WHOLE ANIMAL VOLUME REGULATION IN THE ATLANTIC HAGFISH, MYXINE GLUTINOSA, EXPOSED TO 85 % AND 115 % SEA WATER.

Tes Toop and David H. Evans,
Department of Zoology, University of Florida, Gainesville, FL 32611

Previous studies have shown that hagfishes (Class Agnatha) osmoconform over the narrow range of salinities tolerated (for review see M.W. Hardisty, The Biology of Cyclostomes, Chapman and Hall, London. 1979). The Atlantic hagfish, Myxine glutinosa, survives successfully in salinities ranging between 57 % and 130 % sea water, providing that the daily concentration change does not exceed 15 mOsm (Cholette et al, Comp. Biochem. Physiol. 33: 333-346, 1970). McFarland and Munz (Comp. Biochem. Physiol. 14: 383-398, 1965) examined the regulation of body weight and serum composition in the Pacific hagfish, Eptatretus stoutii, during 7 days exposure to a range of low and high salinity waters (40 - 122 % sea water, SW) followed by a 7 day return to 100 % SW. Hagfish in 80 % and 122 % SW survived the salinity perturbations with maximum weight change occurring on the first day. There was a slow readjustment of body volume over 7 days in hyposaline animals, but no volume readjustment occurred in hypersaline fish. Plasma concentrations were isosmotic with the environment. No similar whole body volume regulation study has been performed with M. glutinosa. As a prelude to future studies on changes in natriuretic peptide receptor populations in different salinities, we investigated the ability of M. glutinosa to survive sudden hypo- and hypersaline stress and its capability, if any, of regulating whole body volume. In addition, we wanted to know at what time during the experiment maximum weight perturbation occurred.

Fifteen hagfish (mean mass: 40.8±2.5 g) were placed in tared plastic tubs (approximately 1 liter) with drainage holes drilled around the lid and base. Hagfish were then submerged in 100 % SW (922±7.7 mOsm) and allowed to recover from handling. During the next 24-48 h each hagfish within its tub was weighed until we were confident that reproducible weights could be obtained. These weights were averaged for the pre-experimental weight. Five hagfish were then transferred into each salinity (either 85 % or 115 % SW, previous observations had shown that these were the maximum and minimum salinities comfortably tolerated by the hagfish). The remaining five hagfish were maintained in 100 % SW as controls. All hagfish were maintained between 12 - 14 C. Hagfish were weighed at 6, 12, 24, and 36 h, after the start of the experiment and daily thereafter for 13 days. Percent weight gain or loss were calculated. In addition, once the time of maximum weight change had been established, a further set of animals was set up in each salinity for sacrifice at this time. Blood samples were collected and centrifuged, hematocrits and plasma osmolality were obtained, and the remaining plasma frozen at -70 C. Tissue samples were also collected, snap frozen in liquid nitrogen, and stored at -70 C. Data were analyzed using Statview 512 + (Abacus Concepts Inc. 1988). Paired t tests were performed at the $p < 0.05$ significance level to compare weights of individuals each day with their initial weight before treatment. Osmolalities and hematocrits were analyzed using one way ANOVAs ($p < 0.05$); the hematocrit data was first transformed to the arcsin before analysis.

The weight of hagfish in 100 % SW did not differ statistically from the initial weights during the course of the experiment (Figure 1). Hagfish in 85 % SW gained weight rapidly to a maximum of 8 % at 6 h after the initial transfer. Their weight had decreased towards normal levels (2 - 4 %) by 36 h. Weights returned to original levels on the second day, although they were not statistically the same on days 3, 6 and 7. Hagfish in 115 % SW decreased in weight with maximum weight loss (-10 - -8 %) occurring by the end of the first day. By day 4 there was a slight readjustment towards normal levels (-6 - -8 %) but weights were always significantly different from the initial weights. Plasma osmolality and hematocrits were measured at 12 h for 85 % SW hagfish and 3 days for the 115 % SW group. The plasma osmolalities for all three groups were statistically the same as the environmental water for that particular group. Hematocrits for all groups were

significantly different from each other. Mean hematocrits were: 100 % SW, mean = 28±1 (n = 13); 85 % SW, mean = 21±1 (n = 11); 115 % SW, mean = 31±1 (n = 17).

Our results indicate that M. glutinosa is able to compensate for the volume load that occurs after low salinity transfer but is unable to compensate for volume decrease as a result of hypersaline exposure. This finding is similar to that found for E. stoutii, which increased weight by a maximum of 10 % in 80 % SW, but with maximum weight gain occurring less rapidly than in this study, being reached at 24 h after the initial transfer. E. stoutii in 80 % SW approached normal weights at 5 days, indicating that compensation in this species and at this slightly lower salinity appears to take longer than in the present study. E. stoutii in 122 % SW lost approximately 15 % of its body weight that was not compensated during the seven days of the experiment. The higher percentage weight changes for E. stoutii are probably partly due to the slightly more extreme salinities to which these hagfish were exposed. We also confirm that hagfish plasma becomes isosmotic with the environmental water after transfer to different salinities. In its responses to high and low salinity stress, M. glutinosa, together with E. stoutii, are similar to marine invertebrate osmoconformers, which counteract the effects of a water load/salt loss in low salinities more effectively than they compensate for the salt load/ water loss in high salinity water (Oglesby, J. Exp. Zool. 215: 289-301. 1980).

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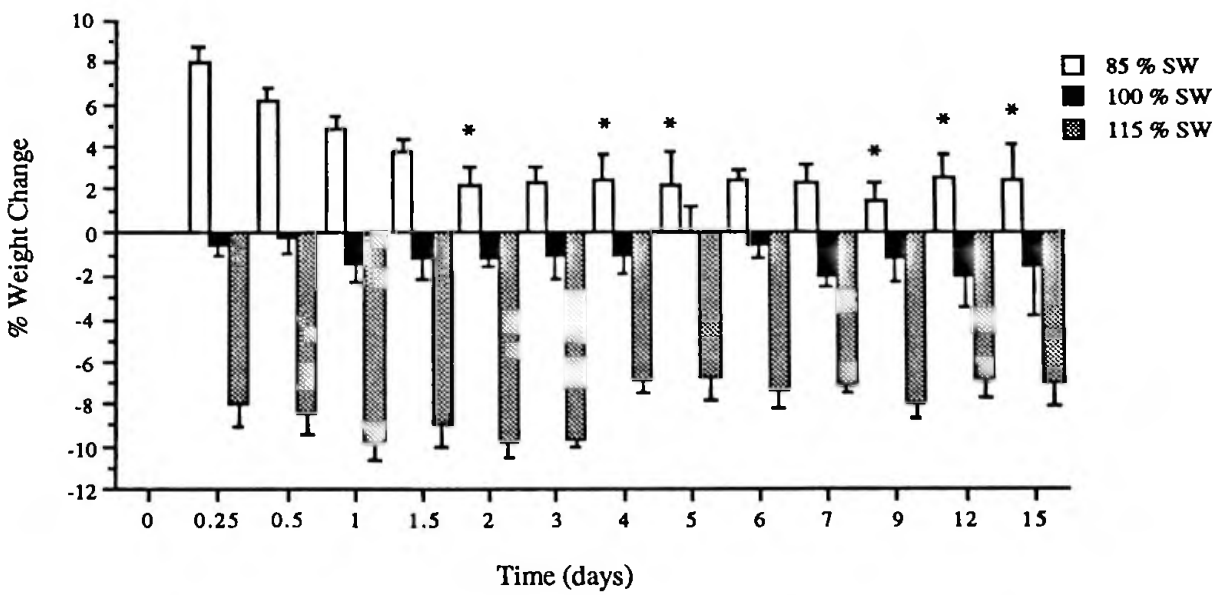

Figure 1. Percent weight change vs time for hagfish exposed to 85 %, 100 %, and115 % SW.
* NOT significantly different from initial weights: $p < 0.05$. Weights of hagfish in 100 % SW were not different from the initial