Biological Sciences

Plasma Glucose Levels for Red Drum *Sciaenops ocellatus* in a Florida Estuarine Fisheries Reserve

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Running head: Wild red drum plasma glucose
Abstract Despite the significant value of the southeastern United States’ red drum (Sciaenops ocellatus) fishery, there is a lack of clinical blood chemistry data. This was the first study to assess plasma glucose values as an indicator of stress response to evaluate variation and the effect of reproductive activity for wild adult red drum in Florida. Red drum (n=126) were collected from NASA’s Kennedy Space Center waters during three reproductive periods in 2011. Samples were obtained from the branchial vessels of the gill arch. Plasma glucose levels were significantly different among reproductive periods, with the highest mean values recorded during the spawning period, September-October (38.23 mg/dL ± 10.0). The glucose range was 17 – 69 mg/dL. Glucose values were lower during all three periods than previous values recorded for cultured or captive red drum studies. This may indicate that fish from this population were under less stress than other populations previously sampled.

Keywords red drum, plasma glucose, reserve, wild fish

Introduction In the Southeastern United States there is a significant recreational catch-and-release fishery and limited commercial fishery for red drum, Sciaenops ocellatus. The species inhabits nearshore and estuarine waters from Massachusetts to the Gulf of Mexico coast (Murphy and Taylor 1990). Recreational landings combined from the state of Florida’s coastlines were estimated at 2.5 million pounds total weight of fish in 2007 (Murphy and Munyandorero 2009). The impact of angling on the health of individual fish is poorly understood. The National Aeronautics and Space Administration’s (NASA) Kennedy Space Center (KSC) Reserve was established in 1962 to safeguard rocket launch operations, and acts as a de-facto marine fisheries reserve. The reserve is off-limits to public anglers and fits the most stringent guideline of a marine protected area: a no-take reserve for all animals residing in its area. Mature resident red drum are known to spawn throughout the fall period within the reserve (Stevens and Sulak 2001; Reyier et al. 2008). Reyier et al. (2011) stated that such a high incidence of red drum estuarine spawning may mostly be facilitated by mild winter water temperatures in East-central Florida which is recognized as a climactic transition zone that has temperature winters compared to other estuaries of the south U.S. Atlantic and Gulf of Mexico. The KSC Reserve minimizes many of the challenges to growth and survival experienced by red drum elsewhere in the southeastern U.S., such as alleviating intense angling pressure, particularly during the spawning period.

Stress responses of red drum have been studied in cultured fish (Robertson et al. 1987) with an emphasis on the use of glucose as a reliable index (Wedemeyer and Yasutake 1977; Thomas and Robertson 1991). Physiology
studies of wild fish are usually lethal and glucose profiles in wild fish are currently lacking. The only study on short-term physiological responses from angling using plasma glucose concentrations involved cultured and wild S. ocellatus with values averaging 38 mg / dL for wild fish and 31 mg / dL for hatchery produced fish (Gallman et al. 1999).

Stress causes increases in plasma glucose concentrations, generated initially by catecholamine-mediated glycogenolysis, and at later stages by cortisol-mediated gluconeogenesis (Pankhurst 2011). If a “normal” range of plasma glucose concentration can be defined, these data can be used to aid in the determination of the health status of a population of interest. A wide range of factors influence plasma glucose concentration causing increased variance. Elevations in glucose concentrations can be induced by pollutants, exercise, hypoxia, handling, restraint, or transportation (Strange and Schreck 1978; Barton 1980; Thomas et al. 1980). Other factors that influence glucose values are the sex of the fish and maturity level, time of day, nutritional state, and water temperature (Robertson et al. 1987; Pankhurst 2011).

The goal of this study was to establish a glucose data range for wild red drum in the KSC Reserve, and to assess its application for health assessment or as a stress indicator in free-ranging red drum. An additional objective was to determine if there were changes in blood glucose concentrations in wild red drum associated with reproductive activity.

**Materials and Methods**

**Fish collection.** Red drum were caught inside NASA’s Kennedy Space Center (KSC) Reserve of Merritt Island National Wildlife Refuge, (28°32’59.76”N 80°35’40.38”W), the oldest fully protected no-take fisheries reserve in the United States. The Reserve is a shallow estuary, with a mean depth of 1.5 meters, of which 33 km² of estuarine waters was used as the study area. Large surf rods and 30 lb. test fishing line were used to decrease angling time, and barbless circle hooks with cut mullet (Mugil spp.) were used as bait. Only fish over 65 cm standard length were used for this project. At least 50% of male fish that reach 51 cm fork length on the Atlantic coast of Florida are sexually mature and most are at age 1 or 2, while females have a 50% maturity of 90 mm fork length and most are sexually mature at age 3 and all by age 6 (Murphy and Taylor 1990). Fish were caught during the three reproductive periods of 2011, defined as pre-spawning (May), spawning (September and October), and post-spawning (December). Angling time in minutes was recorded for each fish as the time the fish was hooked to when it was landed. Fish were quickly netted, de-hooked, and then placed in lateral recumbency for blood collection.
Plasma collection

Red drum were manually restrained on an inverted v-tray with a biologist holding the operculum away from the body wall to allow access to the gills. A 4 ml blood sample was extracted from the branchial vessels at the base of the first or second gill arch on the left side of the fish using a sterile 1 ½”, 20 gauge needle, and drawn into a lithium heparin Vacutainer (BD, Franklin, NJ) tube labeled with an individual identification number. The time interval from angling time to blood sample collection was recorded. All fish were then released alive at their capture location.

Laboratory glucose assay. Blood samples were placed on ice until it was returned to the laboratory in no more than six hours and centrifuged for a minimum of 10 minutes at 3000 g to separate the plasma and cell fractions. Plasma was then aliquoted into 1.8 ml cryovials and stored frozen at -70 °C until further processing. Plasma samples were thawed on ice and glucose levels measured by an absorbance assay, according to the instructions provided in the Invitrogen, Amplex Red Glucose / Glucose Oxidase Assay Kit (A22189). Samples were diluted 20 X with buffer to keep the concentrations within the limit of the kit. The four sample plates were analyzed using a microplate reader (BioTek Synergy HT, Winooski, VT).

Statistical analyses

To explore differences in mean glucose, concentrations were compared among the three sampling periods (pre-spawning, spawning, and post-spawning) using a one-way analysis of variance (ANOVA). A regression between glucose value and angling time to blood sample collection was performed on all fish sampled. Mean comparisons were conducted using the Bonferroni procedure. A p-value was considered significant if lower than 0.05. The limit for statistical significance was 0.167 for the Bonferroni correction. The statistical analyses were conducted in R (R Core Team 2014). All values reported are means ± 1 SD.

Results

Mean angling time was less than seven minutes per fish. Time to blood sample collection was less than 8.5 minutes total, including angling time. Males used in this study were all above the 50% maturity length of 51 mm fork length and only 11% of females sampled were over 90 cm total length. Sex was undetermined in 35. Fish caught outside of the spawning period were more difficult to determine without the use of a blood sample for hormone analysis or internal gonad identification. Glucose values recorded ranged from 17 – 69 mg / dL. The pre-spawning period fish (n=38) collected in May 2011 had mean glucose concentrations of 35.7 mg / dL ± 6.8.
Spawning period fish (n=45) collected in September and October 2011 had mean glucose concentrations of 38.2 mg/dL ± 10.0. The post-spawning period fish (n=42) collected in December 2011 had mean glucose concentrations of 29.0 mg/dL ± 6.1. The regression between blood sample collection time and glucose values was not a strong correlation [R² = 0.003].

A one-way ANOVA examining plasma glucose concentrations by period indicated a significant change [F(2,123)= 15.76, p < 0.001]. Visual inspection of the residuals did not reveal any violations of the model assumptions. The Bonferroni mean comparison test revealed that mean plasma glucose concentrations during the spawning period [(t(86)=5.20, p < 0.01] and pre-spawning period t-spawning [(t(78)=4.64, p < 0.01] were significantly higher than post-spawning. However, mean plasma glucose concentrations during spawning were not significantly different than those obtained during the pre-spawning period [(t(82)=1.34, p = 0.18] (Figure 1).

Discussion

This study reports plasma glucose concentrations for wild caught red drum in the KSC Reserve during three reproductive periods. Temporal differences were observed with the fish obtained during the post-spawning period exhibiting significantly lower plasma concentrations of glucose when compared to samples obtained from fish during either the pre-spawning or spawning periods. Since sex was not able to be distinguished for 35 fish it was unclear how to categorize their lengths according to the 50% fork length measurement for males and females. Also only 11% of the females in this study were over 90 cm, meaning the other females or unknown sex fish could have been reproductively immature fish causing a higher range in glucose values. However it is currently unknown what the differences would be for immature red drum versus mature red drum and if there are any significant differences. A population of fish exhibiting a large range of values for plasma glucose is not uncommon (Robertson et al. 1987; Gallman et al. 1999). Capture and handling stress can affect glucose values, typically inciting a hyperglycemic response. Since the fish captured in this study all had angling times of less than seven minutes and all blood samples were collected under eight and a half minutes, the values collected could reflect near baseline values for wild caught fish of this species. The mean plasma glucose concentrations during all three periods, 35.7, 38.2 and 29.0 mg / dL, were all lower than the basal values reported by Robertson (1987) who concluded that the resting values for juvenile cultured red drum were 45.6 ± 8.3 mg / dL. The red drum sampled in our study could have lower glucose values because the fish were less stressed due to the KSC Reserve preventing exposure to constant angling pressure.
This is the first study to collect plasma glucose concentrations during three reproductive periods in wild red drum. Information concerning plasma glucose concentrations during reproduction are valuable because they can help provide an understanding of the biology of the species in its natural state. Plasma glucose concentrations can be used to aid in the determination of the health status of the larger population when compared to other wild red drum populations in the species range. Gallman et al. (1999) recorded a mean of 38.21 mg/dL on average for wild caught red drum similarly sized, 47.7 cm, during August to October in South Carolina. This average is the same as the spawning glucose concentrations from this study (38.23 mg/dL for samples obtained in between September and October). The glucose values for red drum collected in the KSC Reserve are the lowest reported for wild red drum anywhere in its range. These low glucose values suggest that levels for this population are within the healthy range for the species.

This study produced glucose concentrations for angled wild red drum in Florida from a no-take fisheries reserve. The lack of fishing in the KSC reserve likely contributes significantly to the low glucose values recorded for all red drum in this study. There was a temporal effect on plasma glucose values, with post-spawning fish having the lowest values compared to fish during either pre-spawning or spawning periods. Since the population of red drum in the IRL has been documented to spawn in the estuarine waters, having a reserve where the fish have a reprieve from the intense angling pressure of surrounding areas is beneficial for reproduction. These collected glucose concentrations could be used for future comparisons to wild red drum populations during their reproductive periods to assess their health status.

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References


FIGURE 1.- Mean glucose (mg / dL) in captured wild red drum was lower during post-spawning than either pre-spawning or spawning. Solid circles show glucose (mg / dL) by period captured in Kennedy Space Center’s Reserve modeled by a one-way ANOVA; error bars give the 95% confidence interval of the prediction. Also shown are the raw data (hollow circles).