism of air-exposed decapods has been studied mainly in semiterrestrial and terrestrial crustaceans that have become adapted to these conditions through evolution. Except for the robber crab *Birgus latro*, which relies on purinotelism (Greenaway and Morris, 1989), terrestrial species are generally ammoniotelic (for a review, see Greenaway, 1991). However, terrestrial species differ with regard to the predominant form of excreted ammonia (gaseous NH_3 or NH_4^+) and the route and mechanisms involved in this excretion. For example, discontinuous excretion of gaseous ammonia is found in some terrestrial isopods (Wieser and

Schweizer, 1970; Kirby and Harbaugh, 1974; Wright and O'Donnell, 1993) and highly terrestrial crabs (Greenaway and Nakamura, 1991; Varley and Greenaway, 1994), unusual ammonia release *via* the urine and reprocessing of this ammonia-enriched urine in the branchial chambers has been reported in the ghost crab *Ocypode quadrata* (De Vries and Wolcott, 1993) and enhancement of active NH_4^+ excretion through the gill epithelium by recycling urine salts was found in terrestrial crabs (Wolcott, 1991). Under prolonged dry conditions, ammonia production may be switched off, as observed by Wood *et al.* (1986) in *Cardisoma carnifex*. In land crabs, urate is of minor importance in total excreted nitrogen. However, it represents a common nitrogenous metabolite, as shown by large urate deposits in the haemocoel (Gifford, 1968). In *Gecarcoidea natalis*, these urate salts are intracellular and originate from *de novo* biosynthesis (Linton and Greenaway, 1997*a*,*b*).

In contrast, the nitrogen metabolism of aquatic crustaceans under aerial conditions has received little attention. In response to either short-term emersion (deFur and McMahon, 1984; Vermeer, 1987) or long-term air exposure (Regnault, 1992; Schmitt and Uglow, 1997), an increase in blood ammonia levels is generally observed in marine decapods. This blood ammonia overload is assumed to be the result of an impediment to ammonia excretion during emersion, even if the rate of ammonia production is lowered as a result of a general metabolic depression (Regnault, 1994). An increase in blood urate content was also observed in air-exposed *Cancer pagurus*, but this was considered to be the result of internal hypoxia rather than uricogenesis enhancement (Regnault, 1992).

While most of these studies concerned sublittoral and fully aquatic decapods, it is of interest to investigate the effects of air exposure on nitrogen metabolism in marine intertidal species, since they inhabit the transition area between aquatic and aerial domains. The intertidal crab *Carcinus maenas*, which can survive prolonged air exposure (Truchot, 1975), was used as a model for this study. For comparison, the sublittoral portunid crab *Necora puber*, which is rarely found in the intertidal zone and is not able to tolerate long-term emersion (Johnson and Uglow, 1985), was also studied.

To examine changes in nitrogen metabolism of *C. maenas* and *N. puber* under aerial conditions, these species were submitted to experimental emersions of 72 h and 18 h, respectively. Nitrogen excretion rates (ammonia, amines, urea) during prolonged emersion and a subsequent reimmersion were measured. Changes in blood ammonia content (extracellular compartment) and muscle ammonia content (intracellular compartment) were recorded throughout the air exposure and the recovery periods. Changes in blood urate content were also followed, since this nitrogenous metabolite is usually present in terrestrial and semiterrestrial crabs. Blood lactate levels were also investigated to determine the emersion period that *C. maenas* and *N. puber* could tolerate before resorting to anaerobic metabolism.

Materials and methods

Crabs

Carcinus maenas (L.) (70–110 g wet mass) and *Necora puber* (L.) (50–100 g) were collected in summer using baited pots in the low intertidal area and the sublittoral zone, respectively, at Roscoff (N. Brittany, France). Animals were kept in running sea water (open system) at ambient temperature (18–19 °C) and salinity 33–34 ‰ for 1 week before use; they were fed every 2 days with pieces of thawed fish (*Trachurus trachurus*). Only male crabs in intermoult stage were used for experiments.

General experimental design

Emersion

Crabs were emersed in a series of five 60 l tanks each containing a rack of eight polyvinylchloride (PVC) boxes (width 13 cm; height 18 cm). The tanks were filled with sea water and fed continuously with running sea water from an open system. Sea water was allowed to circulate through the totally immersed boxes, the bottoms of which were perforated with small holes and the tops of which were covered with a PVC grid. After they had been fed, crabs were settled individually into the boxes and covered with a 2 cm thick layer of polystyrene chips previously soaked in sea water. They were kept in these conditions for 24 h. Forty crabs were used for each experiment.

Experiments were started by draining four of the five tanks; the fifth contained control crabs, which remained immersed. Animals were exposed progressively to air without handling stress. The layer of polystyrene chips which initially floated at the top of boxes followed the seawater level as the tanks were drained, finally covering the emersed crabs. These moistened chips offered crabs protection against dehydration and direct light, as does seaweed in their natural habitat. They also reduced visual disturbance caused by the movements of observers. Emersions were performed at room temperature (18±1 °C) under natural photoperiod and lasted for 4–72 h for *C. maenas* and for 4–18 h for *N. puber*. The blood and muscle of emersed crabs was sampled only once to avoid secondary effects such as haemolymph loss and subsequent haemoconcentration and any effects of stress due to cheliped removal. In consequence, between three and five air-exposure experiments were carried out to record temporal changes in the variables studied throughout the whole emersion period.

Control crabs were kept immersed in running sea water throughout the air-exposure experiment. Their blood was sampled at the time that the experimental crabs were deprived of environmental water. This control value (C) is the pre-emersion value. In contrast to emersed crabs, blood could be taken from control crabs up to three times during a 24 h period since crabs quickly compensated for the reduced blood volume by drinking, provided that the blood withdrawn represented less than 2.6 % of body mass (Greco *et al.* 1986).

Preliminary experiments indicated that *N. puber* survive a prolonged emersion at 18 °C better during the night than during

the day. Therefore, emersion experiments were performed between 16:00 h and 10:00 h, for this species. In these conditions, 90 % of the crabs survived and recovered fully from emersion.

Reimmersion

Duplicate groups (two groups of eight crabs each) of *C. maenas* and *N. puber* were emersed for 72 h and 18 h, respectively, as described above but without any sampling disturbance. At the end of these air-exposure periods, the blood of all the crabs was sampled. The crabs were then reimmersed, by refilling the tanks with running sea water, and kept in these conditions during the period required for complete recovery (⩽24 h). Blood from crabs of one of the two groups was sampled after 1 h of reimmersion, then after 6 h, 12 h and 24 h. Blood from crabs of the other group was sampled following 3 h, 9 h and 24 h of reimmersion. Successive blood sampling of reimmersed crabs was assumed to be no more harmful than for control crabs.

Four other groups of crabs were similarly emersed and reimmersed and used to provide muscle samples. One group was sampled at the end of the air-exposure period, the others were sampled after 6 h, 12 h or 24 h of reimmersion.

Nitrogen excretion

Excretion in normoxic water

Both species were treated similarly. Crabs that had not been fed for 24 h were settled into 10 l PVC containers filled with 7.5 l of fresh sea water; this was aerated continuously, and water temperature was 18±1 °C. At this temperature and in sea water (33–34 ‰, pH 8), NH_4^+ accounts for 97.4 % of total ammonia (Bower and Bidwell, 1978); therefore, loss of gaseous ammonia (NH_3) was considered to be negligible.

In a preliminary study, no changes in ammonia content of sea water as the result of bacterial activity or algae were observed in control tanks (without any crabs) over the usual experimental period (<12 h).

Because nitrogen excretion by crabs is affected by handling stress (Hunter and Uglow, 1993), excretory products released during the first 2 h were not taken into account. The ammonia, amine and urea content of the sea water at the end of this 2 h period was taken as the value at time zero (T_0) for each of the three nitrogenous compounds. After T_0, sea water was sampled for its ammonia content every 2 h over an 8 h period. At the end of this period, sea water was also sampled for determination of amine and urea content. The mean ammonia excretion rate recorded in these conditions represented the pre-emersion rate. As emersed crabs were not fed during an experiment, the ammonia excretion rate of control crabs kept unfed for up to 24 h (*N. puber*) or 72 h (*C. maenas*) was determined in a similar manner.

Excretion during emersion

For estimation of nitrogen excretion rate during emersion, crabs of each species were emersed in boxes of the same size as described above but with unperforated bottoms. A small volume (30 ml) of filtered (0.45 μm) sea water was added to the bottom of each box (the water layer was less than 3 mm deep). This sea water was sampled (1 ml) for determination of its ammonia content every 4 h during the first 12 h of emersion, then every 24 h for *C. maenas*. For *N. puber*, it was sampled after 18 h of emersion only. The amine and urea contents of this sea water were determined after 18 h and 24 h of emersion for *N. puber* and *C. maenas*, respectively.

Excretion during reimmersion

After the maximum emersion period (either 18 h or 72 h), during which crabs were not disturbed by any sampling, crabs were transferred into 10 l PVC containers filled with 7.5 l of fresh and continuously aerated sea water. Sea water from each container was sampled (20 ml) for its ammonia content before introducing the crabs and exactly 5 min after reimmersion. The sea water was then sampled following 1 h, 3 h and 6 h of reimmersion. After this period, the sea water was totally renewed in order to avoid the effects of high environmental ammonia levels on ammonia excretion. The amounts of ammonia excreted by crabs between 6 h and 12 h of reimmersion were measured. The sea water was again renewed, and ammonia excretion was measured over the next 12 h. Following every renewal of sea water, a new T_0 value was recorded after 1 h.

Amine excretion during reimmersion was determined following the first 5 min of reimmersion and during the next hour only.

Measurements

Seawater analysis

Duplicate seawater samples (5 ml) were analysed immediately for their ammonia content using the hypochlorite method (Solorzano, 1969) adapted to small seawater volumes. For ammonia excreted during emersion, duplicate 500 μl samples of sea water were diluted 10-fold and treated in a similar manner. Samples were diluted when necessary with the sea water used for filling the experimental containers. This reference sea water was used as a blank and for making standard $(NH_4)_2SO_4$ solutions (2.5–25 μmol l^{-1}).

Amine content was determined using the method of North (1975). According to North (1975), compounds that give a maximum fluorescence when the reaction is performed at pH>9 represent amino acids and amides. Standard glycine solutions (1–10 μmol l^{-1}) were used for calibration.

Urea content was measured using the method of Newell *et al.* (1967) as modified by Davenport and Sayer (1986) for small samples. Standard urea solutions (10–100 μg l^{-1} urea-N) were used for calibration.

Blood and muscle analysis

Prebranchial haemolymph (approximately 500 μl) was collected through the arthropodial membrane at the base of the fourth pereiopod using a 1 ml sterilized syringe and a 21 gauge needle. Blood samples were transferred into refrigerated Eppendorf tubes, immediately shaken and kept on ice. Two

100 µl subsamples of clear haemolymph were diluted (5–20-fold) with double-distilled water for measurement of ammonia content using the microdiffusion method of Clinch *et al.* (1988), as previously (Regnault, 1992). Standard $(NH_4)_2SO_4$ solutions (10–60 µmol l^{-1}) in double-distilled water were used for calibration. Two 50 µl subsamples of haemolymph were analysed for urate content using a Sigma kit (685). Finally, duplicate haemolymph subsamples were deproteinized on ice with 10 % trichloroacetic acid, centrifuged (10 000 ***g*** at 4 °C for 15 min), and the supernatant was analysed for L-lactate content using a Sigma kit (826-UV) in which the glycine–hydrazine buffer was modified as recommended by Graham *et al.* (1983).

One cheliped was removed by forced autotomy, and the propodus muscle was dissected out rapidly on ice. Muscle tissue (100 mg) was weighed quickly in a refrigerated Eppendorf tube and homogenized (2×30 s) in 9 vols of ice-cold double-distilled water using a micro-crusher. Following centrifugation (10 000 ***g*** at 4 °C for 15 min), supernatants were diluted (10- to 50-fold) using double-distilled water and analysed immediately by microdiffusion for ammonia content.

Expression of results and statistics

Ammonia and urea excretion rates were expressed as µmol g^{-1} wet mass h^{-1}. Ammonia and urate contents of blood are expressed as µmol l^{-1}. Muscle ammonia content and blood lactate content are expressed as mmol l^{-1}. Values are expressed as means ± S.E.M. The significance of differences between means was assessed (P<0.05) using the Wilcoxon Mann–Whitney U-test. When possible, these statistical results were confirmed using Student's t-tests, either paired (intra-group comparisons) or unpaired (inter-group comparisons) as appropriate.

Results

Carcinus maenas

Nitrogen excretion

The mean ammonia excretion rate of control crabs (mean mass 80.3±3.1 g) in normoxic sea water was 0.20±0.05 µmol g^{-1} wet mass h^{-1} (N=10). Urea excretion rate was 0.014±0.004 µmol g^{-1} wet mass h^{-1} (N=10). Nitrogen excreted as amines (fluorescamine-positive compounds at pH 9.2; see Materials and methods) was detected at trace levels only. Thus, NH_4^+-N accounted for 88.4 % of total excreted nitrogen and urea-N accounted for 11.6 %. In control crabs kept unfed for 72 h, ammonia excretion rate was 0.088±0.015 µmol g^{-1} wet mass h^{-1} (N=10).

When *C. maenas* was exposed to air, its rate of nitrogen excretion was reduced markedly. During the first 12 h of air exposure, the mean ammonia excretion rate decreased significantly (P<0.05), reaching 0.024±0.007 µmol g^{-1} wet mass h^{-1} (N=10). It decreased significantly again in the following hours, representing 3 % of the pre-emersion rate after 48 h of emersion. NH_4^+-N remained the main nitrogenous excretory product (97.5 % of total excreted nitrogen) during the whole air-exposure period (72 h). Amine-N and urea-N constituted 1.55 % and 0.95 % of total excreted nitrogen, respectively. Thus, 1.0 mg of nitrogen was excreted by a 100 g crab during a 72 h air exposure, whereas 16.84 mg of nitrogen would be excreted by this crab kept immersed for the same period.

Following reimmersion of *C. maenas*, a very large ammonia release (approximately 96 µmol for a 100 g crab) was observed during the first 5 min (Fig. 1A). This sudden large ammonia output corresponded to an ammonia excretion rate of 11.5±1.7 µmol g^{-1} wet mass h^{-1} (N=10) or almost 60 times the pre-emersion rate. During the next hour, the mean ammonia excretion rate returned to slightly above the pre-emersion value, but was significantly (P<0.05) higher than the ammonia excretion rate of immersed crabs unfed for 72 h. Ammonia excretion rate then increased significantly (P<0.05) and remained high over an 11 h period, peaking at 0.586±0.052 µmol g^{-1} wet mass h^{-1} (N=10) by 6 h of reimmersion. At this time, ammonia excretion rate was approximately 3.5 times the pre-emersion rate. A mean ammonia excretion rate (0.237±0.021 µmol g^{-1} wet mass h^{-1}, N=10) significantly higher than the value of immersed and unfed crab (P<0.05) was observed over the last 12 h of reimmersion. No significant amine release was observed within either the first 5 min of reimmersion or the next hour.

Blood ammonia levels

In control crabs, the mean ammonia concentration of prebranchial haemolymph was 81.2±6.9 µmol l^{-1} (N=37). It remained at this level during the whole experimental period, as shown by immersed crabs bled again 48 h later (Fig. 2A).

During the first 12 h of air exposure, blood ammonia concentration increased significantly (P<0.05) at a rate of 4.47 µmol l^{-1} h^{-1}, reaching 134.8±10.2 µmol l^{-1} (N=32). Blood ammonia content then remained stable (approximately 120 µmol l^{-1}) until the end of the 72 h air-exposure period. At this time, haemolymph ammonia content was 117.8±17.6 µmol l^{-1} (N=26).

Following reimmersion, blood ammonia content decreased significantly (P<0.05) from 118.4±26.7 µmol l^{-1} (N=16) to 74.5±13.4 µmol l^{-1} (N=8) within the first hour (Fig. 2A). This value was similar to that observed in control crabs. Blood ammonia content then increased steadily (P<0.05) at a rate of 48.7 µmol l^{-1} h^{-1}, reaching 317.9±37.4 µmol l^{-1} (N=8) after 6 h of reimmersion. Blood ammonia content remained at this level during the next 6 h, then decreased. After 24 h of reimmersion, blood ammonia content (150.7±17.6 µmol l^{-1}, N=16) was still higher (P<0.05) than the pre-emersion value.

Muscle ammonia levels

The mean muscle ammonia content was 2.99±0.27 mmol l^{-1} (N=14) in control crabs. In air-exposed crabs, muscle ammonia content showed a tendency to increase during the first 12 h (Fig. 3A). At this time, muscle ammonia concentration was 4.49±0.82 mmol l^{-1} (N=12); although this value was higher than the pre-emersion value, the difference was not significant

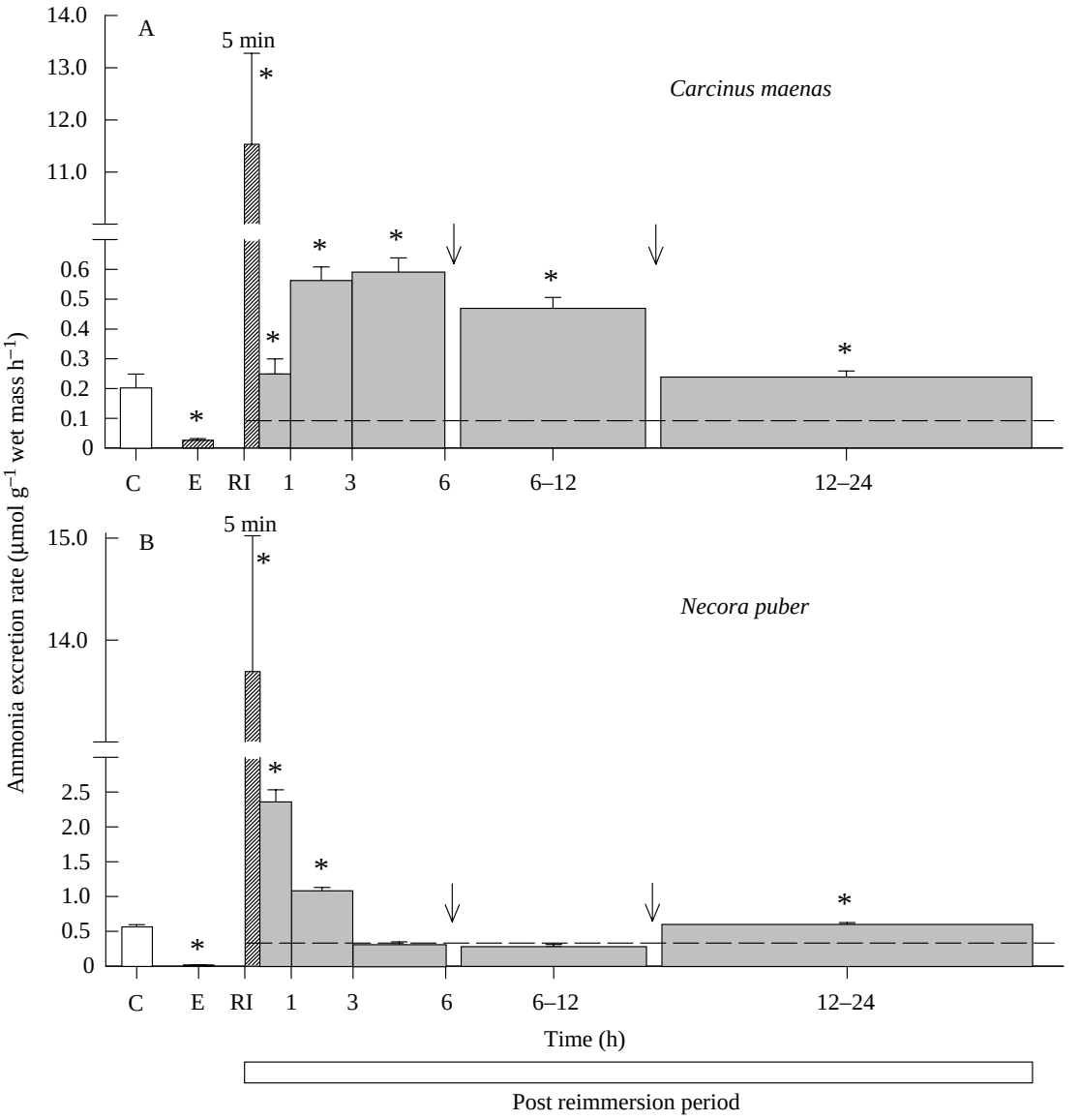

Fig. 1. Ammonia excretion rate (μmol g^{-1} wet mass h^{-1}) of *Carcinus maenas* (A) and *Necora puber* (B) during an experimental emersion (E) of 72 h (A) and 18 h (B) and following reimmersion (RI). The experiment was carried out at 18 °C. Control immersed crabs (C) represent the pre-emersion value. Dashed lines indicate the ammonia excretion rate of control crabs unfed for either 72 h (A) or 24 h (B). Arrows indicate renewal of sea water during the post-reimmersion period. Values are mean ± S.E.M. (N=10). The mean wet mass of the crabs was 80.3±3.1 g (*C. maenas*) and 77.8±5.3 g (*N. puber*). Significant changes (P<0.05) between unfed controls (dashed line) and experimental crabs are indicated by an asterisk.

(0.05<P<0.1). However, this increase appeared to be transient since muscle ammonia content returned to the pre-emersion level within the next 12 h of air exposure. Muscle ammonia content was 3.29±0.35 mmol l^{-1} (N=19) after 72 h of imposed emersion.

Reimmersion of crabs induced a significant increase (P<0.05) in muscle ammonia content from 3.18±0.43 mmol l^{-1} (N=8) to 5.15±0.68 mmol l^{-1} (N=8) within the first 6 h. Muscle ammonia content then returned to control levels. The value observed after 24 h of reimmersion (3.44±0.59 mmol l^{-1}, N=8) was not significantly (P>0.05) different from the pre-emersion value.

Blood urate levels

Blood urate content of control crabs was 78.9±8.3 μmol l^{-1} (N=15) (Fig. 4A). In air-exposed crabs, blood urate level was maintained during the first 4 h of emersion. A slightly lower urate content (67.4±3.9 μmol l^{-1}, N=14) was observed after 12 h of emersion. Blood urate level then increased at a rate of 0.72 μmol l^{-1} h^{-1} until the end of the air-exposure period. However, blood urate concentration was significantly higher (P<0.05) than the pre-emersion value after 72 h of air exposure only; at this time, blood urate content was 109.6±5.9 μmol l^{-1} (N=20).

In *C. maenas* exposed to air for 72 h without any sampling

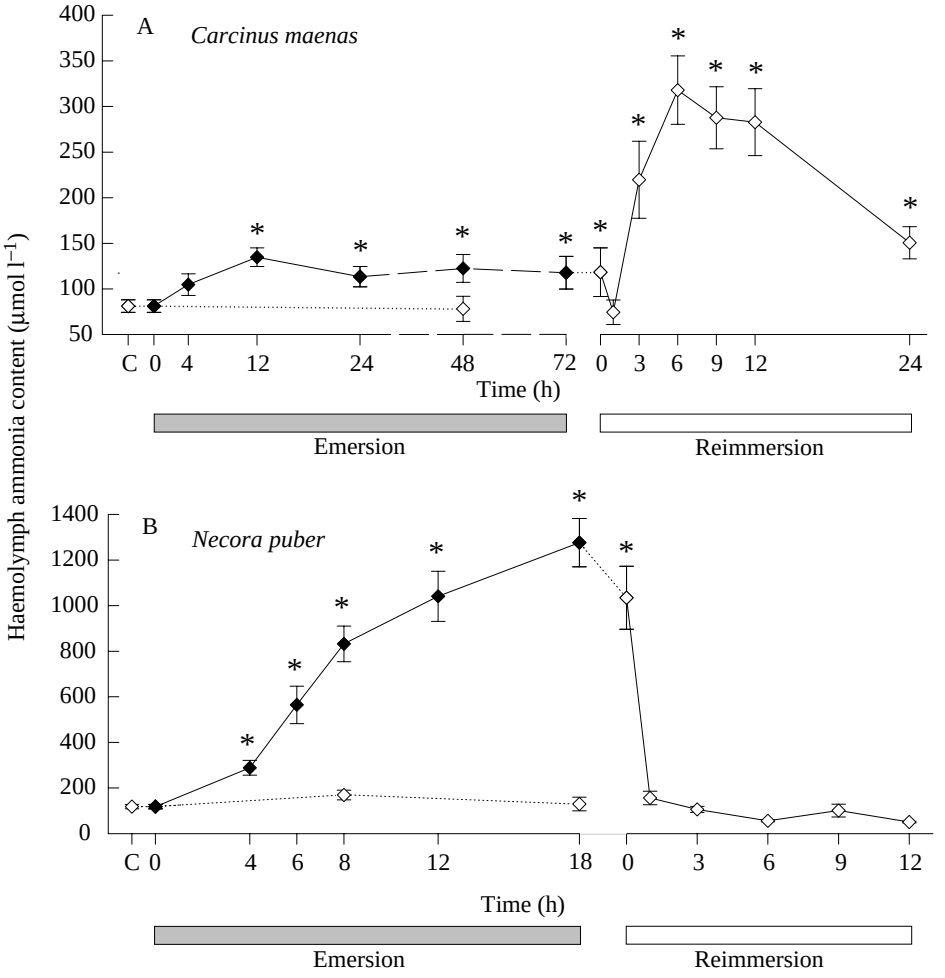

Fig. 2. Changes in haemolymph ammonia content (µmol l^{-1}) during prolonged air exposure and subsequent recovery. (A) *Carcinus maenas* exposed to air for 72 h and reimmersed for 24 h; values are means ± S.E.M., *N*=16–37 for the air-exposure period and *N*=8 during the reimmersion period. (B) *Necora puber* exposed to air for 18 h and reimmersed for 12 h; values are means ± S.E.M., *N*=10–35 during air exposure and *N*=8 during reimmersion. For both species, haemolymph ammonia content of control immersed crabs at the onset of air exposure (C, pre-emersion value) and during the emersion period (dotted line) is indicated. Significant changes ($P<0.05$) between control (C) and experimental crabs are indicated by an asterisk.

disturbance, blood urate content was 115.9±7.2 µmol l^{-1} (*N*=16). Following reimmersion, blood urate content did not change during the first hour (122.5±10.8 µmol l^{-1}, *N*=8), but had returned to the pre-emersion level after 3 h of reimmersion (85.9±5.6 µmol l^{-1}, *N*=8) (Fig. 4A).

Necora puber

Nitrogen excretion

The mean ammonia excretion rate of *N. puber* (mean mass 77.8±5.3 g, *N*=10) in normoxic sea water was 0.552±0.041 µmol g^{-1} wet mass h^{-1} (*N*=10). Urea excretion rate was 0.014±0.001 µmol g^{-1} wet mass h^{-1} (*N*=10), and nitrogen excreted as amines was detected at trace levels only. Thus, NH_4^+-N accounted for 94.9 % of total excreted nitrogen and urea-N accounted for 5.1 %. In control crabs kept unfed for 24 h, the ammonia excretion rate was 0.316±0.028 µmol g^{-1} wet mass h^{-1} (*N*=10).

In air-exposed crabs, the mean ammonia excretion rate was 0.017±0.001 µmol g^{-1} wet mass h^{-1} during the whole air-exposure period, representing 3.2 % of the pre-emersion rate. NH_4^+-N remained the main nitrogenous excretory product (97.2 % of total excreted nitrogen), urea-N and amine-N representing 2.55 % and 0.25 %, respectively. Thus, 0.46 mg of nitrogen was excreted by a 100 g crab during an 18 h air exposure, whereas 12.31 mg of nitrogen would be excreted by this crab kept immersed for the same period.

At reimmersion, *N. puber* released a large amount of ammonia (approximately 114 µmol for a 100 g crab) within the first 5 min of reimmersion (Fig. 1B). This ammonia release corresponds to an ammonia excretion rate of 13.7±1.3 µmol g^{-1} wet mass h^{-1} (*N*=8) or 25 times the pre-emersion rate. Following this initial output, high ammonia excretion rates were recorded for the next 3 h: 2.35±0.18 µmol g^{-1} wet mass h^{-1} (*N*=8) during the first hour of reimmersion and 1.07±0.05 µmol g^{-1} wet mass h^{-1} (*N*=8) over the next 2 h. These excretion rates were respectively four and two times higher than the control value. The mean ammonia excretion rate recorded between 3 and 12 h of approximately 0.30 µmol g^{-1} wet mass h^{-1} was not significantly different from the rate of ammonia excretion of unfed controls. During the last 12 h of reimmersion, ammonia excretion rate was 0.596±0.029 µmol g^{-1} wet mass h^{-1} (*N*=8) and was not significantly different from the pre-emersion level but was significantly higher than the value for unfed controls.

During the first 5 min following reimmersion, a release of 42.6 µmol of amine-N for a 100 g crab was observed. Amine release proceeded, although at a lower level (11.2 µmol of amine-N for a 100 g crab), during the next hour.

Blood ammonia levels

The ammonia concentration of prebranchial haemolymph of control crabs was 118.3±9.4 μmol l^{-1} (*N*=16) and did not change significantly during the experimental period (Fig. 2B).

In air-exposed crabs, blood ammonia content increased markedly. A significant (P<0.05) increase was observed after 4 h of emersion (288.6±32.1 μmol l^{-1}, *N*=10), indicating that ammonia levels had increased at a rate of 42.6 μmol l^{-1} h^{-1}. This rate of increase was maintained during the whole experimental period, except between 4 and 8 h of air exposure, when a higher rate (136 μmol l^{-1} h^{-1}) was observed. At the end of the 18 h air exposure, the mean ammonia content of the blood was 1276.6±106.2 μmol l^{-1} (*N*=35). Even higher blood ammonia concentrations (2500–3000 μmol l^{-1}) were observed in some *N. puber* emersed for 18 h; these individuals recovered fully when reimmersed.

Following reimmersion, removal of the blood ammonia load was extremely rapid. A blood ammonia content not significantly different from the pre-emersion level was observed after 1 h of reimmersion. Recovery of blood ammonia concentration was completed within 3 h, since ammonia content was 105.9±13.3 μmol l^{-1} (*N*=8) at this time.

Muscle ammonia levels

Control crabs had a muscle ammonia content of 7.24±1.02 mmol l^{-1} (*N*=8). During air exposure, muscle ammonia content increased, although it was significantly higher (P<0.05) than the pre-emersion value only at the end of the air-exposure period (Fig. 3B). At this time, mean ammonia concentration in muscle was 10.42±0.99 mmol l^{-1} (*N*=10). Individual values as high as 17.4 mmol l^{-1} were recorded.

Following reimmersion, muscle ammonia content was determined in three crabs after 6 h. At this time, muscle ammonia content was not significantly different from control values (5.49±0.33 mmol l^{-1}). This suggests that recovery of muscle ammonia content was achieved within a few hours.

Blood urate levels

The mean blood urate content of control crabs was 98.4±6.8 μmol l^{-1} (*N*=16). In response to air exposure, blood urate content increased steadily (P<0.05) at a rate of 46.5 μmol l^{-1} h^{-1} for the first 8 h of emersion (Fig. 4B), after which the rate of increase was lower. Blood urate concentration reached a value of 659.3±19.7 μmol l^{-1} (*N*=36) at the end of the air-exposure period.

Following reimmersion, the blood urate content of *N. puber* decreased steadily during the first 6 h of reimmersion. The rate

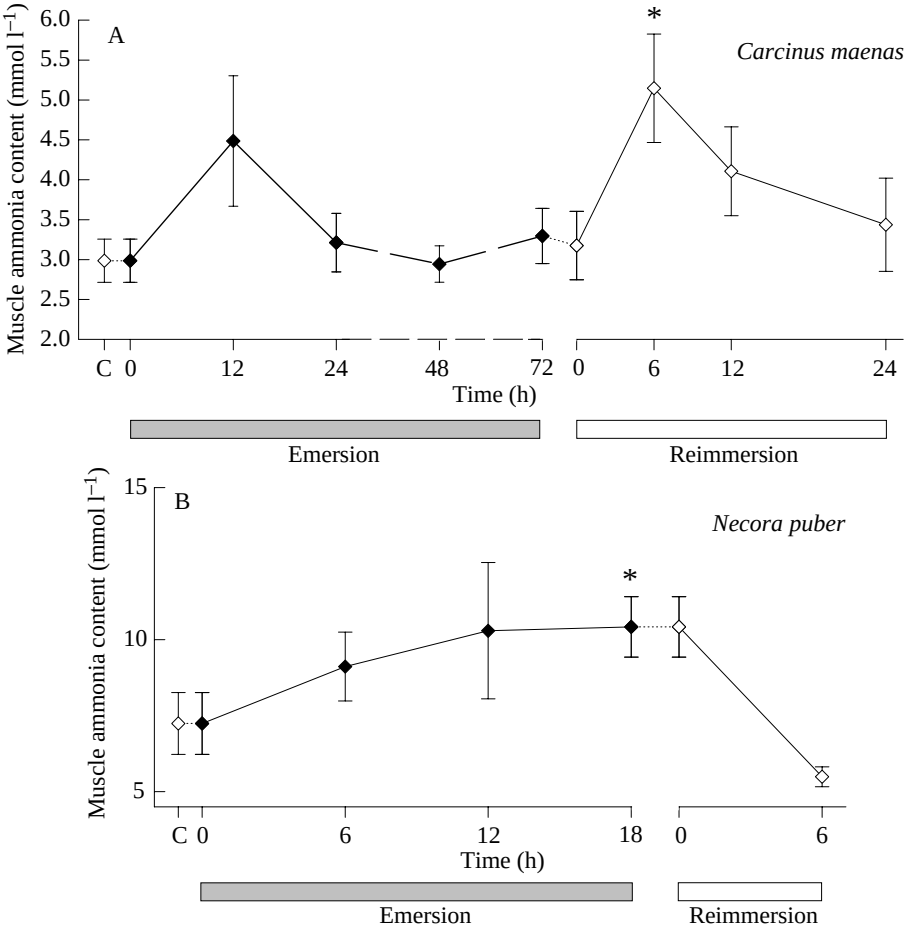

Fig. 3. Changes in muscle ammonia content (mmol l^{-1}) during prolonged air exposure and subsequent recovery. (A) *Carcinus maenas* emersed for 72 h and reimmersed for 24 h; values are means ± S.E.M., *N*=12–19 during air exposure and *N*=8 during reimmersion. (B) *Necora puber* exposed to air for 18 h and reimmersed for 6 h; values are means ± S.E.M., *N*=8–10 during air exposure and *N*=3 during reimmersion. For both species, the pre-emersion value (C) was the muscle ammonia content of control immersed crabs at the onset of air exposure. Significant changes (P<0.05) between control (C) and experimental crabs are indicated by an asterisk.

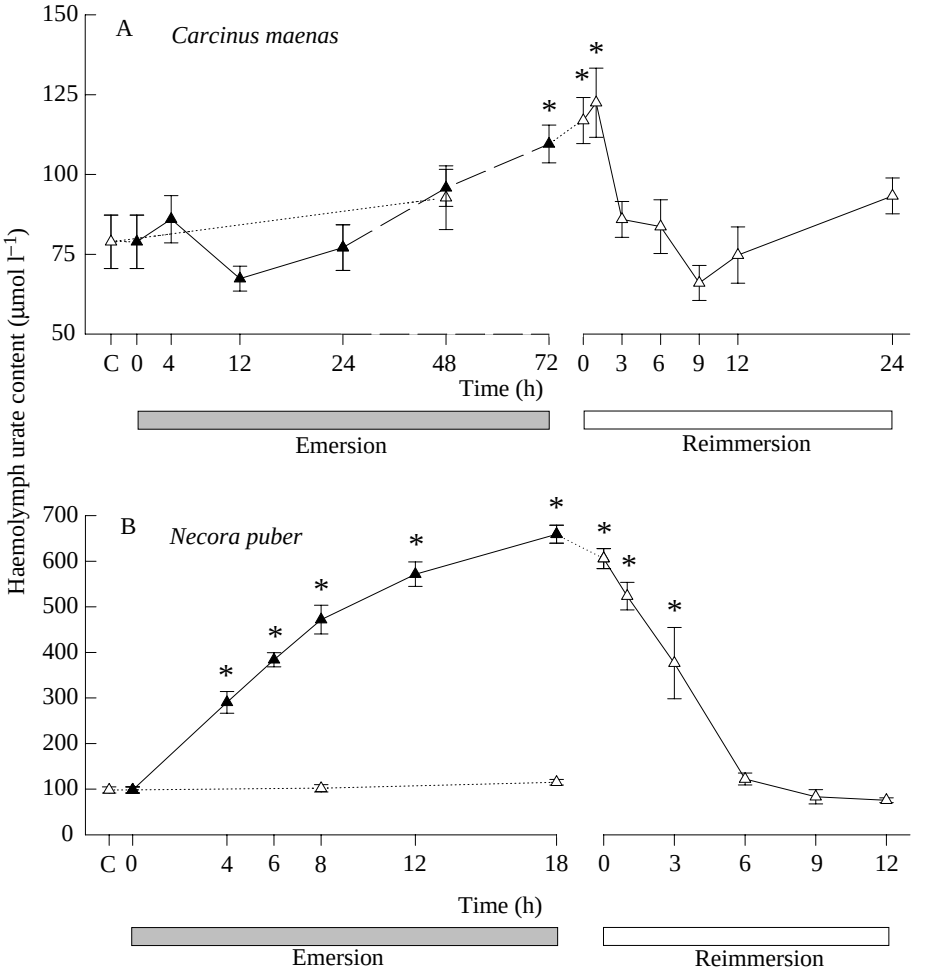

Fig. 4. Changes in haemolymph urate content (µmol l^{-1}) during prolonged air exposure and subsequent recovery. (A) *Carcinus maenas*; values are means ± S.E.M., *N*=14–20 during air exposure and *N*=8 during reimmersion. (B) *Necora puber*; values are means ± S.E.M., *N*=10–35 during air exposure and *N*=8 during reimmersion. Other details are as in Fig. 2.

of decrease was higher than the rate of increase observed during emersion. Blood urate content was not significantly different from control values at 122.3±13.1 µmol l^{-1} (*N*=8) after 6 h of reimmersion and 83.3±15.6 µmol l^{-1} (*N*=8) after 9 h of reimmersion. In this species, recovery of blood urate level was completed within 6 h.

Nitrogen balance

In both species, nitrogen balance was calculated for a 100 g crab. This was aimed at comparing the presumed nitrogen overload resulting from emersion with the total nitrogen released during the recovery period. Using the time required for complete recovery of all variables studied, a recovery period of 12 h for *N. puber* and 24 h for *C. maenas* was used for this calculation.

The presumed nitrogen overload was estimated as the difference between the amount of nitrogen excreted by an emersed crab during the whole air-exposure period and the amount excreted by an immersed control during an equivalent period. The presumed nitrogen overloads were 15.8 and 11.8 mg of nitrogen for *C. maenas* and *N. puber*, respectively.

The total nitrogen released during the recovery period was the sum of the amount of nitrogen released within the first 5 min of reimmersion and the amount excreted in excess during the subsequent recovery period. This latter nitrogen excess was calculated by subtracting the amount of nitrogen excreted by a control crab unfed for either 24 h or 72 h, according to the species (see Fig. 1), from the amount of nitrogen excreted by a reimmersed crab. Total nitrogen release was 12.7 mg for *C. maenas* and 6.6 mg for *N. puber*.

The calculated nitrogen balance indicated that the total nitrogen released during the recovery period represented 80 % of the presumed nitrogen overload in *C. maenas* and 57 % in *N. puber*.

Blood lactate levels in C. maenas *and* N. puber

In *C. maenas*, blood lactate content was 0.344±0.031 mmol l^{-1} (*N*=10) in control crabs. Compared with this pre-emersion value, blood lactate content decreased within the first 12 h of emersion (Fig. 5). At 12 h, blood lactate content (0.143±0.013 mmol l^{-1}, *N*=10) was significantly lower ($P<0.05$) than the pre-emersion value. It remained stable during the following 24 h. At the end of the imposed air exposure (72 h), haemolymph lactate content was 0.327±0.015 mmol l^{-1} (*N*=10), a value not significantly different from the pre-emersion value.

In *N. puber*, blood lactate content of control crabs was 0.194±0.017 mmol l^{-1} (*N*=8). A marked increase ($P<0.05$) in

blood lactate levels was observed within the first 4 h of emersion. The high rate of increase was maintained over the whole emersion period, and blood lactate level finally reached 63.1±3.9 mmol l^{-1} (N=10) in crabs exposed to air for 18 h.

Discussion

Nitrogen excretion

Both *C. maenas* and *N. puber* showed a high degree of ammoniotelism, since approximately 88 % and 95 %, respectively, of nitrogen was excreted as ammonia, when immersed. Little nitrogen was excreted as urea and amines. In both species, ammonia excretion was rapidly and strongly reduced by air exposure, but the excretion of amines and urea did not increase significantly. This indicated that no major shift in pathways of nitrogen catabolism occurs in response to prolonged emersion. This is corroborated by studies on the nitrogen excretion of *Cancer pagurus* emersed for 18 h (Regnault, 1994) and of semiterrestrial crabs, which remain ammoniotelic, although exceptions do exist (for a review, see Greenaway, 1991). *Birgus latro* is known to excrete urate in the faeces (Greenaway and Morris, 1989), although most land crabs do not excrete urate but store it in detectable amounts in intracellular compartments (Linton and Greenaway, 1997*a*,*b*). According to these authors, urate synthesis and its storage represent a nitrogen reserve rather than a storage form of nitrogen in response to an impediment to ammonia excretion. Preliminary measurements of urate levels in the whole body of *C. maenas* indicated that total urate levels did not increase after a 72 h exposure to air (F. Durand, unpublished data). This is not surprising given the high activity of uricase in this species (Dykens, 1991).

During the first 12 h of emersion, the ammonia excretion rate was reduced to 12 % and 3 % of pre-emersion values in *C. maenas* and *N. puber*, respectively. This cannot be attributed to a total loss of branchial water since some branchial water is still present at this time (Depledge, 1984; Henry, 1994; Regnault, 1994). Furthermore, some exchanges between branchial water and the sea water covering the bottom of the box could occur by capillary transfer *via* the surface of the pericardial sac (Drach, 1939; Mason, 1970). In *C. maenas* exposed to air for a much longer period than *N. puber*, the ammonia excretion rate decreased further when air exposure was prolonged. A decrease in ammonia excretion when crabs are deprived of environmental water could be the result of several processes. (i) A reduction in the exchange area between the haemolymph and branchial water caused by the collapse of gill lamellae under these conditions. This will be prevented in *C. maenas* both by expansion of the marginal canal of the lamellae (Drach, 1930) and by the presence of inter-lamellar bridges (Johnson and Uglow, 1985). Since ammonia excretion rates of both species were reduced to a similar low value during the first 12 h of air exposure, a reduction of the exchange area alone could not be responsible for the low rates of ammonia excretion recorded. (ii) Ammonia accumulation in branchial water (up to 1.9 mmol l^{-1} in *Cancer pagurus* exposed to air for 18 h; Regnault, 1994) will oppose the diffusion and/or electrochemical gradients required for passive ammonia release (Kormanik and Cameron, 1981; Kormanik and Evans, 1984; Greenaway, 1991), thus impairing this mode of ammonia excretion. (iii) An increase in the ammonia content of the seawater layer on the bottom of the box could inhibit excretion. The ammonia content of this water was 1.1 mmol l^{-1}

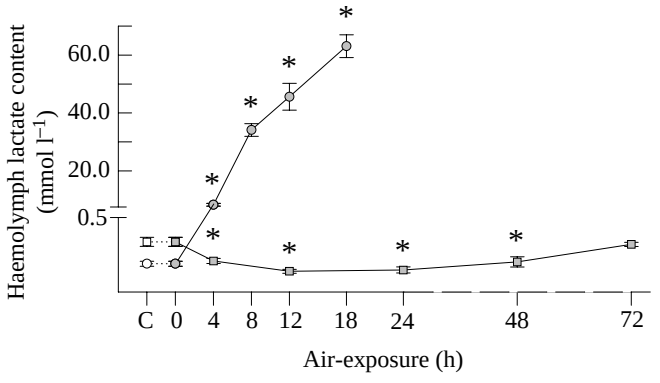

Fig. 5. Changes in haemolymph lactate content (mmol l^{-1}) of *Carcinus maenas* (squares) exposed to air for 72 h and of *Necora puber* (circles) exposed to air for 18 h; values are means ± S.E.M., N=10 for *C. maenas* and N=8–10 for *N. puber*. C, pre-emersion value. Significant changes (P<0.05) between control (C) and experimental crabs are indicated by an asterisk.

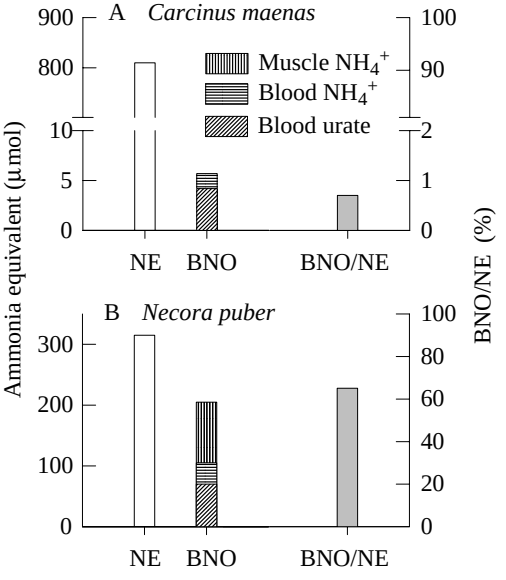

Fig. 6. Comparison between the nitrogen excess (NE, µmol), the body nitrogen overload (BNO, µmol) and the BNO/NE ratio (%) in *Carcinus maenas* (A) and *Necora puber* (B). NE, nitrogen excreted by a reimmersed crab (excluding the initial nitrogen burst) above control levels; BNO, nitrogen accumulated as ammonia and urate (1 urate is equivalent to $4NH_4^+$) in the blood and muscle of an emersed crab. Haemolymph and muscle were assumed to represent 30 % and 25 % of body mass, respectively. For both species, values were calculated for a 100 g crab.

(*N. puber*) and 2.3 mmol l^{-1} (*C. maenas*) at the end of emersion. Crabs were not surrounded by this water but, because exchanges between it and the branchial water are possible, its high ammonia level might contribute to the reduction in the rate of ammonia excretion.

In semi-terrestrial crabs, urine recycling in the branchial chamber favours ammonia excretion by the maintenance of active Na^+/NH_4^+ exchanges (Wolcott, 1991). *C. maenas* has been observed to reprocess its urine in the branchial chamber (Harris and Santos, 1993), although the effects of this process on ammonia excretion have not been investigated. This process nevertheless might be responsible for the low ammonia excretion rate of *C. maenas* after 48 h of air exposure.

In crabs, the main function of the antennal gland is in hydromineral regulation. The bladder is a small compartment (no more than 2 % of body mass in *C. maenas*; Riegel *et al.* 1974) and little ammonia is excreted in the urine (Regnault, 1987). This is also true for terrestrial and semi-terrestrial crabs (Gifford, 1968; Wolcott and Wolcott, 1987; Greenaway and Nakamura, 1991; Dela-Cruz and Morris, 1997). Urine secretion continues during emersion, although at a lower rate (Harris and Santos, 1993); however, the ammonia from the urine would have been mixed with the water from the bottom of the box and was taken into account during the estimation of ammonia excretion during emersion.

The nitrogen burst at reimmersion

In both species, a very large ammonia release (approximately 100 µmol for a 100 g crab) was observed during the first 5 min of reimmersion. Such a large ammonia output at reimmersion has been observed previously in *Cancer pagurus* (Regnault, 1994). This ammonia burst, which corresponded in the present study to an ammonia excretion rate 25 times higher (*N. puber*) and 60 times higher (*C. maenas*) than the pre-emersion rate, could not represent a realistic metabolic rate. Handling stress, which was observed to increase the ammonia excretion rate by 1.5- to twofold (F. Durand, unpublished data), together with an increase in the metabolic rate (a maximal fivefold increase), could not explain such a large enhancement of ammonia excretion. Consequently, this ammonia output must represent the draining of an extracellular compartment, as discussed previously by Regnault (1994). In *C. maenas*, the glycosaminoglycan (GAG) content of gills was observed to increase steadily throughout a 72 h air exposure (Regnault and Durand, 1998). Enrichment of gill tissue with these anionic polymers, which are major components of the extracellular matrix, suggests that GAG synthesis in response to air exposure might preserve this tissue from dehydration and favour NH_4^+ storage in the extracellular matrix. Since GAGs behave like any cation exchanger, the NH_4^+ would be liberated at reimmersion when the electrolyte concentration and pH of the branchial water changed. Thus, the gill extracellular matrix rather than branchial water stores would be implicated in the sudden release of ammonia observed at this time.

Nitrogen balance

From the calculations of nitrogen balance, 57 % and 80 % of the presumed nitrogen overload was released by *N. puber* and *C. maenas*, respectively, during the recovery period. This discrepancy may be due to a possible underestimation of nitrogen excretion during emersion as a result of gaseous NH_3 excretion. Release of gaseous NH_3 from pleon fluid is known in terrestrial isopods (Wright and O'Donnell, 1993). In two highly terrestrial crabs, *Geograpsus grayi* and *Ocypode quadrata*, NH_3 volatilization occurs in the branchial chambers (Greenaway and Nakamura, 1991; De Vries and Wolcott, 1993), but in most amphibious crabs, little or no ammonia is excreted in gaseous form (Wood and Boutilier, 1985; Linton and Greenaway, 1995; Dela-Cruz and Morris, 1997). Thus, even if gaseous ammonia excretion cannot be discounted for *C. maenas*, this possibility is extremely unlikely in the marine species *N. puber*, which does not naturally experience prolonged air exposure. Consequently, the above discrepancy is unlikely to be due to the production of gaseous NH_3 during emersion.

In *Cancer pagurus*, a reduction in the rate of nitrogen metabolism to approximately 30 % of its pre-emersion level in response to air exposure was considered to be the result of metabolic depression (Regnault, 1992). This was also observed, to a larger extent, in *Cardisoma carnifex*, which appeared to cease its ammonia production rather than turn to excretion of gaseous ammonia (Wood *et al.* 1986). Assuming that this adaptive process operates in *C. maenas* and *N. puber*, the rate of ammonia production would be reduced to 80 % and 55–60 % of the pre-emersion level, respectively, according to our calculated balance. The 80 % decrease in the rate of oxygen consumption of emersed *N. puber* observed by Johnson and Uglow (1985) suggests a more profound decrease in general metabolism than that implied by the decrease in the rate of ammonia production (this study). Moreover, the shift of *N. puber* to anaerobic metabolism, as shown by the increased blood lactate content, indicates that the depression of metabolic rate was not fully effective. In contrast to *N. puber*, the 20 % reduction in the rate of nitrogen production observed in *C. maenas* was in agreement with the 20–30 % decrease in the rate of oxygen consumption observed in this species during air exposure (Newell *et al.* 1972; Wallace, 1972). In spite of this moderate decrease in metabolic rate, *C. maenas* did not employ anaerobic metabolism. However, the slight decrease in nitrogen metabolism observed in *C. maenas* during prolonged air exposure could be the result of a reduction in general activity (quiescence of emersed crabs) as well as a depression of metabolic rate *per se*. Thus, resorting to a strategy of metabolic depression seems unnecessary for this species, which is known for its ability to survive in both sea water and air (Taylor and Butler, 1978).

Blood and muscle ammonia content

In both species, blood ammonia content increased during the first 12 h of air exposure, although the amplitudes of these increases were not equivalent: the rate of increase was 10–30

times higher in *N. puber* than in *C. maenas*. This general pattern indicates that ammonia production did not cease during air exposure, despite the reduction in the rate of ammonia excretion. An increase in blood ammonia levels in response to air exposure has been observed previously in several species following 4–18 h of air exposure (deFur and McMahon, 1984; Schmitt and Uglow, 1997; Regnault, 1992).

After the first 12 h of air exposure, blood ammonia level increased further in *N. puber*, whereas it remained stable for the next 60 h of emersion in *C. maenas*. This indicated that *C. maenas*, in contrast to *N. puber*, can regulate its blood ammonia level. This is only apparent after a prolonged air exposure and, consequently, has not been reported for other marine species. It is also of interest to note that some specimens of *N. puber* had a very high blood ammonia content which, nevertheless, was not lethal since these specimens recovered totally following reimmersion.

Muscle ammonia content increased in response to air exposure in *N. puber*. Few studies have investigated the muscle ammonia content of crustaceans (Vincent-Marique and Gilles, 1970; Henry and Cameron, 1981; Shinagawa *et al.* 1995), and changes in response to air exposure have not been reported to our knowledge. In the amphibious fish *Periophthalmus cantonensis*, ammonia accumulated in muscle when the fish were emersed or exposed to high environmental ammonia levels (Iwata, 1988). Such an increase presumably indicates that muscle tissue can store ammonia during air exposure as it does during muscular work. In the present study, muscle ammonia content increased continuously in *N. puber* during the emersion period, and muscle tissue appeared to be a site of ammonia storage. In contrast, the slight transient increase in muscle ammonia level observed in *C. maenas* indicates that muscle tissue may be a site of temporary ammonia storage but also a site of ammonia detoxification, for example by the synthesis of nitrogenous compounds. In *C. maenas*, this suggestion is corroborated by the pattern during reimmersion: (i) an increase in muscle ammonia content early in recovery (Fig. 3A), (ii) an increase in blood ammonia content (Fig. 2A) and (iii) simultaneously elevated ammonia excretion rates (Fig. 1A). These patterns suggest that nitrogenous compounds in muscle were used as metabolic substrates in the first 12 h of reimmersion. Neither emersed nor immersed *C. maenas* exhibited the high levels of blood ammonia observed during the first 12 h of recovery. While muscle ammonia content had returned to the pre-emersion level after 12 h of reimmersion, blood ammonia content remained higher than its pre-emersion level even after 24 h, suggesting that nitrogen catabolism was still elevated. Non-essential amino acids could be good candidates for the products of this process. An increase in the muscle amino acid pool in response to either emersion or environmental ammonia overload has been observed in an amphibious fish (Iwata, 1988) and in the isopod *Porcellio scaber* (Wright *et al.* 1996). In addition, the activity of the NADH-dependent glutamate dehydrogenase (the main route for ammonia incorporation in non-essential amino acids) was observed to increase in muscle of *Cancer pagurus* following 12 h of air exposure (Regnault, 1992). In contrast, *N. puber* exhibited little or no ability to regulate the ammonia level, either in blood or in muscle, and ammonia storage as amino acids in this species seems to be unlikely during emersion.

Blood urate and lactate levels

In *N. puber*, blood urate content increased markedly throughout the period of air exposure and, although surprisingly high values, given the solubility of urate, were recorded (approximately 660 μmol l^{-1}), similarly high blood urate levels have been observed previously in crustaceans (Henry and Cameron, 1981). An increase in blood urate levels was observed, although at a lower rate, in *Cancer pagurus* emersed for 18 h (Regnault, 1992). Regnault (1992) suggested that blood urate accumulation was the result of the inhibition of urate breakdown rather than the enhancement of uricogenesis. Uricase is known to be inhibited when oxygen availability is reduced and, consequently, urate accumulates in the blood under hypoxic conditions (Dykens, 1991). Urate accumulation in the blood of *N. puber* suggests that this species rapidly developed internal hypoxia. This is corroborated by the rapid increase in its blood lactate content which, after 4 h of air exposure, was 9 mmol l^{-1}, a value similar to that observed after the same period in *Cancer productus* (deFur and McMahon, 1984). The very high rate of increase in blood lactate levels observed in *N. puber* shows that this species switches very rapidly to anaerobic metabolism to meet its energetic needs when emersed.

In contrast, the blood urate content of *C. maena*s did not increase significantly until 72 h of air exposure, at which point it was lower than that recorded in this species after an imposed environmental hypoxia (Lallier *et al.* 1987). Moreover, lactate did not accumulate in the blood of air-exposed crabs. Although *C. maenas* developed some internal hypoxia late in the emersion period, it did not resort to anaerobic metabolism and probably adjusted its metabolic rate to the oxygen availability. Metabolic rate depression, which is observed in several intertidal species in order to avoid anoxia (Storey and Storey, 1990), would be a possible response of *C. maenas* when facing a prolonged air exposure.

Recovery of the body nitrogen overload

Two different patterns of recovery were observed in the two species studied. In *Necora puber*, ammonia excretion rate was enhanced during the first 3 h of reimmersion, but during the next 9 h of reimmersion the rate was similar to that of immersed *N. puber* unfed for 24 h. Both blood and muscle ammonia contents decreased within a few hours of reimmersion, indicating that total ammonia clearance from these two body compartments was achieved rapidly. The slower decrease in blood urate level indicates that urate breakdown occurred gradually and that uricase activity was not impeded, suggesting that the oxygen debt resulting from air exposure was rapidly repaid (deFur and Mc Mahon, 1984; Varley and Greenaway, 1992).

Carcinus maenas exhibited a totally different pattern of

response during the recovery period. In the first hour following the initial burst release of ammonia, its ammonia excretion rate was similar to the pre-emersion rate. However, this rate was significantly higher than that of controls kept immersed and unfed for 72 h. In comparison with these controls, the rate of ammonia excretion of reimmersed crabs during the first hour of reimmersion would lead to a total clearance of ammonia from the haemolymph. The enhancement of the rate of ammonia excretion in the subsequent hours suggests that unexcreted ammonia was stored as nitrogenous compounds rather than as free NH_4^+.

Since ammonia excreted after the initial nitrogen burst originated from internal body compartments, it is of interest to compare the excreted nitrogen excess excluding the initial burst (NE) with the body nitrogen overload (BNO, see Results for definitions). The BNO is the nitrogen (ammonia and urate) that accumulated above the control level in the blood and muscle of emersed crabs throughout the whole air-exposure period.

In *N. puber*, a BNO of 205 μmol was calculated. Thus, blood and muscle nitrogen accounted for 65 % of the excreted NE (315 μmol; Fig. 6B). The remaining 35 % might indicate a large ammonia accumulation in other body compartments, since haemolymph and muscle together account for only 55 % of the total body mass. The large ammonia output and the brief recovery period for internal ammonia levels both suggest that the whole body of *N. puber* was overloaded with ammonia.

In *C. maenas*, the BNO (6 μmol) accounted for less than 1 % of NE (810 μmol; Fig. 6A). This observation, together with changes in blood and muscle ammonia levels during both the emersion and recovery periods, supports the suggestion that most of the unexcreted ammonia during the imposed emersion was used for amino acid synthesis.

Conclusion

In *Necora puber*, the 40–45 % decrease in the rate of nitrogen production observed during air exposure indicates that its first defence against emersion-induced effects is a switch to hypometabolism or metabolic rate depression, to which many intertidal marine invertebrates are known to resort. However, in *N. puber*, anaerobic metabolism was required to meet energetic demands, showing that metabolic depression was not fully effective. In consequence, the rate of nitrogen metabolism, although lowered, remained at a detectable level. In spite of this, *N. puber* was unable to prevent the internal ammonia overload that developed during emersion. Ammonia accumulation was observed in both blood and muscle but also in the whole body, as shown by the BNO/NE ratio, and it is likely that a general ammonia intoxication resulted from prolonged air exposure. In this study, *N. puber* resorted to a survival strategy rather than to adaptation processes and exhibited little or no ability to regulate nitrogen metabolism in the aerial environment.

In contrast to *N. puber*, the rate of nitrogen metabolism in *C. maenas* was slightly reduced during imposed air exposure for periods as long as 72 h. This reduction could be the result of either the generally reduced activity exhibited by quiescent emersed *C. maenas* or a metabolic rate depression *per se*, which remains questionable in view of the known ability of *C. maenas* to breath in both sea water and air. Moreover, *C. maenas* could regulate its nitrogen metabolism under these conditions. The mechanisms and metabolic pathways involved in this regulation, in which amino acid synthesis appears to have a key role, as suggested by the BNO/NE ratio and the recovery events, remain to be investigated. Besides the obvious and previously demonstrated adaptations of its respiratory and osmoregulatory processes, the ability of *C. maenas* to regulate its nitrogen metabolism during emersion confirms that such adaptations are also necessary for crustaceans to evolve a terrestrial existence.

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