Effects of Copper Exposure on Water Balance

by LOUIS A. COURTOIS
California Department of Fish and Game
Water Pollution Control Laboratory
Rancho Cordova, Calif. 95670
and
ROGER D. MEYERHOFF
Department of Fisheries
Oregon State University
Corvallis, Ore.

Monitoring the various fluid compartments of an animal can reveal different physiological responses to stress. Volumes of the fluid compartments (plasma volume, total body water, etc.) reflect the state of hydration of the animal. Dehydration causes a decrease in the fluid compartment and overhydration causes an increase. Measurement of plasma volume has received considerable attention over the past one hundred years. GREGERSEN and RAWSON (1959) reviewed the major research on this subject, and concluded that no matter which technique was utilized, if used correctly, disagreement between initial plasma volume estimates was minimal.

Estimation of plasma volume in fish has only been accomplished in the past 20 years and is based upon two techniques: (1) dilution of an injected tracer substance, and (2) monitoring change in hematocrit following blood letting. Evans blue is a tracer dye which has been utilized extensively for many vertebrate species. The spectral absorption curve of this compound, however, is not the same in the plasma from all species of animals (ALLEN et al., 1953) and needs to be standardized prior to estimating volumes. CONTE et al. (1969) compared three different tracer materials to estimate plasma volume in Salmo gairdnerii. Results showed variation in estimated volumes depending upon the material used. Two factors which could easily account for this discrepancy were the distilled water rinse used and the wavelength at which the spectrophotometer was set. SMITH and BELL (1964) also used Evans blue but utilized saline for rinse and dye formulation, resulting in consistent estimates.

AVTALION et al. (1972) proposed a new method for determination of blood volume in aquatic animals. First, an initial hematocrit was established, followed by letting 20 to 50% of the presumed blood volume. A second hematocrit was then taken. This technique produced estimates of plasma volume consistent with those based on tracer dilution but placed the fish in a state of extreme stress.

Salmonids have been analyzed extensively by the foregoing methods, either while still under anesthesia, or a few hours post-anesthesia. A longer recovery period for the animal post-anesthesia is necessary to ensure a valid estimation of plasma volume, otherwise the animal could still be recovering from the anesthetic stress (HOUSTON et al., 1969). The purpose of the

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present investigation is twofold: first to establish the plasma volume of a free-swimming unanesthetized adult striped bass, Roccus saxatilis; and second to determine if ambient exposure to copper modified plasma volume by affecting water balance.

Materials and Methods

Striped bass, R. saxatilis, used in this investigation were captured in fyke traps on the Sacramento River near Freeport, Sacramento County, California (COURTOIS, 1974a). The fish were transported to the laboratory and held in flow-through tanks at 19.0 ± 0.5°C for a maximum of one week prior to testing. Prior to estimation of plasma volume, each fish was surgically prepared by implantation of a polyethylene cannula (0.58 mm I.D. and 0.96 mm O.D.) in the aorta.

Surgical Procedure

Each fish was individually anesthetized with a solution of MS 222 (0.05-0.1 gm/l) to a depth of 8-10 opercular movements/minute. It was then placed in a dorsal recumbancy on a surgery table (COURTOIS, 1974b) and draped with a moistened terry towel. A pump provided continuous irrigation of the gills with the recirculated anesthetic mixture. A technique similar to that of SMITH (1966) was used to probe for the dorsal aorta. The surgically prepared fish was returned to a flow-through tank and allowed to recover for 24 hours prior to estimating plasma volume.

Test Procedure

The fish was given one large injection of Evans blue solution (1 mg/ml in 0.85% NaCl), the amount depending upon the body weight of the fish (1 ml/kg). An equal volume of blood was removed prior to injecting Evans blue. Blood samples (1.0 ml) were removed at timed intervals, transferred to centrifuge tubes and spun at 6,200 rpm for 15 minutes. Serum was pipetted off and 0.5 ml transferred to a 5.0 ml volumetric flask and diluted with 4.5 ml of 0.85% NaCl. The optical density (O.D.) of the prepared sample was then read against a blank on a Spectronic 20 set at 610 mμ. Comparison of the O.D. to a previously-run standard curve gave the amount of Evans blue present in the serum sample. Once the plasma volume was established under control conditions, each fish was exposed to $CuSO_4$ (anhydrous) by direct addition of the salt to the holding tank water (1 mg/l final concentration of the salt). Blood samples were withdrawn at timed intervals following exposure to the salt (Table 1). Time required for uniform mixing of the salt in the water had been previously established at five minutes. Effects of copper exposure on blood volume were followed as long as blood samples could be drawn.

Results and Interpretation

The dye concentrations established during the period of time less than fifteen minutes were not used for calculation of plasma volume because this has been shown to be a period of rapid mixing of dye (SMITH, 1966). The plasma volume of unanesthetized adult striped bass was found to be between 2.50 and 2.85% of the body weight (Table 1). These calculations are based on the method presented by CONTE et al. (1963). This is the first report of plasma volume for this species and compares favorably with estimates for other teleosts (SMITH, 1966).

TABLE 1.

Freshwater Acclimated Adult Striped Bass Plasma Volume Analysis at 19.0 ± 0.5°C. Pre- and Post-Copper Exposure Volumes are Compared.

Parameter	Fish #602	Fish #603
Pre-copper exposure		
Gross wet weight (gm)	4906	1146
Fork length (cm)	80.6	47.0
Weight Evans blue injected (mg)	5.2653	1.4500
Estimated Evans blue concentration at time zero (ug/ml)	37.4	50.6
Estimated plasma volume (ml)	140.78	28.65
Plasma volume (as % body weight)	2.86	2.50
Post-copper exposure		
Exposure time (minutes)	15	6
Estimated plasma volume (ml)	188.04	39.61
Plasma volume (as % body weight)	3.83	3.45
% Increase in plasma volume (%)	0.97	0.95

Exposure of test fish to ambient copper resulted in an expansion of the plasma volume (Table 1). The magnitude of expansion

was similar in both fish tested (0.95-0.97%). Volume estimates were carried out as long as blood samples were available, but in both cases there was loss of patency of the cannula soon after the copper exposure, which prevented further blood sampling.

Expansion of the plasma volume reported here represents a new concept in mode of action and physiological response to a heavy metal. The results can be explained in terms of passive osmotic diffusion (LOEWY and SIEKEVITZ, 1970)--water moving down a gradient from an area of high concentration (the surrounding environment) across the gill membrane into an area of low concentration (the internal body fluid compartments). Copper would have to be affecting the exterior border of the gill membrane to produce the effect. The magnitude of change of the plasma volume (approximately 1.0% of the body weight) is large enough to be detrimental to the fish if left unchecked. If copper is indeed producing this effect, than a salt-water-acclimated fish should display the converse situation, dehydration or reduction in plasma volume following copper exposure. Further research into the phenomenon is currently being completed.

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