SALINITY ADAPTATIONS IN THE EURYHALINE GREEN CRAB, *CARCINUS MAENAS*

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Euryhaline marine crustaceans are osmotic and ionic conformers when adapted to high salinity (33 ppt); their hemolymph osmotic and ionic concentrations passively reflect those in the surrounding seawater. Hemolymph osmotic concentration is made up primarily of salts (NaCl), but intracellular osmotic concentration contains high amounts of small organic molecules (free amino acids and quaternary ammonium compounds). These organisms can invade the more dilute waters of the estuary because they possess the ability to activate physiological and biochemical mechanisms that allow them to regulate hemolymph osmotic and ionic concentrations above those in the ambient water. However, these organisms still undergo significant hemodilution during the acute phase of low salinity acclimation, and this can result in tissue water gain and cell swelling. Cell volume is usually readjusted by reducing the intracellular concentration of these free amino acids (FAA) and quaternary ammonium compounds. These are released intact from the cells into the hemolymph and are believed to be metabolized by some as of yet unidentified tissue. Therefore, low salinity adaptation involves a coordinated response between ion regulation and cell volume regulation.

Carcinus maenas, the green shore crab, is a euryhaline marine species that is an osmoconformer at high salinity but that makes the transition to osmoregulation at a salinity of approximately 26 ppt. When transferred from 33 to 12 ppt, the hemolymph undergoes a dilution of about 200 mOsm in the initial 24 hr post-transfer (Table 1). This initiates the cell volume regulatory response, as evidenced by the increase in FAA in the hemolymph, as these substances are released from the intracellular space. The FAA are measured indirectly by assaying for the concentration of total ninhydrin-positive substances, or TNPS. Ninhydrin reacts with ammonia, amino acids, and quaternary ammonium compounds and can be detected colorimetrically. In crustacean tissues, the overwhelming majority of intracellular organic osmotic effectors is known to be the FAA. Hemolymph ammonia, as measured by the phenol-hypochlorite method on deproteinized samples, does not change, indicating that the FAA are neither metabolized prior to release into the hemolymph nor in the hemolymph itself.

Table 1. Time course of changes in hemolymph osmotic concentrations, and concentrations of total ninhydrin positive substances (TNPS) and ammonia in *C. maenas* acclimated to 33 ppt and transferred to 12 ppt. Mean $\pm$ SEM (N = 6). T = 12°C.

Treatment	Osmolality (mOsm kg^{-1} H_2O)	TNPS (mM)	Ammonia (uM)
33 ppt acclimated	915 $\pm$ 5	2.26 $\pm$ 0.5	179 $\pm$ 28
33-12 ppt acute transfer			
T1 hr	905 $\pm$ 8	2.75 $\pm$ 0.5	176 $\pm$ 27
T4 hr	834 $\pm$ 6	4.14 $\pm$ 0.7	185 $\pm$ 15
T12hr	751 $\pm$ 16	3.84 $\pm$ 0.5	163 $\pm$ 33

T24 hr	713 ± 29	6.57 ± 0.9	192 ± 18
T48 hr	696 ± 40	7.80 ± 0.9	196 ± 19
T96 hr	674 ± 37	5.11 ± 0.3	119 ± 14
T7 days	667 ± 30	4.40 ± 0.3	181 ± 23

In all euryhaline marine invertebrates, including crustaceans, FAA are known not to be excreted from the animal; rather, it is believed that high rates of ammonia excretion during low salinity acclimation are indicative of FAA deamination, conservation of the carbon skeleton, and excretion of the amino group. *C. maenas* has a typical profile of ammonia excretion in response to low salinity exposure (Table 2): a significant increase in the rate of excretion almost immediately after low salinity transfer, peak rates occurring at the same time (4-48 hr) that tissue FAA are being released from cells into the hemolymph.

Table 2. Ammonia excretion in intact *C. maenas* acclimated to 33 ppt and at various times after transferred to 12 pt. Mean ± SEM (N=6). T = 12°C.

	Ammonia excretion (n mol gm^{-1} hr^{-1})						
33 ppt	T 2hr	T 4 hr	T 6 hr	T 12 hr	T 24 hr	T 96 hr	T 7day
182 ± 40	438 ± 80	541 ± 120	594 ± 160	688 ± 120	412 ± 30	382 ± 30	306 ± 50

The most likely site of deamination of FAA is the gill. If the FAA are taken up from the hemolymph by the gills and deaminated intracellularly, the carbon skeleton could be used for energy, and the resulting ammonia could be excreted directly into the ambient water without altering hemolymph ammonia concentrations. Initial evidence for this comes from the comparison of the intracellular FAA pools in leg muscle vs gills. At 33 ppt, the FAA pool in leg muscle is about 4-5 times higher than in either G2 or G9 (Table 3). After transfer to 12 ppt, the FAA pool in leg muscle declines rapidly over 24 hr and stabilizes at new, lower levels thereafter. The decline parallels the increases in hemolymph FAA and ammonia excretion. In contrast, the FAA pool in the gills actually increases during the acute phase of low salinity acclimation, possibly indicating uptake of amino acids from the hemolymph.

Table 3. The concentration of total ninhydrin positive substances (TNPS) in leg muscle, anterior (G2) and posterior (G9) gills of *C. maenas* acclimated to 33 ppt and transferred to 12 ppt. Mean ± SEM (N = 6). T = 12°C.

	TNPS (m mol gm^{-1} wet weight)		
Treatment	Leg	G2	G9
33 ppt acclimated	750 ± 137	151 ± 18	176 ± 20
33 to 12 ppt transfer			
T 6hr	603 ± 109	203 ± 30	261 ± 35
T 12 hr	666 ± 102	209 ± 31	246 ± 37
T 24 hr	460 ± 50	294 ± 37	370 ± 57
T 48 hr	406 ± 52	274 ± 46	415 ± 40
T 96 hr	416 ± 32	216 ± 62	368 ± 39
T 7day	365 ± 121	291 ± 16	500 ± 39

Measurements of ammonia excretion from isolated anterior and posterior gills also supports this idea. Anterior (G4) and posterior (G8) were dissected out of crabs acclimated to 33 ppt, gently blotted free of seawater and hemolymph, and placed in cold, millipore-filtered seawater for 30 min in order to determine tissue ammonia production. At 33 ppt, G3 and G9 had about the same rate of ammonia production (Table 4). Anterior and posterior gills taken from crabs that had been exposed to 12 ppt for 2 hr had elevated rates of ammonia production, consistent with the gill as the central site of amino acid deamination during the cell volume regulatory response. Interestingly, the elevated rate of ammonia production persisted only in G3 after crabs were acclimated to 12 ppt for 14 days.

Table 4. Ammonia production from isolated anterior (G3) and posterior (G8) gills of *C. maenas* acclimated to 33 ppt and transferred to 12 ppt. Mean $\pm$ SEM (N = 5). T = 12°C.

	Ammonia production (u mol gm^{-1} hr^{-1})		
	33 ppt acclimated	33 - 12 ppt T 2hr	12 ppt acclimated
G3	0.472 $\pm$ 0.07	0.600 $\pm$ 0.11	0.770 $\pm$ 0.08
G8	0.504 $\pm$ 0.06	0.661 $\pm$ 0.11	0.492 $\pm$ 0.08

These results strongly suggest that during the process of low salinity acclimation, in addition to the osmotic and ionic regulatory ability of *C. maenas*, mechanisms of cell volume regulation are also necessary for survival in low salinity. The green crab appears to have a typical invertebrate cell volume regulatory response: cell swelling followed by reduction of the intracellular FAA pool, loss of cellular water, and restoration of cell volume. The FAA pool is reduced by release of amino acids into the hemolymph, and transport via the hemolymph to the gills where they are taken up and deaminated. The resultant ammonia is excreted directly into the ambient water, and the carbon skeleton is conserved.

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