1   Biological Sciences

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

3   Reserve

4   Carla M Bourtis[1] , Ruth Francis-Floyd[2] , Ashley S. P. Boggs[3] , Eric A. Reyier[1]·, Eric D.

5   Stolen[1]·, Roy P. Yanong[4]·, and Louis J. Guillette, Jr.[3]

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7   [1] Kennedy Space Center Ecological Program, InoMedic Health Applications, Mail Code: IHA-300 Kennedy Space
8   Center, FL 32899
9

10   [2] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida,
11   Gainesville, FL
12

13   [3] Department of Obstetrics and Gynecology and the Hollings Marine Laboratory, Medical University of South
14   Carolina, Charleston, SC
15

16   [4] Tropical Aquaculture Laboratory, Program in Fisheries and Aquatic Sciences, School of Forest Resources and
17   Conservation, University of Florida, Ruskin, FL
18

19   Corresponding author: Carla Bourtis, carla.m.bourtis@nasa.gov

20   Running head: Wild red drum plasma glucose

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22 **Abstract** Despite the significant value of the southeastern United States' red drum (*Sciaenops ocellatus*) fishery,

23 there is a lack of clinical blood chemistry data. This was the first study to assess plasma glucose values as an

24 indicator of stress response to evaluate variation and the effect of reproductive activity for wild adult red drum in

25 Florida. Red drum (n=126) were collected from NASA's Kennedy Space Center waters during three reproductive

26 periods in 2011. Samples were obtained from the branchial vessels of the gill arch. Plasma glucose levels were

27 significantly different among reproductive periods, with the highest mean values recorded during the spawning

28 period, September- October (38.23 mg / dL ± 10.0). The glucose range was $17 - 69$ mg / dL. Glucose values were

29 lower during all three periods than previous values recorded for cultured or captive red drum studies. This may

30 indicate that fish from this population were under less stress than other populations previously sampled.

31 **Keywords** red drum, plasma glucose, reserve, wild fish

32 **Introduction**

33   In the Southeastern United States there is a significant recreational catch-and-release fishery and limited

34 commercial fishery for red drum, *Sciaenops ocellatus*. The species inhabits nearshore and estuarine waters from

35 Massachusetts to the Gulf of Mexico coast (Murphy and Taylor 1990). Recreational landings combined from the

36 state of Florida's coastlines were estimated at 2.5 million pounds total weight of fish in 2007 (Murphy and

37 Munyandorero 2009). The impact of angling on the health of individual fish is poorly understood. The National

38 Aeronautics and Space Administration's (NASA) Kennedy Space Center (KSC) Reserve was established in 1962 to

39 safeguard rocket launch operations, and acts as a *de- facto* marine fisheries reserve. The reserve is off-limits to

40 public anglers and fits the most stringent guideline of a marine protected area: a no-take reserve for all animals

41 residing in its area. Mature resident red drum are known to spawn throughout the fall period within the reserve

42 (Stevens and Sulak 2001; Reyier et al. 2008). Reyier et al. (2011) stated that such a high incidence of red drum

43 estuarine spawning may mostly be facilitated by mild winter water temperatures in East-central Florida which is

44 recognized as a climactic transition zone that has temperature winters compared to other estuaries of the south U.S.

45 Atlantic and Gulf of Mexico. The KSC Reserve minimizes many of the challenges to growth and survival

46 experienced by red drum elsewhere in the southeastern U.S, such as alleviating intense angling pressure, particularly

47 during the spawning period.

48   Stress responses of red drum have been studied in cultured fish (Robertson et al. 1987) with an emphasis on

49 the use of glucose as a reliable index (Wedemeyer and Yasutake 1977; Thomas and Robertson 1991). Physiology

50   studies of wild fish are usually lethal and glucose profiles in wild fish are currently lacking. The only study on short

51   term physiological responses from angling using plasma glucose concentrations involved cultured and wild *S.*

52   *ocellatus* with values averaging 38 mg / dL for wild fish and 31 mg / dL for hatchery produced fish (Gallman et al.

53   1999).

54   Stress causes increases in plasma glucose concentrations, generated initially by catecholamine-mediated

55   glycogenolysis, and at later stages by cortisol-mediated gluconeogenesis (Pankhurst 2011). If a "normal" range of

56   plasma glucose concentration can be defined, these data can be used to aid in the determination of the health status

57   of a population of interest. A wide range of factors influence plasma glucose concentration causing increased

58   variance. Elevations in glucose concentrations can be induced by pollutants, exercise, hypoxia, handling, restraint,

59   or transportation (Strange and Schreck 1978; Barton 1980; Thomas et al. 1980). Other factors that influence glucose

60   values are the sex of the fish and maturity level, time of day, nutritional state, and water temperature (Robertson et

61   al. 1987; Pankhurst 2011).

62   The goal of this study was to establish a glucose data range for wild red drum in the KSC Reserve, and to

63   assess its application for health assessment or as a stress indicator in free-ranging red drum. An additional objective

64   was to determine if there were changes in blood glucose concentrations in wild red drum associated with

65   reproductive activity.

66   **Materials and Methods**

67   **Fish collection.** Red drum were caught inside NASA's Kennedy Space Center (KSC) Reserve of Merritt Island

68   National Wildlife Refuge, (28°32'59.76"N 80°35'40.38"W), the oldest fully protected no-take fisheries reserve in

69   the United States. The Reserve is a shallow estuary, with a mean depth of 1.5 meters,of which 33 km^2 of estuarine

70   waters was used as the study area. Large surf rods and 30 lb. test fishing line were used to decrease angling time,

71   and barbless circle hooks with cut mullet (*Mugil* spp.) were used as bait. Only fish over 65 cm standard length were

72   used for this project. At least 50% of male fish that reach 51 cm fork length on the Atlantic coast of Florida are

73   sexually mature and most are at age 1 or 2, while females have a 50% maturity of 90 mm fork length and most are

74   sexually mature at age 3 and all by age 6 (Murphy and Taylor 1990). Fish were caught during the three reproductive

75   periods of 2011, defined as pre-spawning (May), spawning (September and October), and post-spawning

76   (December). Angling time in minutes was recorded for each fish as the time the fish was hooked to when it was

77   landed. Fish were quickly netted, de-hooked, and then placed in lateral recumbency for blood collection.

78 Plasma collection

79   Red drum were manually restrained on an inverted v-tray with a biologist holding the operculum away

80 from the body wall to allow access to the gills. A 4 ml blood sample was extracted from the branchial vessels at the

81 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

82 lithium heparin Vacutainer (BD, Franklin, NJ) tube labeled with an individual identification number. The time

83 interval from angling time to blood sample collection was recorded. All fish were then released alive at their capture

84 location.

85 **Laboratory glucose assay.** Blood samples were placed on ice until it was returned to the laboratory in no more than

86 six hours and centrifuged for a minimum of 10 minutes at 3000 g to separate the plasma and cell fractions. Plasma

87 was then aliquoted into 1.8 ml cryovials and stored frozen at -70 °C until further processing. Plasma samples were

88 thawed on ice and glucose levels measured by an absorbance assay, according to the instructions provided in the

89 Invitrogen, Amplex Red Glucose / Glucose Oxidase Assay Kit (A22189). Samples were diluted 20 X with buffer to

90 keep the concentrations within the limit of the kit. The four sample plates were analyzed using a microplate reader

91 (BioTek Synergy HT, Winooski, VT).

92 Statistical analyses

93   To explore differences in mean glucose, concentrations were compared among the three sampling periods

94 (pre-spawning, spawning, and post-spawning) using a one-way analysis of variance (ANOVA). A regression

95 between glucose value and angling time to blood sample collection was performed on all fish sampled. Mean

96 comparisons were conducted using the Bonferroni procedure. A *p*-value was considered significant if lower than

97 0.05. The limit for statistical significance was 0.167 for the Bonferroni correction. The statistical analyses were

98 conducted in R (R Core Team 2014). All values reported are means ± 1 SD.

99 **Results**

100   Mean angling time was less than seven minutes per fish. Time to blood sample collection was less than 8.5

101 minutes total, including angling time. Males used in this study were all above the 50% maturity length of 51 mm

102 fork length and only 11% of females sampled were over 90 cm total length. Sex was undetermined in 35. Fish

103 caught outside of the spawning period were more difficult to determine without the use of a blood sample for

104 hormone analysis or internal gonad identification. Glucose values recorded ranged from 17 – 69 mg / dL. The pre-

105 spawning period fish (n=38) collected in May 2011 had mean glucose concentrations of 35.7 mg / dL ± 6.8.

106 Spawning period fish (n=45) collected in September and October 2011 had mean glucose concentrations of 38.2 mg

107 / dL ± 10.0. The post-spawning period fish (n=42) collected in December 2011 had mean glucose concentrations of

108 29.0 mg / dL ± 6.1. The regression between blood sample collection time and glucose values was not a strong

109 correlation [R^2 = 0.003].

110   A one-way ANOVA examining plasma glucose concentrations by period indicated a significant change

111 [$F_{(2,123)}$= 15.76, $p < 0.001$]. Visual inspection of the residuals did not reveal any violations of the model

112 assumptions. The Bonferroni mean comparison test revealed that mean plasma glucose concentrations during the

113 spawning period [$t(86)$=5.20, $p < 0.01$] and pre-spawning period t-spawning [$t(78)$=4.64, $p < 0.01$] were

114 significantly higher than post-spawning. However, mean plasma glucose concentrations during spawning were not

115 significantly different than those obtained during the pre-spawning period [$t(82)$=1.34, $p= 0.18$] (Figure 1).

116 **Discussion**

117   This study reports plasma glucose concentrations for wild caught red drum in the KSC Reserve during

118 three reproductive periods. Temporal differences were observed with the fish obtained during the post-spawning

119 period exhibiting significantly lower plasma concentrations of glucose when compared to samples obtained from

120 fish during either the pre-spawning or spawning periods. Since sex was not able to be distinguished for 35 fish it was

121 unclear how to categorize their lengths according to the 50% fork length measurement for males and females. Also

122 only 11% of the females in this study were over 90 cm, meaning the other females or unknown sex fish could have

123 been reproductively immature fish causing a higher range in glucose values. However it is currently unknown what

124 the differences would be for immature red drum versus mature red drum and if there are any significant differences.

125 A population of fish exhibiting a large range of values for plasma glucose is not uncommon (Robertson et al. 1987;

126 Gallman et al. 1999). Capture and handling stress can affect glucose values, typically inciting a hyperglycemic

127 response. Since the fish captured in this study all had angling times of less than seven minutes and all blood samples

128 were collected under eight and a half minutes, the values collected could reflect near baseline values for wild caught

129 fish of this species. The mean plasma glucose concentrations during all three periods, 35.7, 38.2 and 29.0 mg / dL,

130 were all lower than the basal values reported by Robertson (1987) who concluded that the resting values for juvenile

131 cultured red drum were 45.6 ± 8.3 mg / dL. The red drum sampled in our study could have lower glucose values

132 because the fish were less stressed due to the KSC Reserve preventing exposure to constant angling pressure.

133   This is the first study to collect plasma glucose concentrations during three reproductive periods in wild red

134   drum. Information concerning plasma glucose concentrations during reproduction are valuable because they can

135   help provide an understanding of the biology of the species in its natural state. Plasma glucose concentrations can be

136   used to aid in the determination of the health status of the larger population when compared to other wild red drum

137   populations in the species range. Gallman et al. (1999) recorded a mean of 38.21 mg / dL on average for wild caught

138   red drum similarly sized, 47.7 cm, during August to October in South Carolina. This average is the same as the

139   spawning glucose concentrations from this study (38.23 mg / dL for samples obtained in between September and

140   October). The glucose values for red drum collected in the KSC Reserve are the lowest reported for wild red drum

141   anywhere in its range. These low glucose values suggest that levels for this population are within the healthy range
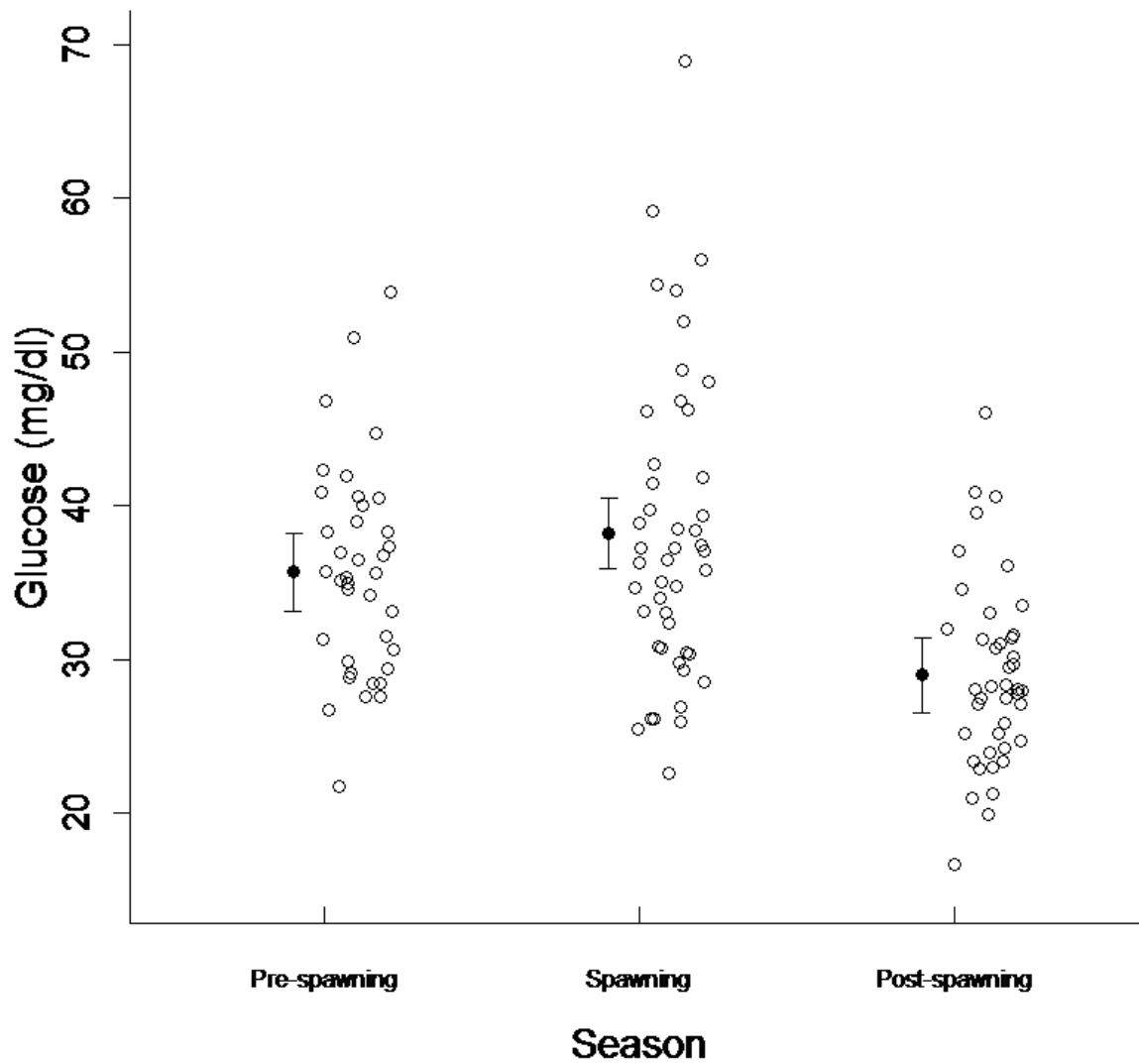
142   for the species.

143   This study produced glucose concentrations for angled wild red drum in Florida from a no-take fisheries

144   reserve. The lack of fishing in the KSC reserve likely contributes significantly to the low glucose values recorded for

145   all red drum in this study. There was a temporal effect on plasma glucose values, with post-spawning fish having the

146   lowest values compared to fish during either pre-spawning or spawning periods. Since the population of red drum in

147   the IRL has been documented to spawn in the estuarine waters, having a reserve where the fish have a reprieve from

148   the intense angling pressure of surrounding areas is beneficial for reproduction. These collected glucose

149   concentrations could be used for future comparisons to wild red drum populations during their reproductive periods

150   to assess their health status.

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204

205   FIGURE 1.- Mean glucose (mg / dL) in captured wild red drum was lower during post-spawning then either pre-

206   spawning or spawning. Solid circles show glucose (mg / dL) by period captured in Kennedy Space Center's

207   Reserve modeled by a one-way ANOVA; error bars give the 95% confidence interval of the prediction. Also shown

208   are the raw data (hollow circles).

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