AMMONIA EXCRETION IN THE ROCK CRAB, *CANCER IRRORATUS*: THE EFFECT OF VARYING AMMONIA GRADIENTS

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Ammonotelic aquatic organisms may excrete ammonia ($NH_3 + NH_4^+$; hereafter referred to as T_{amm}) across the surface of the gills via active exchange mechanisms or by passive diffusion. Since ammonia exists in aqueous systems either as the gas NH_3, or as the ion NH_4^+, diffusion may occur via either form (Kormanik, G. A. and J. A. Cameron, Mar. Biol. Lett., 1981a; J. Comp. Physiol. 141:457-462, 1981b; Am. Zool. 21:1042, 1981c). They suggest that NH_3 is the predominant form in the blue crab and the freshwater catfish while Goldstein et al. (J. Exp. Zool. 219:395-397, 1982) suggest that diffusion of ammonia is predominantly in the form of NH_4^+ in two species of sea water teleost fish. In order to compare the role of NH_3 and NH_4^+ in diffusion, we examined T_{amm} excretion in the rock crab, *Cancer irroratus*, in response to variations in the gradients for NH_3 and NH_4^+.

Rock crabs (100 to 200 grams) were purchased from a local fisherman, maintained in running sea water at 15 ± 1°C and not fed for at least 48 hours prior to use in experiments. To measure T_{amm} excretion, crabs were transferred through a series of sea water baths, for durations of two hours in each bath. Water samples were taken from the baths at the beginning of the experiment and at half-hourly intervals thereafter. T_{amm} in sea water was determined by the phenol-hypochlorite method (Solorzano, L., Limnol. Oceanog. 14:799-801, 1969). Water pH (at 15°C) was determined at half-hourly intervals as well. Blood was removed from the crabs by puncture of a rubber septum glued (cyanoacrylate) over a hole drilled in the dorsal carapace over the heart, prior to the start of exposure to the first sea water bath (SW1), at the end of exposure to the second sea water bath, and at the end of exposure to the third sea water bath (SW2). The blood was kept on ice and the pH of a sample was immediately determined using an IL Blood/Gas Analyzer Model 213. The remaining blood was allowed to clot. The clot was disrupted by forcing the blood repeatedly through a syringe needle. The clot was spun down, and the serum stored frozen until T_{amm} was assayed enzymatically with a Sigma Kit #170-UV.

The NH_3 and NH_4^+ gradients were modified as follows: In Experiment 1 the T_{amm} concentration of the experimental bath was increased over that of the blood by addition of NH_4Cl to ca. 1 mM (actually 1000 ± 10 µM, n = 12). The pH was not modified. In Experiment 2, the T_{amm} concentration of the experimental sea water bath was increased only slightly, by addition of NH_4Cl to ca. 80 µM (actually 78 ± 7 µM, n = 9). The pH was adjusted upwards, to ca. 9, by addition of 5 M NaOH. It was necessary to add more NaOH at half-hourly intervals to maintain the elevated bath pH. Thus by manipulation of T_{amm} concentration and pH, the NH_3 and NH_4^+ gradients could be modified independently of one another. NH_4^+ concentration and the partial pressure of NH_3 (pNH_3) were calculated from the Henderson-Hasselbalch equation (Kormanik & Cameron, 1981a) with values for pK, and solubility for sea water (= 500 mM NaCl) from Cameron and Heisler (J. Exp. Biol. 105:107-125, 1983). Additionally, we determined the transepithelial potential (t.e.p.) during some of these transfer experiments (see Kormanik & Cameron, 1981b, for methods) since the electrical gradient is important to the movement of NH_4^+.

Table 1. Summary of measured T_{amm} (μM) and pH in water and blood from Experiments 1 and 2. N = 7 to 12.

Experiment 1

pH	SW1	high T_{amm} SW	SW2
blood	7.856 ± 0.072	8.033 ± 0.027	8.042 ± 0.009
water	7.73 ± 0.04	7.72 ± 0.04	7.75 ± 0.05
$[T_{amm}]$			
blood	372 ± 59	412 ± 42	327 ± 38
water	24.6 ± 3.2	885 ± 17	34.4 ± 3.6

Experiment 2

pH	SW1	low T_{amm} high pH SW	SW2
blood	8.027 ± 0.027	8.005 ± 0.050	7.928 ± 0.039
water	7.75 ± 0.03	9.03 ± 0.05	7.93 ± 0.02
$[T_{amm}]$			
blood	317 ± 48	311 ± 76	324 ± 42
water	32.8 ± 9.0	36.9 ± 3.7	25.6 ± 5.3

Table 2. T_{amm} excretion in the crab, <u>Cancer irroratus</u>, transferred through a series of sea water baths, where excretion is expressed in $\mu mol \cdot 100g^{-1} \cdot h^{-1}$, n = 9 to 12. (+ sign indicates movement or gradient directed from the blood to the sea water medium.)

Experiment 1

	SW1	high T_{amm} SW	SW2
T_{amm} excretion	+6.09 ± 1.10	-31.6 ± 9.3*	+10.9 ± 2.4**
blood to SW gradients			
NH_4 (in μM)	+348 ± 34	-464 ± 34	+299 ± 46
pNH_3 (in μTorr)	+102 ± 10	-2.5 ± 13	+138 ± 20

Experiment 2

	SW2	low T_{amm}, high pH SW	SW2
T_{amm} excretion	+9.14 ± 2.40	-16.4 ± 2.1**	+9.3 ± 2.2**
blood to SW gradients			
NH_4 (in μM)	+282 ± 35	+297 ± 44	+284 ± 37
pNH_3 (in μTorr)	+128 ± 10	-47.3 ± 34	+104 ± 13

Significantly different compared to the previous bath
* $p < 0.01$
** $p < 0.001$

Table 1 reports the conditions under which T_{amm} excretion rates were examined. All values are reported as averages for the two hour exposure to each bath. In Experiment 1, the T_{amm} of the experimental sea water solution was elevated far above ambient sea water, but the pH was not significantly different ($p > 0.1$) from SW1 or SW2. The ambient sea water T_{amm} concentration was over twice that of the blood. In Experiment 2, the T_{amm} concentration of the experimental solution was elevated only slightly but was still far lower than that of the blood. The value reported in Table 1 (36.8 µM) is the average, and actually lower than the initial bath concentration (78 ± 7 µM, n = 9) since in this experiment T_{amm} disappeared from the bath water (see below). These data indicate the necessity of using measured T_{amm} concentrations for the determination of prevailing gradients rather than nominal values used by other investigators (Goldstein et al., 1982). Additionally, the latter failed to take into consideration the t.e.p. and pNH_3 gradients, thus the gradients they report are suspect.

The pH of the Experiment 2 sea water bath was over one unit higher than either SW1 or SW2. To determine if T_{amm} would be lost from the sea water bath due to volatilization of NH_3 we prepared a bath with 1 mM T_{amm} and high pH (ca. 9), without crabs. The T_{amm} concentration did not change over the several hours we made measurements. Therefore any changes in the concentration of T_{amm} in the bath represent movements into or out of the animal.

T_{amm} excretion and the calculated pNH_3 and NH_4^+ gradients, from blood to water, are reported in Table 2. The t.e.p. we measured was -1.1 ± 0.1 mV (n = 3), inside negative in SW1, which decreased to -1.25 ± 0.3 mV (n = 3) in high T_{amm} SW. The latter would be expected to affect the NH_4^+ gradient only by about 5% in this investigation and can therefore be neglected in the gradient calculations. In Experiment 1 (Table 2), the crabs excreted T_{amm} in SW1. Both the pNH_3 and the NH_4^+ gradients were directed from blood to bath. In the high T_{amm} SW bath, however, the crabs took up T_{amm}, as represented by a decrease in T_{amm} in the bath. In this case, the pNH_3 gradient was essentially abolished (not significantly different from zero, $p > 0.1$), while the NH_4^+ gradient was directed from the bath to the blood. In the final bath, SW2, typical gradients were reestablished, from blood to bath, and T_{amm} excretion again proceeded. Thus with the pNH_3 gradient abolished, net T_{amm} uptake appears to proceed in response to the NH_4^+ gradient. Of what relative importance is the pNH_3 gradient? In Experiment 2, in the SW1 bath, T_{amm} excretion again proceeded down the gradients for pNH_3 and NH_4^+, both directed from blood to bath. In the experimental bath, however, while a substantial NH_4^+ gradient was directed from blood to bath, the pNH_3 gradient was directed from bath to blood. In spite of the direction of the NH_4^+ gradient, the crabs took up T_{amm}. When typical gradients were again established in SW2, from blood to water, T_{amm} excretion again proceeded from blood to water. Thus, even a small pNH_3 gradient can oppose a substantial gradient for NH_4^+. These observations support previous data of Kormanik and Cameron (1981a,b,c) which demonstrated that T_{amm} excretion occurs not only by diffusion of the NH_4^+, but also in the form of the gas, NH_3. (Funded by NSF PCM 83-02621 to DHE and NSF-ROA and UNC-A intramural grant to GAK.)