SALT AND WATER BALANCE IN *HEXAGENIA LIMBATA* (EPHEMEROPTERA: EPHEMERIDAE) WHEN EXPOSED TO BRACKISH WATER

MICHAEL A. CHADWICK[1,2], HEATHER HUNTER[1], JACK W. FEMINELLA[1] AND RAYMOND P. HENRY[1]
[1]Department of Biological Sciences, 331 Funchess Hall, Auburn University, Auburn, AL 36849-5414

[2]Present address: Department of Biological Sciences, 5722 Deering Hall
University of Maine, Orono, ME, 04469-5722

Like most aquatic insects, mayflies rarely occur in saline habitats, probably because of intolerance to elevated salt (NaCl) concentrations ("halophobic" species, sensu Gallardo-Mayenco 1994). However, some mayflies are tolerant to increased salinity. Berner & Sloan (1954) reported *Callibaetis floridanus* (Baetidae) occurring in brackish waters (2-10 ppt), and *Tricorythus* (Tricorythidae) was tolerant of salinities from 0.2 - 3.2 ppt (Goetsch & Palmer 1997). In an Oklahoma stream, *Hexagenia limbata* (Ephemeridae) was unaffected by saline discharge (Magdych 1984). Recently, a population of *H. limbata* was found in seasonally saline reaches (0-25 ppt) of the Mobile River (Chadwick & Feminella 2001).

Occurrence and survival of freshwater insects in brackish water prompt basic questions about what physiological mechanisms allow insects to persist in saline habitats. Aquatic invertebrates employ 2 physiological mechanisms of salinity adaptation, osmoregulation and cell volume regulation (reviewed by Pierce & Amende 1981, Bradley 1985). Osmoregulation allows the animal to maintain its hemolymph osmotic concentration, thus maintaining a relatively constant internal state. Alternatively, cell volume regulation occurs in animals that cannot regulate the osmotic concentration of their hemolymph. Hemolymph osmotic concentration increases with increasing ambient salinity, which results in an osmotic gradient that reduces intracellular water and causes cells to shrink. By increasing their internal osmotic pressure through synthesis of organic compounds (e.g., amino acids and sugars), cells can restore normal volume by regaining lost water. Our work examined how increased salinity affected total body water balance and hemolymph osmoregulation for a salinity-tolerant population of *H. limbata* from the Mobile River delta.

Late instars (19-28 mm total length) were collected live from Dead Lake, a distributary of the Mobile River, SW Alabama. Nymphs were brought to the laboratory in chilled, aerated river water (0 ppt salinity), where they were transferred to tanks containing aerated river water, and held at room temperature for at least 7 d before being used in experiments.

To assess water balance, nymphs were exposed to 1 of 4 salinity treatments (0, 5, 8, 12 ppt, n = 5), with 1 nymph per treatment replicate. Initially, nymphs were blotted dry and weighed individually on a microbalance (Sartorius 1712 MP8) to the nearest 0.01 mg. Nymphs were then randomly placed into a salinity treatment, and reweighed 1, 2, 4, 8 h after the start of the experiment to quantify water loss or gain (as whole-animal change in wet mass). A Kruskal-Wallace test ($\alpha = 0.05$) was used to examine relative mass change (initial—reweighed mass) across the time periods for each salinity treatment.

All nymphs survived the 8-h experiment. Individual wet mass varied from 62.16 to 164.78 mg. No difference in whole animal wet-mass change over time was found for any salinity treatment (0 ppt: $F_{4,20} = 2.12$, $p = 0.1163$; 5 ppt: $F_{4,20} = 2.55$, $p = 0.0709$; 8 ppt: $F_{4,20} = 2.15$, $p = 0.1122$; 12 ppt: $F_{4,20} = 0.06$, $p = 0.9927$). Further, no discernible trend in wet-mass change among salinity treatments was observed (Fig. 1).

If nymphs at higher salinities regulated their cell volume, then these insects would have initially lost cellular water because of osmotic gradients, unless they regulated their hemolymph osmolality. That no wet-mass differences were detected at any time suggest that these insects regulate hemolymph osmolality rather than cell volume. However, this interpretation must be taken with caution because of unknown variation in wet-mass associated with water adhering to surfaces, especially the gills and within the ali-

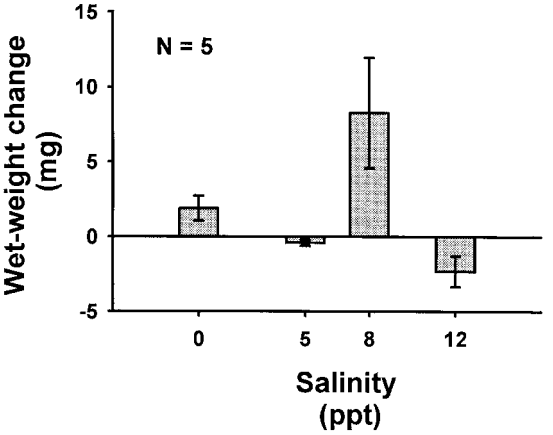

Fig. 1. Mean (±SE) whole animal wet-mass change for *H. limbata* nymphs after 8 h of exposure to 4 salinity treatments.

mentary canal. Nevertheless, in terms of mortality, survival of an 8-h exposure to elevated salinity is similar to what *H. limbata* would experience in tidal portions of the Mobile River. Thus, these results suggest individuals within this population can endure the osmotic stress associated with brief saline periods with little appreciable change in water balance.

A 2nd experiment was conducted to assess the effects of elevated salinity on hemolymph osmolality. Live nymphs not used in the water balance experiment were divided equally among 4 salinity levels (0, 5, 8, 12 ppt), where they were acclimated for 7 d. Hemolymph was then extracted by making a cut above the terminal filament and allowing hemolymph (~10 μL) to drip into sample vials. Osmolality was analyzed with a Wescor 5100c vapor pressure osmometer, and 1-way ANOVA was used to assess differences in osmolality among treatments ($\alpha = 0.05$). A Dunnett's *t* test was used to compare pairwise differences in osmolality between each salinity treatment and the 0 ppt control.

Acute change in salinity from 0 to 12 ppt resulted in 100% mortality over the 7-d acclimation period, but, like the 1st experiment, there was 100% survivorship within the other 3 treatments (0, 5, 8 ppt). Among treatments where nymphs survived**,** hemolymph osmolality differed significantly ($F_{2,27} = 19.74$, $p < 0.001$). However, only the 8-ppt treatment differed significantly from the 0 ppt control. Mean osmolality increased with increasing salinity (Fig. 2), but the osmotic pressure (i.e., difference between ambient osmotic concentration and hemolymph concentration) decreased with increasing salinity.

In the treatments < 8 ppt, nymphs were able to regulate their hemolymph osmotic concentration at levels hyperosmotic to the ambient water. At 8 ppt, hemolymph was essentially isosmotic. Results of both experiments suggest that at salinities > 8 ppt nymphs lose ability to osmoregulate and begin to osmoconform, with mortality ensuing under exposure to increased salinity. The failure of freshwater organisms to survive in high-salinity environments is believed to result from an inability to increase cellular concentrations of organic compounds, such as amino acids and sugars, to match increases in hemolymph salt concentrations (Gainey & Greenberg 1977). Euryhaline insect larvae (e.g., mosquitoes) can survive up to 20 ppt, and do so by synthesizing proline, serine and trehalose (Garrett & Bradley 1987). It is possible that nymphs of *H. limbata* lack a similar ability, and are therefore limited to environmental salinities in which they are osmoregulators.

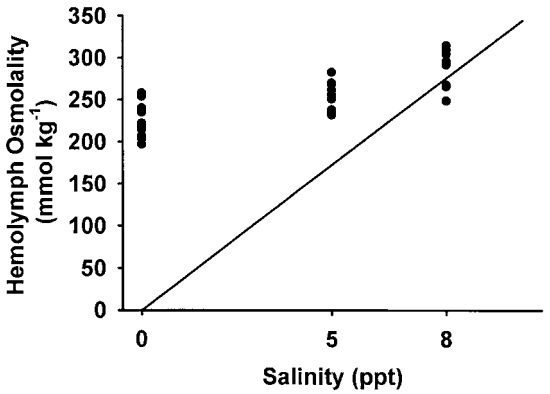

Fig. 2. Hemolymph osmolality for *H. limbata* nymphs at 3 salinity treatments. The solid line represents isotonic conditions.

Summary

Hexagenia limbata nymphs were shown to regulate hemolymph osmolality at salinities < 8 ppt. Nymphs survived for extended periods (7 d) in isosmotic conditions (~8 ppt). However, individuals exposed to salinities > 8 ppt could not survive for extended periods, but they could tolerate these conditions for 8 h, a time period that approximates tidal inundation.

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