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Analysis of refractometry for determining total plasma protein in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) at various salinities

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Abstract

Total plasma protein (TPP) is a broad clinical indicator of health, stress, and well being. A simple and rapid technique for determining TPP is refractometry, which measures the refractive index of all dissolved materials in solution. It was hypothesized that plasma dissolved solids in fish held at increasing salinity levels would result in plasma samples with a higher refractive index and affect true TPP readings. Efficacy of refractometry over a range of salinities was evaluated by comparing TPP values obtained by refractometry with those obtained by a dye-binding assay (Biuret method) specific to proteins. Blood analytes were evaluated in hybrid striped bass *Morone chrysops* × *M. saxatilis* (HSB) acclimated to nominal salinities of 0, 10, 20, and 30 g L^{-1}. Hybrid striped bass acclimated to hypertonic environments exhibited higher ($P < 0.05$) electrolytes, TPP, and osmolality than those acclimated to low salinity, but were still within the normal reference interval for HSB. Although salinity affected TPP determined by both methods, the observed increase in TPP values over the range of salinities was significantly greater in samples analyzed by refractometry. Total plasma protein concentrations measured by refractometry were always higher and the difference in absolute values determined by the two methods appears to be caused by a factor not measured in this investigation. It is concluded that refractometry is not well suited for analyzing accurate TPP in HSB or determining relative changes in TPP across a range of salinities.
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Keywords: Refractometry; Plasma protein; Salinity; Hybrid striped bass

1. Introduction

Total plasma protein (TPP) concentration relative to a reference interval is used as a broad clinical indicator of health, stress, and well being of terrestrial and aquatic organisms. When a reference interval is known, routine measurement of TPP can be used as a tool for management decisions and monitoring the status of both wild and cultured populations.

A variety of methods exist for determining protein concentrations of plasma or serum. Colorimetric procedures are generally the preferred choice; however, they are expensive, time consuming, and not easily performed in the field. Because of ease, rapid mode of operation, and small amount of material required, Alexander and Ingram (1980) suggest refractometry as an alternative for use with fish.

Refractometry measures the refractive index of all dissolved materials in solution. Under stable conditions, plasma concentrations of inorganic ions, glucose, amino acids, and other dissolved organics are relatively

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constant within a species; however, protein can vary from animal to animal (Alexander and Ingram, 1980). The working assumption is that variation in the refractive index of plasma is caused by changes in protein concentration, although Hille (1982) proposes that other blood constituents can also cause variations.

Refractometry for determination of total serum or plasma protein has been reported for mammals (Barry et al., 1960), birds (Morgan et al., 1975), crustaceans (Leavitt and Bayer, 1977), and fish including striped bass (Hunn and Greer, 1990) and hybrid striped bass (HSB) *Morone chrysops*×*M. saxatilis* (Hrubec et al., 1997a). However, blood dissolved solids in most aquatic animals are directly influenced by dissolved solids in the aquatic medium. Therefore, refractometry may be more suitable for terrestrial animals than fish living in environments which vary in dissolved solids (George, 2001).

Normal ranges for hematologic and serum biochemical profiles exist for the euryhaline HSB in a uniform environment, however changes in environment can affect blood parameters (Hrubec et al., 1996, 1997a,b). Species exposed to increasing salinities exhibit higher ionic concentrations and altered hematological parameters (Ram Bhaskar and Srinivasa Rao, 1989; LeaMaster et al., 1990). Moreover, transfer between fresh and saline water may result in quantitative changes in plasma protein (Eddy, 1981).

It was hypothesized that plasma dissolved solids in fish held at increasing salinity levels would result in plasma samples with a higher refractive index and affect true TPP readings. Therefore paired samples of plasma were analyzed for TPP using both refractometry and the Biuret method in fish held at increasing salinities. Moreover, paired analyte values between serum and plasma are not as steadfast in fish as in mammals (Hrubec and Smith, 1999), therefore total protein in paired serum and plasma samples was also evaluated.

2. Materials and methods

2.1. Fish and experimental systems

Juvenile HSB (*M. chrysops*×*M. saxatilis*) obtained from Keo Fish Farm (Keo, Arkansas) were transported to Harbor Branch Oceanographic Institution (Fort Pierce, Florida) and reared from 20 to 280 g. Fish were maintained at 21–28 °C, 1–3 g L^{-1} salinity, and fed a commercial HSB diet containing 40% digestible protein, 12% lipid and 3.6 kcal kg^{-1} protein (Burris Mill and Feed, Franklinton, Louisiana).

A randomized complete block design was employed with each of two blocks serving as replicates. Fish were transferred to one of four recirculating systems with nominal salinities of 0, 10, 20, and 30 g L^{-1} (Table 1) prior to sampling. Recirculating systems consisted of 900-L culture vessels with micro-particulate, biological, and charcoal filtration (Filstar Canister, Aquatic Ecosystems, Apopka, Florida) and ultraviolet light sterilization. Fish were maintained at ambient temperature (21±1.7 °C) and photoperiod (12-h light:12-h dark) and fed the above diet at 1% body weight day^{-1} divided between two equal feedings.

Culture vessels were stocked with 16 fish each (mean weight 280±39 g) taken from a pool of approximately 600 fish. At initiation of the experiment, fish were directly transferred into the systems containing 0 and 10 g L^{-1} salinities. Fish within the 20 and 30 g L^{-1} treatments were initially transferred into 12 g L^{-1} salinity and slowly acclimated to nominal salinities with daily incremental increases. Acclimation periods were 2 and 7 days for 20 and 30 g L^{-1} treatments, respectively. Salinity adjustments were made by mixing fresh- and saltwater well sources. The seasonal well maximum of 28 g L^{-1} necessitated the addition of NaCl to raise salinity to 30 g L^{-1}. Well analyses indicated calcium levels of 56 and 358 mg L^{-1} and an alkalinity of 175 and 180 mg L^{-1} as $CaCO_3$ for fresh- and saltwater, respectively.

Dissolved oxygen, salinity, and temperature were measured twice daily with a YSI 85 meter (YSI, Inc., Yellow Springs, Ohio). Total ammonia–nitrogen (TAN) and nitrite–nitrogen (NO_2–N) were measured once daily on a Hach DR/890 colorimeter (Hach Co., Loveland, Colorado).

2.2. Sample collection and analysis

Blood samples were drawn after 14 days at the target salinity. Fish were not fed 24 h prior to sampling. Fish within each treatment were anesthetized in 80 mg L^{-1} tricaine methanesulfonate (MS-222, Western Chemical,

Table 1
Mean (SD) values of temperature, dissolved oxygen, salinity, total ammonia–nitrogen, and nitrite–N for hybrid striped bass *Morone chrysops*×*M. saxatilis* held at nominal salinities of 0, 10, 20, or 30 g L^{-1}

Parameter	Nominal salinity (g L^{-1})			
	0	10	20	30
Temperature (°C)	20.4 (1.5)	20.9 (1.6)	20.7 (1.7)	21.1 (2.0)
Dissolved oxygen (mg L^{-1})	8.0 (0.5)	7.6 (0.6)	6.7 (0.5)	6.8 (0.5)
Salinity (g L^{-1})	1.0 (0.0)	10.3 (0.1)	20.0 (0.2)	30.3 (0.2)
Total ammonia–N (mg L^{-1})	0.3 (0.3)	0.6 (0.3)	0.6 (0.3)	0.5 (0.2)
Nitrite–N (mg L^{-1})	1.9 (0.9)	1.8 (0.9)	0.6 (0.4)	0.4 (0.1)