EFFECT OF SEAWATER OSMOLALITY ON HEMOLYMPH LEVELS OF METHYL FARNESOATE IN THE GREEN SHORE CRAB *CARCINUS MAENAS*

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Similar to other euryhaline crabs, the green shore crab *Carcinus maenas* regulates its hemolymph osmolality. In hypoosmotic seawater below 27 ppt salinity (~800 mOsm/kg) these crabs osmoregulate and maintain hemolymph osmolality above that of the seawater, whereas in seawater between 27 ppt salinity and 32 ppt salinity (full-strength seawater, ~920 mOsm/kg) they osmoconform. Transfer of *C. maenas* to hypoosmotic seawater also causes a chronic rise in hemolymph levels of methyl farnesoate (MF), a juvenile hormone analog found in crustaceans (Lovett, D.L. et al., Comp. Biochem. Physiol. 128A:299-306, 2001). MF levels begin to rise in animals transferred to seawater below 20 ppt salinity, and the rise is inversely related to salinity. While this relationship suggests that MF may have a role in osmoregulation, the rise in MF levels might reflect a general response to osmotic stress. Furthermore, it is not clear whether the response to low salinity seawater is due to the low ionic or low osmotic conditions of hypoosmotic seawater.

To investigate these issues, male green crabs were acclimated to 5, 27, 32, 40 or 50 ppt salinity seawater in recirculating tanks with biological filters. Other animals were acclimated to 27 ppt (820 mOsm/kg) seawater and then some were transferred for 2 days to 5, 20, or 32 ppt salinity seawater, while others were transferred for 2 days to 5 ppt or 20 ppt seawater containing mannitol. Sufficient mannitol was added to increase the osmolality of these solutions to that of 27 ppt seawater and full-strength seawater, respectively. Mannitol (a solute that does not cross cell membranes) was added to increase the osmotic concentration of the seawater without altering the ionic concentration. The osmolality of the saltwater solutions and the hemolymph was measured using a vapor pressure osmometer. Hemolymph levels of MF were measured using HPLC (Borst, D.W., and Tsukimura, B., J. Chromatography 545:71-78, 1991).

Table I. Effects of acclimation salinity on hemolymph osmolality and methyl farnesoate (MF) concentration in the green shore crab *Carcinus maenas*, acclimated to 5, 27, 32, 40 or 50 ppt salinity seawater for 2 weeks. Mean ± SE indicated. For MF concentrations, means with same letter are not significantly different ($\alpha > 0.05$; ANOVA, $F_{4,47} = 4.853$, $P = 0.0023$).

Composition of acclimation medium	Osmolality of medium (mOsm/kg)	N	Osmolality of hemolymph (mOsm/kg)	Conc. of MF in hemolymph (ng/mL)
5 ppt	174 ± 2	10	548 ± 20	31.0 ± 11.9
27 ppt	820 ± 1	15	896 ± 6	6.4 ± 1.7[a]
32 ppt	918 ± 1	10	960 ± 7	5.3 ± 1.7[a]
40 ppt	1213 ± 2	10	1180 ± 8	1.1 ± 0.7[a]
50 ppt	1487 ± 10	9	1462 ± 6	4.9 ± 1.0[a]

Table II. Effects of various seawater solutions on hemolymph osmolality and methyl farnesoate (MF) concentration in the green shore crab *Carcinus maenas*. Animals were acclimated to 27 ppt salinity seawater for 18 days. Some crabs were then transferred for 2 days to 5, 20, or 32 ppt salinity seawater, while others were transferred to solutions of 5 or 20 ppt seawater containing mannitol. Mannitol was added to increase the osmotic concentration of these solutions to that of 27 and 32 ppt seawater, respectively. Mean ± SE indicated. For MF concentrations, means with same letter are not significantly different ($\alpha > 0.05$; ANOVA, $F_{4,30} = 10.726$, $P < 0.0001$).

Composition of medium	Osmolality of medium (mOsm/kg)	N	Osmolality of hemolymph (mOsm/kg)	Conc. of MF in hemolymph (ng/mL)
5 ppt	111 ± 5	5	511 ± 28	33.6 ± 6.3[a]
20 ppt	538 ± 4	6	775 ± 12	7.5 ± 3.0[b]
32 ppt	845 ± 3	6	884 ± 10	2.9 ± 1.1[b]
5 ppt + mannitol	818 ± 6	9	963 ± 9	19.9 ± 4.8[a]
20 ppt + mannitol	985 ± 7	9	928 ± 10	3.7 ± 1.5[b]

As can be seen in Table I, crabs acclimated to hypoosmotic (5 ppt salinity) seawater were osmoregulating, since the osmolality of their hemolymph was substantially higher than that of the seawater. In contrast, animals maintained in seawater at the crab's isoosmotic point (27 ppt salinity) and in hypersaline seawater (40 and 50 ppt salinity) were osmoconforming, since the osmolality of their hemolymph was similar to that of their seawater solutions. Hemolymph levels of MF were elevated only in crabs that were osmoregulating. Crabs maintained in hypoosmotic seawater (5 ppt salinity) had MF levels (31 ng/ml) that were nearly five-fold higher than the levels (6.4 ng/ml) observed in animals acclimated to isoosmotic seawater (27 ppt salinity). The MF levels of crabs acclimated in full-strength seawater (32 ppt) and in hypersaline seawater (40 ppt and 50 ppt salinity) were similar to those observed in animals maintained in isoosmotic seawater. Thus, osmotic stress *per se* is not sufficient to elevate MF levels, since this response was observed only in animals maintained in hypoosmotic conditions and not in those maintained in hyperosmotic conditions.

Table II shows that the response to hypoosmotic seawater appeared to be due largely to hypoionic conditions rather than the hypoosmotic conditions. Crabs maintained in hypoionic seawater made isoosmotic by the addition of mannitol (5 ppt salinity seawater plus mannitol) still had elevated levels of MF. In contrast, MF levels were not elevated in crabs maintained in a saltwater solution that was nearly isoionic with seawater at the crab's isosomotic point (20 ppt salinity seawater plus mannitol). Hence, it does not appear that the addition of mannitol affected MF levels. Therefore, when crabs were transferred to dilute seawater, it was the low concentration of specific ion(s), and not low total osmolality of the external medium, that elicited an increase in hemolymph levels of MF. Further studies are in progress to determine which specific ion(s) elicit an increase in MF levels when they are present in low concentrations.

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