DEVELOPMENT OF METHODS FOR NON-LETHAL HEALTH ASSESSMENT OF THE RED DRUM (*SCIAENOPS OCELLATUS*) INSIDE NASA’S KENNEDY SPACE CENTER NO-TAKE FISHERIES RESERVE

By

CARLA M. GARREAU

A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012
To my angel, Cookie
ACKNOWLEDGMENTS

I would like to thank my major advisor Ruth Francis-Floyd for her advice, constructive criticism and guidance throughout my graduate experience. I would like to thank Lou Guillette, Jr. for your belief in me that I could work full-time and complete a graduate degree. I appreciate all of our field talks and your patience teaching me about endocrinology. I am extremely grateful for all the time Eric Reyier has spent teaching me fish scientific names in the field, quizzing me regularly, and his efforts helping me create the project design for this research. I aspire to have his work ethic to always get the job done proficiently and professionally no matter what the task at hand. I am thankful for Roy Yanong who helped guide me through my academic journey.

David Westmark taught me how to bleed a red drum, and without him this project would not have been such a painless process for all the fish involved, thank you! Conducting my field sampling would not have been possible without the help of the “Aquatics Team”, Doug Scheidt, Russ Lowers, Eric Reyier, Karen Holloway-Adkins, and Shanon Gann for their tireless perseverance in the field. I would also like to express thanks to Carlton Hall, Donna Oddy and Tim “Lucky Charm” Kozusko for assisting me in the field and laboratory. I greatly appreciated the statistics help from Eric Stolen and his patience in helping me grasp multivariate analysis.

I wish to express my gratitude to thank Angie Saul and Chad Young for allowing access to the Stock Enhancement Research Facility broodstock as my initial patients for this protocol and also all the staff who helped with the pilot study, Josh Taylor, Josh Lunde, Mat Rhodes, Kerry Mesner, Micah Alo and Chris Young. I much appreciated the assistance of Patrick Thompson and Heather Maness from the University of Florida Aquatic Animal Health Program for their assistance with the Abbott i-STAT analyzer. I
am immensely grateful to Ashley Boggs from MUSC, and Heather Hamlin from University of Maine, for their help teaching me how to run radioimmunoassays in the laboratory and their patience with my learning curve for understanding the results.

Finally I am eternally grateful to my entire family, especially my parents for encouraging me to pursue my graduate degree and continuing to reassure me to work hard and that it would all be worth it in the end. I wanted to thank my dad for teaching me how to fish, my brother for going fishing with his little sister when we were kids, and to my mom for encouraging me to keep on “fishing” for my dreams. My parents taught me to be an independent and self-reliant young adult and I credit them for doing a wonderful job raising me to work hard and play hard and to combine the two to discover my dream career as a marine biologist. I want to thank Spiros for always believing in me and being a great support for me to lean on when times were difficult juggling work, school, family and friends, you helped me steer the course in the turbulent seas of a grad student’s life. Lastly, this thesis is dedicated to my angel, Cookie, who was the best friend a girl could have for 14 years. She provided me with unconditional love from grade school to graduate school, being the most supportive friend I’ve ever had until the end of her precious life.

This research was funded in part by Inomedic Health Applications, Aquatic Animal Health Program of the University of Florida, the Medical University of South Carolina and Hollings Marine Lab. All research was performed under the authorization of the special activity license No. SAL-09-0512A0SR. All animal handling was in accordance with the Merritt Island National Wildlife Refuge Special Use Permit No. 2011SUP001 and NASA’s Kennedy Space Center IACUC Project No. GRD-11-077.
# Table of Contents

ACKNOWLEDGMENTS .................................................................................................................. 4

LIST OF TABLES .......................................................................................................................... 8

LIST OF FIGURES ......................................................................................................................... 9

LIST OF ABBREVIATIONS ............................................................................................................. 10

ABSTRACT .................................................................................................................................. 11

CHAPTER

1 RED DRUM (*SCIAENOPS OCELLATUS*) PHYSIOLOGY AND ENDOCRINOLOGY ................................................................. 13

   Life History .................................................................................................................................. 13
   Red Drum Spawning ....................................................................................................................... 14
   NASA’s Kennedy Space Center Reserve ..................................................................................... 16

   Health Assessments .................................................................................................................... 18
   Wildlife Health Assessments ....................................................................................................... 18
   Specimen Banking ......................................................................................................................... 19
   American Alligator Health Assessment ....................................................................................... 20
   Marine Mammal Health Assessments ......................................................................................... 20

   Fish Health Assessments ........................................................................................................... 23
   FWC Fish Assessment ................................................................................................................. 25
   SERF Health Index ....................................................................................................................... 26
   Collection Method ....................................................................................................................... 26
   Handling Techniques .................................................................................................................... 27
   Sedation Techniques .................................................................................................................... 28
   Bleeding Techniques .................................................................................................................... 28
   Reproductive Health ..................................................................................................................... 30
   Sex Hormones ............................................................................................................................... 30

   Stress Response .......................................................................................................................... 32
   Acute and Chronic Stress .............................................................................................................. 33
   Cortisol ....................................................................................................................................... 34
   Glucose ....................................................................................................................................... 36
   Objectives and Hypotheses ......................................................................................................... 37

2 HEALTH ASSESSMENT OF ADULT RED DRUM ....................................................................... 41

   Study Objectives .......................................................................................................................... 42
   Materials and Methods ................................................................................................................. 44
   Fish Capture Methods ................................................................................................................. 44
   Plasma Collection ......................................................................................................................... 44
External examination protocol ................................................................. 45
Sex Identification ...................................................................................... 46
Tagging and Handling .............................................................................. 46
Water Quality ............................................................................................ 46
Condition Factor ....................................................................................... 46
Steroid Assays ........................................................................................... 47
Glucose Analyses ....................................................................................... 48
i-STAT Analyzer ....................................................................................... 49
Statistical Analyses ................................................................................... 49
Results ........................................................................................................ 50
  Morphometrics & Condition Factor .......................................................... 50
  Water Quality ........................................................................................... 51
  Health Index .............................................................................................. 51
  Parasites ................................................................................................... 51
  Plasma Glucose Concentrations ............................................................... 52
  Plasma Cortisol Concentrations ............................................................... 52
  Plasma 11-KT Concentrations ................................................................. 53
  Plasma E2 Concentrations ....................................................................... 53
  Sex Identification ..................................................................................... 54
  Recaptures ................................................................................................. 54
Discussion ................................................................................................... 55
  Predicted Outcomes ................................................................................ 55
  Stress Response ....................................................................................... 62
  Water Quality ........................................................................................... 63
  Further Research ..................................................................................... 64

LIST OF REFERENCES ................................................................................. 74

BIOGRAPHICAL SKETCH .......................................................................... 82
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Health index scoring system developed for red drum by the Stock Enhancement Research Facility (Dukeman et al. 2006).</td>
<td>40</td>
</tr>
<tr>
<td>2-1</td>
<td>Morphometric data and condition factor for each reproductive period</td>
<td>72</td>
</tr>
<tr>
<td>2-2</td>
<td>Plasma values for each reproductive period</td>
<td>72</td>
</tr>
<tr>
<td>2-3</td>
<td>Water quality in the KSC Reserve during each sampling period</td>
<td>73</td>
</tr>
<tr>
<td>2-4</td>
<td>Predicted sex for undetermined red drum caught in the KSC Reserve</td>
<td>73</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1-1</td>
<td>Kennedy Space Center security zone de-facto no-take fisheries reserve is outlined in yellow.</td>
<td>39</td>
</tr>
<tr>
<td>2-1</td>
<td>Kennedy Space Center security zone de-facto no-take fisheries reserve.</td>
<td>68</td>
</tr>
<tr>
<td>2-2</td>
<td>Collecting a 4ml blood sample from the branchial vessels in the gill of a wild caught red drum.</td>
<td>69</td>
</tr>
<tr>
<td>2-3</td>
<td>Sex determination of wild caught red drum in the field.</td>
<td>69</td>
</tr>
<tr>
<td>2-4</td>
<td>Health index score of wild caught red drum (± S.D.) presented by sampling period.</td>
<td>70</td>
</tr>
<tr>
<td>2-5</td>
<td>Plasma cortisol concentrations of wild caught red drum (± 1 S.E.) presented by reproductive period and by sex.</td>
<td>70</td>
</tr>
<tr>
<td>2-6</td>
<td>Plasma 11-KT concentrations of wild caught red drum (± S.D.) presented by reproductive period and sex.</td>
<td>71</td>
</tr>
<tr>
<td>2-7</td>
<td>Plasma E$_2$ concentrations of wild caught red drum (± S.D.) presented by reproductive period and sex.</td>
<td>71</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-KT</td>
<td>11-ketotestosterone</td>
</tr>
<tr>
<td>DFA</td>
<td>discriminant function analysis</td>
</tr>
<tr>
<td>$E_2$</td>
<td>$17\beta$-estradiol</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme immunoassay</td>
</tr>
<tr>
<td>FIM</td>
<td>fisheries independent monitoring</td>
</tr>
<tr>
<td>FIT</td>
<td>Florida Institute of Technology</td>
</tr>
<tr>
<td>FL</td>
<td>fork length</td>
</tr>
<tr>
<td>FWC</td>
<td>Florida Fish and Wildlife Conservation Commission</td>
</tr>
<tr>
<td>GEA</td>
<td>gross external abnormality</td>
</tr>
<tr>
<td>GPS</td>
<td>global positioning system</td>
</tr>
<tr>
<td>HERA</td>
<td>Health and Risk Assessment project</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IRL</td>
<td>Indian River Lagoon</td>
</tr>
<tr>
<td>KSC</td>
<td>Kennedy Space Center</td>
</tr>
<tr>
<td>MINWR</td>
<td>Merritt Island National Wildlife Refuge</td>
</tr>
<tr>
<td>MPA</td>
<td>marine protected area</td>
</tr>
<tr>
<td>NASA</td>
<td>National Aeronautics and Space Administration</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>PIT</td>
<td>passive integrated transponder</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>SERF</td>
<td>Stock Enhancement Research Facility</td>
</tr>
<tr>
<td>SL</td>
<td>standard length</td>
</tr>
<tr>
<td>TL</td>
<td>total length</td>
</tr>
</tbody>
</table>
Despite significant value of the Florida red drum (Sciaenops ocellatus) fishery, a lack of sex and stress hormone data are available. Current non-lethal health assessment programs do not collect this information. This was the first study to assess sex and stress hormones for adult red drum in Florida, with the goal of developing protocols defining “health” and providing baseline data. This project incorporated the Stock Enhancement Research Facility (SERF) external health index with blood chemistry analysis of glucose, cortisol, 11-ketotestosterone (11-KT) and 17β-estradiol (E2). Red drum (n=126) were collected from NASA’s Kennedy Space Center waters, the oldest fully protected no-take fisheries reserve in the United States, during three different reproductive periods to evaluate seasonal variation and effect of reproductive activity on stress response.

Fish in all periods scored near or above the “healthy” level for the SERF health index. The lower scores were attributed to wild fish having more parasites than cultured fish. On average condition factor ranked all fish as excellent-exceptional. Glucose,
cortisol, and E$_2$ levels were significantly different among reproductive periods. Cortisol values ranged between 0.93 – 1.25 ng/ml, well below typical 10 ng/ml found in teleosts. 11-KT was significantly elevated during the reproductive period for both sexes. Blood collection occurred in less than three minutes and may have minimized the glucose and cortisol response associated with handling. Results from this study illustrate the potential value of future comparisons of red drum near the study area, the range of the species, and as a model for other sciaenids.
CHAPTER 1
RED DRUM (SCIAENOPS OCELLATUS) PHYSIOLOGY AND ENDOCRINOLOGY

Life History

The red drum, *Sciaenops ocellatus*, (family Sciaenidae) are part of an important inshore recreational fishery in Florida (Adams and Tremain 2000). Florida is recognized as the “Fishing Capital of the World” based on a 2006 National Survey of Fishing, Hunting and Wildlife-Associated Recreation that shows Florida is the number one recreational fishing state. Florida attracts about 2.8 million anglers annually. In 2007, the Indian River Lagoon (IRL) was calculated to have a commercial fishing value of $3.8 million dollars and $1.5 billion dollars for recreational fishing (Hazen and Sawyer 2008).

Red drum are a long-lived fish, some exceeding 30 years of age in Florida Atlantic waters (Murphy and Taylor 1990). The species inhabits nearshore and estuarine waters from Massachusetts to the Gulf of Mexico coast. Annual commercial landings of red drum in Florida averaged nearly 1.0 million pounds from the early 1960’s until 1986 (Winner et al. 1999). Declines in adult red drum resulted in closure of the commercial fishery in Florida in 1986 (Murphy 2009). At that time, the recreational fishery became regulated by a slot size of 18 – 27 inches (457 - 685 mm) and a daily bag limit of one fish (Johnson and Funicelli 1991). These measures were put in place by state and federal managers to safeguard the spawning stock biomass and help ensure stable annual recruitment (Murphy 2009).

The recreational fishing effort has more than doubled on both the Atlantic and Gulf coasts since 1989 (Winner et al. 1999). Large juvenile size classes (200 - 600 mm) comprise the majority of all red drum fishery landings in Florida waters (Adams and Tremain 2000). It is important for fisheries managers to have an understanding of the
use of essential fish habitat area of red drum to protect this species. Recreational landings combined from the Gulf and Atlantic in Florida were estimated at 2,499,324 fish in 2007 (Murphy 2009). The latest red drum stock assessment conducted in 2008 indicated that overfishing was not occurring on either coast of Florida (Murphy 2009). In February 2012 the Florida Fish and Wildlife Conservation Commission divided the state into three fishery management areas for red drum: Northwest, Northeast, and the South. The bag limit was increased to two fish per day in the Northwest and Northeast regions, which indicates success in the management of the species. It is difficult to assess exploited fish populations and to evaluate the risk involved in fishery management decisions and difficult to determine when management actions are truly working to sustain stocks (Murray et al. 1999).

**Red Drum Spawning**

Red drum have demonstrated a pattern in spawning site selection by returning year after year to spawn in similar locations, since red drum eggs and planktonic larvae have been collected repeatedly in parts of its range including Texas, Alabama, Mississippi, and Florida (Johnson and Funicelli 1991). Starting in late August through October, the majority of red drum produce numerous planktonic larvae in nearshore waters near estuarine inlets (Perez-Dominguez and Holt 2002). Larval red drum, however, have been found commonly in July and early August and occasionally in June in the IRL, Florida (Reyier et al. 2008). After a few weeks in the plankton, the larvae settle in seagrass beds within estuaries that serve as primary nursery habitat for juvenile red drum. Sexually mature red drum have been reported in bays and estuaries in Texas, North Carolina and Florida, despite its usual offshore spawning. Recent data has shown that adult red drum reside and spawn within certain estuaries including
Pamlico Sound, North Carolina (Ross et al. 1995), Savannah River and Atlamaha River Estuaries, Georgia (Lowerre-Barbieri et al. 2008), Tampa Bay and IRL System, Florida (Murphy and Taylor 1990; Reyier et al. 2011), and Chandeleur Sound Louisiana (Comyns et al. 1991). Reyier et al. (2011) stated that estuarine reproduction is an important life strategy for the species in east-central Florida. Management of estuarine-spawning red drum may depend in part on factors such as identification, preservation, and management of spawning and nursery areas, and preferred estuarine habitats for the various life history stages (Johnson and Funicelli 1991). The proportion of the population involved in this inshore spawning activity is currently unknown; however these fish may be at greater risk from intensive inshore recreational fishing harvest.

Red drum spawning in the southern portion of the species range tends to occur later in the year and is more protracted beginning as early as August and extending into December in the northern Gulf of Mexico, Texas and Tampa Bay. Spawning behavior has been observed between mid-September and mid-February in the Everglades National Park (Jannke 1971). Perez-Dominguez and Holt (2002) found that the benefit of evolving an estuarine-dependent lifestyle increases the growth potential of fry and juveniles in fluctuating environments. Red drum larvae are very tolerant of temperature fluctuations from 22-27°C (Perez-Dominguez and Holt 2002).

Juveniles have strong site affinity to their inshore estuarine habitat, but growing evidence demonstrates that adult red drum are also utilizing estuarine habitats throughout their life and can complete their life cycle there without the need for an offshore residency period. Reyier et al. (2011) found that acoustically tagged and monitored red drum in the IRL, Florida, exhibit strong site fidelity from winter through
early summer with movement increasing within its range during the fall spawning months; however the majority of tagged fish remained within the lagoon year-round. The non-migratory behavior of this population seems to be unique to the northern IRL region as most mature adults from other areas emigrate to nearshore waters with maturity (Reyier et al. 2011). Johnson and Funicelli (1991) believe that red drum may display some degree of homing or imprinting instincts, which has been observed in other fish species, facilitating their ability to return to spawn at their place of origin. Red drum eggs were collected in Mosquito Lagoon during October and early November in clumps suggesting they were from spawning aggregations (Johnson and Funicelli 1991). Adams and Tremain (2000) observed that estuarine creeks are critical nursery habitats used by juveniles and suggested that the use of warmer creek waters during winter months was crucial for survival when lagoon temperatures declined.

Murphy and Taylor (1990) studied red drum in Florida and found that males matured at smaller sizes and younger ages than did females, on both the west and east coasts. They estimated the lengths at which 50% of fish were mature on the Atlantic coast for males at 511 mm and females at 900 mm. The average observed sizes for males were significantly larger at ages 1 and 2 on the Atlantic coast than the Gulf Coast. Red drum in Florida appear to grow more rapidly than red drum in Mississippi, South Carolina and Texas (Murphy and Taylor 1990).

**NASA’s Kennedy Space Center Reserve**

Mature resident red drum are known to spawn throughout the fall period within the National Aeronautics and Space Administration’s (NASA) Kennedy Space Center (KSC) Reserve (Stevens and Sulak 2001; Reyier et al. 2008). The study area includes 33 km$^2$ of estuarine waters within the KSC boundary and a *de-facto* marine fisheries reserve.
that was established in 1962 to safeguard rocket launch operations (Figure 1-1). This is the oldest fully protected no-take fisheries reserve in the United States (Roberts et al. 2001) and is managed by Merritt Island National Wildlife Refuge (MINWR). A no-take reserve is an area where extractive activities are banned except for limited scientific and educational purposes (Bartholomew and Bohnsack 2005). Few no-take reserves exist in the United States (Murray et al. 1999). The KSC reserve area is a shallow estuary, with a mean depth of 1.5 meters. There are a few dredged areas with depths up to 15 m that were created during construction of rocket launch pads and roadways, as well as a navigational channel that is 3-4 m in depth. This estuary is isolated from the Atlantic Ocean by barrier islands which have only five widely spaced inlets, the closest being Sebastian Inlet, approximately 95 km south of the study area (Reyier et al. 2011). Many marine fishes utilize estuaries for a crucial life history component due to highly productive waters that serve as feeding and nursery grounds for the species that comprise 75 percent of U.S. commercial landings (Chambers 1992). The estuary has extensive seagrass beds of shoal grass (*Halodule wrightii*), manatee grass (*Syringodium filiforme*) and salt marsh that provide refuge from predators and foraging opportunities for red drum throughout their life cycle.

The KSC Reserve minimizes many of the challenges to growth and survival experienced by red drum elsewhere in the southeastern U.S. These may include intense angling pressure, reduced habitat quality caused by coastal development, and storm water runoff contaminants from anthropogenic sources. Previous research has demonstrated that sportfish within the KSC Reserve achieve a larger mean size and exist in higher densities than those in adjacent public areas (Johnson and Funicelli
Red drum have strong site fidelity (Reyier et al. 2011) and those from KSC Reserve waters spend a large portion of their adult lifespan in the reserve. Red drum spawning in the KSC Reserve may have the positive benefit of being protected from adjacent public waters, protecting a portion of the spawning stock from exploitation (Bohnsack 1993). Stevens and Sulak (2001) did a mark-recapture study with red drum in public waters surrounding the KSC Reserve and found that large adult sportfish were being protected in the reserve area. It was functioning as a replenishment zone for the reproductively active population much like a marine protected area (MPA). The KSC Reserve is off-limits to public anglers and fits the most stringent guideline of an MPA which is a no-take reserve for all animals residing in its area.

**Health Assessments**

**Wildlife Health Assessments**

Marine animals are faced with health threats including infectious agents (viruses, parasites, bacteria, and fungi), entanglements, catch and release stress, and exposure to, or accumulation of toxic pollutants. Health assessments on marine animals have been developed to improve our understanding of the biology and overall health and vigor of wildlife populations. Tracking and analyzing health trends in populations of marine animals suggest they can function as sentinel species and provide a useful tool for evaluation of the well-being of aquatic ecosystems (Bossart 2011). Marine mammals and reptiles have been extensively studied by using specific health assessment protocols that are proving valuable in comparing populations from different geographic regions. Data may include presence or absence of disease, contaminants or physiological metrics. The protocol for wild animal sampling is trending toward non-lethal assessments, and incorporation of methods that facilitate the return of animals to
the population being studied. This in turn allows for long-term sampling of the same
animal, if tagged with a unique identification number, facilitating assessment of changes
over time. Representative sample sizes are used as a proxy so the populations' health
status can be extrapolated from assessments conducted on individual animals. Ranges
for 'normal' values of various hematological parameters, such as hematocrit, packed
cell volume, and glucose are used to assess the overall health of the sentinel species
using the animals as bioindicators. If a range of these known data are available it adds
to the decision in classifying a health status. Several efforts currently underway, are
intended to correlate select health metrics with exposure to pollution, biotoxins, and
disease by use of live-capture techniques and release techniques thus decreasing the
need for lethal sampling (Kucklick et al. 2010a).

Specimen Banking

A very important aspect of health assessment protocols is specimen banking,
which ensures that geographic and temporal trends can be examined retrospectively
when questions arise or conditions change. Traditional specimen banking has relied
heavily on lethal sampling, but recent studies have proven the value of non-lethally-
collected marine mammal tissue samples for assessment of pollutant exposure
(Kucklick 2010b). The National Institute of Standards and Technology (NIST)
developed uniform collection protocols for marine mammal tissues which included
blood, blubber, and organ tissues. Marine mammal assessment data includes: body
condition, notation of lesions/wounds, body weight, eye exam, analysis of blood, skin,
and reproductive parameters. Also implantation of a passive integrated transponder
(PIT) tag before the animal is released will facilitate access to data from the same
animal in the future. NIST also supports monitoring of sea turtle health and developed a
uniform protocol to collect blood and scute samples from live-capture and release (Kucklick 2010b). Aguirre and Lutz (2004) state that marine turtles can serve as sentinels of ecosystem health for benthic environments in Florida. However to determine the health status of an individual or a population, normal functional physiology and disturbed or pathological physiology must be distinguished (Aguirre and Lutz 2004).

**American Alligator Health Assessment**

Hamlin et al. (2010) were able to determine the seasonal and environmental influence on androgen cycles in the adult male American alligator, *Alligator mississippiensis*, in Florida, at Merritt Island National Wildlife Refuge (MINWR), a site with known heavy metal contamination. Non-lethal methods were used, including blood sampling. These techniques facilitate mark-recapture studies that may help identify seasonal changes in health parameters. Boggs et al. (2011) also studied the American alligator using non-lethal techniques and were able to ascertain seasonal variation in plasma thyroid hormone concentrations from two locations in Florida. Wildlife sentinels may more accurately reflect the real world exposure conditions of contaminants or select pathogens (Hamlin and Guillette 2011).

**Marine Mammal Health Assessments**

Bossart (2011) explains that marine mammals are excellent sentinel species due to their long life spans, long-term coastal residency, high trophic level diet, and fat stores that contain anthropogenic toxins. Goldstein et al. (2006) noted that it is important to include physical examination, history, and diagnostic testing as part of the health assessment of marine mammals. Atlantic bottlenose dolphins, *Tursiops truncatus*, have been studied in Sarasota Bay, Florida since 1970 and annual health
assessments continue there and in other locations in the southeastern United States (Wells et al. 2004). A large data set of blood parameters has been collected and integrated to create an objective, quantitative and comparable health assessment scoring system. Four different designations have been developed for assessment of wild dolphin population health status. Parameters from baseline blood values were used to assign a health status score. These range from apparently good health to a serious medical condition that requires treatment (Wells et al. 2004). Some concerns with this type of scoring system include variations in analytical techniques used by different laboratories and missing values can bias health scores down. Further, the scoring system does not include body condition (Wells et al. 2004).

Bottlenose dolphins have also been studied in a collaborative effort in Charleston, South Carolina, and the IRL, Florida as part of a Health and Risk Assessment (HERA) project from 2003-2005. Bossart et al. (2008) explained that the goals of the HERA project were to develop standardized tools for health and risk assessment. These included physical exam, sampling of teeth for age determination, skin/blubber, ultrasound to detect pregnancy, hematology, serum chemistry, gastric, urinalysis, blowhole and fecal cytology. Hematology was used to evaluate relative leukocyte counts, hemoglobin, red blood cell counts, mean corpuscular platelet volume, mean corpuscular hemoglobin, white blood cell counts, and total platelets all of which were determined by an automated analyzer. Serum chemistry included analyses for sodium, potassium, chloride, bicarbonate, anion gap, blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, magnesium, glucose, total direct and indirect bilirubin, cholesterol, triglycerides, iron and fibrinogen. Specific immunological tests and antibody titers were
also run from collected blood samples. Data from these dolphins can be compared to previously collected data through nine different collaborative projects that are involved with HERA. Conclusions include insights on sexually transmitted tumors that are occurring in these dolphin populations. Multiple abnormalities in serum chemistries, protein electrophoresis, and immunological parameters were found in dolphins with tumors when compared to healthy dolphins (Bossart et al. 2008).

Dolphins were considered healthy when they were categorized to be free of disease by Bossart (2008). An example of establishing normal parameters includes the serum range of glucose for wild bottlenose dolphins which is 62-139 mg/dL (Varela et al. 2006). This becomes a reference range for veterinarians to use in assessment of wild and captive dolphins. The HERA project researchers caution that interpretation of the immune system data may not be possible yet. Data on wild dolphins has still not been well defined and further research is needed to define the complex immune function and overall health of dolphins (Bossart et al. 2008).

The Florida manatee, *Trichechus manatus latirostris*, is a federally endangered marine mammal that has been extensively studied in Florida for over 25 years. Subsequently, a large health database has been assembled from non-lethal health assessments conducted on this species in Florida (Bonde et al. 2004). Long-term recapture data allows researchers to notate differences in captive and wild populations over time. Harvey *et al.* (2007) noted differences in blood plasma values measured in wild and captive manatees during routine diagnostic health assessments. Differences in blood plasma values for these populations were attributed to many variables including diet, time from last feeding, water chemistry, environmental stressors, presence of
bacterial contaminants, body condition, capture stress and seasonal variations. Differences between captive and wild populations are expected, but poorly defined for many species. When conducting health assessments on a wild population, since many intrinsic factors will be out of the researchers’ control, it is imperative to abide by a strict protocol without deviation to attempt to eliminate sampling errors in the field.

**Fish Health Assessments**

The use of fish as biological indicators of estuarine conditions has many advantages. Useful health indicators are capable of detecting responses to synergistic, sub-lethal stress which can affect the fitness of an organism with population-level consequence such as condition index, organ function, liver glycogen content or liver somatic index (Leamon et al. 2000). Fish health assessment is playing an increasing role in both fishery management and environmental monitoring policy. Hematologic and plasma chemistry data can provide insight into organ function, general metabolic homeostasis and immune function, once baseline information has been established. Fish can provide a more sensitive assessment of the link between human activities and their ecological consequences (Schlacher et al. 2007). One concern with biological indicators such as blood parameters is that normal ranges for a particular fish species must be determined prior to being used in a monitoring program (Leamon et al. 2000). A comparison of the range and relationship of these indicators between proximate but separate non-impacted locations would present the natural range of values for the species. Baseline blood parameter data from unfished stocks can vastly improve estimates of population parameters for harvested species (Murray et al. 1999). Leamon et al. (2000) stated that data obtained from a non-impacted location study site, such as a reserve with no fishing allowed, could function as a standard for evaluating other sites.
All of the above reasons make the KSC reserve an excellent study location for determining baseline blood values of wild red drum. Red drum are suitable study animals for immune responses of natural populations due to their life history as sedentary juveniles through adults that remain in the same estuarine habitat for four to five years (Evans et al. 1997). This would be an important component of a health index valuable for future use and comparisons with other red drum stocks.

Fish health can be assessed using morphological, hematological and immunological examination as well as by using experimental disease challenges (Maita 2007). The extent to which natural environmental variables and anthropogenic stressors influence the immune response of marine teleosts to antigenic challenges in their natural environment remains unknown (Evans et al. 1997). However non-lethal health assessments currently have limited adaptations for work in fishery science. Fish health assessments conducted in the field are commonly conducted by post-mortem necropsy potentially removing healthy animals from wild stocks. Red drum throughout the state of Florida, including the IRL, have been tested for total mercury content since it is known to bioaccumulate in fish tissue. Human fish consumption has been correlated with mercury content in fish (Adams and Onorato 2005). Data from this study were utilized by the Florida Department of Health to issue a health advisory suggesting limits to human consumption of red drum in the Florida Keys- Florida Bay area. Currently the legal recreational slot size for red drum is an effective filter to prevent human consumption of fish with potentially higher concentrations of mercury caused by bioaccumulation.
FWC Fish Assessment

The Florida Fish and Wildlife Conservation Commission (FWC) has conducted health evaluations on wild fish populations during their Fisheries Independent Monitoring (FIM) program since 1990. Fish are usually captured using a trammel net or bag seine. These methods result in fish being in the net for at least 10-25 minutes before they are moved to a live well for further processing. While fish are struggling in the confined space of the net or live well, their primary and secondary stress responses become elevated over the duration of the capture.

Currently evaluations conducted through the FIM program are focused on gross external abnormalities (GEA) such as lesions, wounds, infections or other obvious signs of injury. If no external GEA are observed the fish is counted and released. If a fish does exhibits a GEA, then it is culled and sent to the FWC Fish and Wildlife Research Institute for necropsy and further analysis. Length and weight measurements are recorded for fish that are going to be sent in for further processing and Fulton's condition factor is calculated later in the laboratory. Condition factor has been accepted as a gross health index of general fish condition and is thought to provide information on energy reserves and the potential for an animal to tolerate toxicant challenges or other environmental stressors (Grund et al. 2010). Tissues collected include: otoliths and dorsal spines used for age determination, fins for genetic sampling, reproductive organs for gonad histology, muscle sample for determination of mercury concentrations, and stomach contents for dietary determination. After the fish is evaluated, a potential cause of death is assigned to determine if anthropogenic causes are involved and to see if any mitigation can be performed to prevent future fish kills or GEAs. FWC does
not have any non-lethal field methods to examine internal health of wild fish populations or GEAs.

**SERF Health Index**

The Stock Enhancement Research Facility (SERF), operated by FWC in Port Manatee, Florida, has developed a health index for juvenile red drum which they use to assess the health status of their fingerlings. The health index relates all quantitative and qualitative data associated with external evaluations. Health challenges of cultured fish are often related to water quality and stocking densities (Dukeman et al. 2006). Fish are netted from their tanks and euthanized with a lethal dose of MS-222 to be processed for the assessment. This metric creates an assessment using the external condition of the fish (i.e., physical abnormalities, parasite load, microbial infections, and condition factor). The health index provides a score in the range of 0-50 for each fish examined (Table 1-2). When a red drum scores a value below 45, it is determined to have had compromised health, whereas a score of 45 and above is considered in the “healthy” range. If fish score below 45, water quality, stocking density and feeding regime are examined to determine the cause of the compromised health of the fish.

**Collection Method**

Many stress hormones have a short latency period making a baseline determination difficult or impossible. Handling of any kind will result in changes of stress hormones occurring within a few minutes. Collecting fish from a wild setting involves many variables that are not a concern in a laboratory setting. These may include how long it takes to collect the fish, variable water quality, water temperature, unknown age and sex of the fish being targeted. Techniques used to collect wild fish include hook and line, trammel netting, seining, and long-lining.
The ideal capture method for non-lethal testing would be the quickest way to get fish on board with minimal stress followed by rapid release. Many species of fish can be captured by hook and line fairly easily; however a disadvantage of this technique is the risk of gut hooking the fish or causing structural damage to the mouth or jaw. Trammel nets, seining and long-lining all require the fish to be entangled in a net or on a hook for at least 10-20 minutes before the animal would be retrieved for assessment. Using long-lining as a method for fish collection, Pankhurst and Sharples (1992) reported that snapper, *Pagus auratus*, that were in the set for one and half hours had cortisol values of 22 ng/ml and that these increased to 58 ng/ml within sixty minutes of landing. A study by Lowerre-Barbieri et al. (2003) of hook and line catch and release fishing on a wild spawning aggregation of common snook was conducted to determine effect on reproductive output. Findings from this study conducted on the Atlantic coast of Florida found that the stress of capture and release did not appear to cause the common snook to cease spawning based upon recapture data that evaluated ovarian development. The study also concluded that females implanted with sonic tags continued to spawn even after encountering considerable handling and stress involved with the surgery to implant the tags.

**Handling Techniques**

Once a fish is captured, a few options are available in the field for handling the animal. Restraint options include placement in a cooler with water and aeration, restraint in a sling, or use of an inverted v-tray without water. The option of placing the fish in water would be ideal, however contamination needs to be considered especially in a remote field setting where only the surrounding ambient water is available for rinsing, and parasites could be moved between fish.
Another consideration is ambient air and water temperature. Since field sampling in Florida can mean hot weather and even hotter water temperatures, this parameter needs to be monitored and controlled to be kept within ambient water ranges. If fish are confined in a small container and water temperature is high, the dissolved oxygen concentration can plummet rapidly compromising the fish’s well-being.

**Sedation Techniques**

Once the fish is in hand, some researchers employ the use of anesthesia, tricaine methanesulfonate (MS-222), which currently is the only US Food and Drug Administration approved anesthetic for fish; however it can have effects on blood chemistry values. Thomas and Robertson (1991) found that in red drum when MS-222 is used at a dosage of 80 mg/l for two and half to six minutes, it can block the plasma cortisol biochemical response to handling stressors. Further, MS-222 cannot be used on legally edible wild fish that could be caught by the public unless they are held for a 21 day withdrawal period before being released (US Food and Drug Administration 1997). Since field sampling often is a catch and release practice, use of anesthetics is not feasible given current legal constraints and concerns for anesthetic residue in edible tissue. A few main reasons to use anesthetics are to sedate and immobilize the animal and to eliminate pain or discomfort to the animal. Manual restraint needed for venipuncture and evaluation of external morphometric measurements does not typically require the use of an anesthetic.

**Bleeding Techniques**

Maita (2007) explained that measurement of blood parameters, such as hematocrit value, hemoglobin concentration, red blood cell counts, and plasma components are tools that can be used to monitor fish health. Plasma components
commonly assessed are total protein, urea nitrogen, glucose, total cholesterol, triglyceride, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. These components are used to infer health by comparing it to data gathered previously and deciding if the current values are within a healthy range. If values obtained are outside of the ranges previously detected it could mean that the current health status of the animal is in decline. These plasma components measure the fish’s response to stressors and everyday life. An advantage of using blood samples for health assessments is that these parameters are routinely measured using commercially available kits and equipment and several analyses can be measured from the same animal using a small amount of blood. An advantage of using blood collection techniques as a measure of fish health is that they usually can be carried out without killing the fish (Maita 2007). A disadvantage is that many factors can confound hematological data such as differences in species, age, sex, water quality, water temperature, and handling methods. All these variations can make comparisons between studies difficult and make setting normal ranges, or reference ranges, challenging (Maita 2007). Ways to mitigate these variations are to create a static protocol to collect samples that is repeatable with boundaries on fish length, weight, time of year to sample, time of day, and a standard collection method.

Blood is usually collected from one of four locations in the fish, caudal vein, heart, dorsal aorta, or the branchial vessels. There are advantages and disadvantages associated with each site. Collecting a blood sample from either the caudal vein, by cardiac puncture or from the dorsal aorta leaves a chance for other constituent body fluids to enter the sample. Also cardiac puncture is typically only performed on sedated
fish that are going to be euthanized. Using the branchial vessels in some species of larger fish is quick and efficient. The choice of blood collection techniques will depend on the fish species, size, body morphology, protected status, available restraint methods, and lethal or non-lethal outcome.

Reproductive Health

The health and success of a species ultimately relies on successful reproduction. Understanding baseline values for sex hormones is necessary to ascertain what sex hormone ratios could potentially impact reproductive success of red drum (Roberts et al. 1999). Effective management practices require an understanding of a species’ reproductive biology. When a species of fish is under threat from excessive fishing pressure during its spawning period this can lead to detrimental effects on larval output (Coleman et al. 1996). An approach to measuring fishing pressure is to examine fish after undergoing capture, noting the duration of capture, and evaluating their stress hormones. Stress can unfavorably affect reproductive physiology in fish by causing changes in reproductive hormone concentrations, fecundity, egg size, and survival of eggs and larvae (Billard et al. 1981; Campbell et al. 1994; McCormick 1998).

Sex Hormones

Seasonal changes in gonadal steroid hormone concentrations have been well documented in many freshwater teleost species. Less information is available on gonadal steroid hormone concentrations for marine species (Prat et al. 1990). Wild white sturgeon have been studied by Webb et al. (2002) for potential classification of sex and stage of gonadal maturity using plasma hormones, 11-ketotestosterone (11-KT) and 17β-estradiol (E₂). They reported that fish between 96 -152 cm fork length with values of 5 ng/ml or higher of 11-KT were classified as male, and those with values of
2mg/ml or higher of $E_2$ were classified as females. Their conclusion was that the use of plasma sex steroids provided an accurate and less invasive method for calibrating current population models. They were able to use plasma hormone analysis to replace the need for surgical gonadal biopsies on these sexually monomorphic fish.

Red drum are also sexually monomorphic. Mature males may be recognized if a drumming sound is heard, or if milt is seen extruding from the vent during spawning season. Traditionally fisheries research has required the sacrifice of individuals for accurate sex identification which is necessary for population dynamics studies and stock assessments. A study on cultured and captive red drum by Kucherka et al. (2006) evaluated circulating sex steroid concentrations of (11-KT) and ($E_2$). The key results were that a minimum concentration of 11-KT, 1.0 ng/ml, is the threshold to distinguish male sex of fish during summer or early fall, of cultured adult red drum. It is imperative to be able to correctly identify the sex of red drum to delineate seasonal differences in blood plasma parameters. These parameters may vary based on their sex, since plasma steroid profiles vary during different stages of the gonadal cycle in both males and females (Kucherka et al. 2006). Males had values of 0.7 ng/ml of 11-KT in early fall and 1.0 ng/ml during late fall while females had values of 0.5ng/ml in early fall and late fall respectively. According to Hamlin et al. (2007) few studies have been conducted using fish to define the relationship between stress and possible changes in sex steroid concentrations. By contrast, general stress responses to numerous environmental conditions have been studied in a range of fish species, especially long-lived species of high economic importance (Hamlin et al. 2007).
Stress Response

The definition of “health” for wild populations of fish is a nebulous term. Fish are exposed to stressors on a daily basis both in their natural habitat and under aquaculture conditions (Bayunova et al. 2002). Bonga (1997) defines stress as a response evoked in conditions that cause discomfort, fright, pain, or a sensory perception of an adverse stimulus or its effects. It is a common misconception among fishery biologists that stress in itself is detrimental to the fish. Stress is a necessary adaptive mechanism that allows the fish to cope with stressors and maintain homeostasis (Barton 2002). The stress response of fish is comparable to that of mammals and conforms to a general vertebrate pattern (Bonga 1997). The effects of stressors can disturb the homeostasis of an animal and cause a coordinated set of behavioral and physiological responses that attempt to enable the animal to overcome the threat (Bonga 1997).

Stress responses in fish are part of an integrated process which can be broken down into four components. The primary stress response, the immediate reaction of the animal, can be measured by the animal’s corticosteroid concentrations. The secondary responses are the immediate actions and effects of hormones at the blood and tissue level. These may include increases in cardiac output, oxygen uptake, mobilization of energy and changes in osmoregulation. Some tertiary responses, such as changes in blood physiology, are seen over longer periods of time and may inhibit growth, reproduction, and the immune response (Bonga 1997). The quaternary responses are seen long term at the population level as reproductive outputs, die-offs and changes in birth rates.
**Acute and Chronic Stress**

According to Pankhurst (2011), under most circumstances, recovery from acute stress will occur in most fish species over a period of six hours or less. In some species elevated plasma cortisol concentrations can persist for days if the stressor is chronic or severe (Hamlin et al. 2007). Magnitude of change in plasma cortisol concentration is affected by sampling techniques, size, season, age and rearing temperature in white sturgeon (Hamlin et al. 2007). The typical cortisol and glucose response following exposure to a stressor would be a rapid increase, reach a peak, then a slow decrease to resting concentrations after the stressor has been eliminated. Chronic stress in cultured fish causes an increase in cortisol and may cause negative physiological conditions such as impaired immune function (Tort et al. 1996), increased oxygen radical production (Ruane et al. 2002) and reproductive impairment (Pankhurst and Van Der Kraak 1997).

Stressors may negatively affect reproduction; however there is a growing amount of evidence that states otherwise. Cortisol is a normal endocrine component of the reproductive system but it can suppress reproduction in some cases (Hamlin et al. 2007). This relationship has been studied in Siberian sturgeon (*Acipenser baeri*), and the findings were that plasma concentrations of E₂, testosterone and 11-KT did not decrease with elevated plasma cortisol concentrations following acute handling stress. This is consistent with observations in many other species of fish. Wingfield (1994) explained that studies on wild populations show that severe environmental conditions and the demands of reproduction are not necessarily stressful if they are predictable. Fish have predictable reproductive periods and most usually have site fidelity year after year. Some harsh environmental conditions could include adverse water quality such
as extreme temperatures or salinities, but if seasonal patterns occur annually within the
tolerance range of a fish then these seemingly extreme conditions may be well
tolerated. Consequently, stress caused by a situation that is reoccurring on an annual
basis may be minimal or non-existent for wild populations.

Cortisol

Stress physiology studies on fish have focused on the aquaculture industry with
the goal of maximizing production (Barton 2002). Stressors initiate a comprehensive
endocrine response in fish characterized by hypersecretion of catecholamines and
cortisol which then induces a variety of secondary effects previously mentioned. Stress
responses of red drum have been studied in cultured fishes (Robertson et al. 1987) with
an emphasis on use of plasma cortisol and glucose as reliable indices (Wedemeyer and
Yasutake 1977). Cortisol is the predominant corticosteroid in an acute stress response
in red drum. Cortisol combines mineralocorticoid and glucocorticoid functions in fish
which reflect the relationship between energy metabolism and hydromineral control.
The mineralocorticoid functions provided by cortisol include the promotion of
differentiation of the chloride cells in the gills, intestine and kidneys. The glucocorticoid
functions affect carbohydrate, protein and lipid metabolism (Bonga 1997). Stress can
be acute or chronic, each state having different effects on the endocrine system of the
animal.

In a chronic stress response, cortisol concentrations may remain elevated, but
below peak concentrations for prolonged periods of time. Basal concentrations were
reported as low as 5 ng/ml in red drum (Barton and Iwama 1991). Chronic stress
responses in cultured fish have been assessed with handling disturbance, heavy
metals, organic pollutants, rapid temperature changes, acidic water, and confrontations
with predators (Bonga 1997). Barton and Iwama (1991) reviewed corticosteroid values in sixteen fish families with varying sample sizes, including data on pre-stress and post-stress values which ranged from <1 ng/ml to 2000 ng/ml depending on the various species, stressor and conditions. A laboratory study on Atlantic cod by Morgan et al. (1999) reported that fish stressed for half an hour three times a week for ten weeks by capture/confinement had higher plasma cortisol concentrations than undisturbed control fish. Fish in both groups were however, able to be spawned and there was little difference in the production of eggs, fertilization rates, and hatching success of larvae.

Due to sampling complications, less information on stress and its physiological and endocrine effects in natural settings is available for wild stocks of fish (Pankhurst 2011). Thomas and Robertson (1991) stated that common aquaculture procedures such as netting, handling, disease treatments and transportation are stressful events for fish and maybe associated with increased susceptibility to disease and a reduced capacity to maintain homeostasis. Pankhurst and Sharples (1992) found that to date, the lowest values measured for cortisol in wild fish were 1.7 ng/ml in snapper, Pagrus auratus, which were captured by net and sampled underwater by scuba divers in less than ten minutes. The length of time from capture to blood sample collection is also an important factor to consider for sample design.

An important factor to remember when sampling wild fish is that there is a short time lag that precedes the increase in corticosteroids. Changes caused by the acute stress response can be minimized if sampling occurs rapidly (Pankhurst 2011). Rapid hook-line capture is a suitable field protocol to combat the issue of increasing corticosteroids soon after the perturbation. Basal cortisol values in teleost fishes are
typically <10 ng/ml; however, a number of species have much higher values in described “unstressed fish” (Pankhurst 2011). For example, cortisol values in Salmonidae have been reported at 544 ng/ml Oncorhynchus mykiss, and 122 ng/ml Coregonus lavaretus. Reported cortisol values for Cyprinidae have been Carassius auratus, 66 ng/ml, and for Percichthyidae, Morone saxatillis, 250 ng/ml were recorded values (Barton and Iwama 1991).

Variability in corticosteroid concentrations can be due to a wide range of factors besides stress. The sex of the fish and maturity, time of day, time since last feeding, and seasonal variations all influence corticosteroid concentrations (Pankhurst 2011). Establishing causal relationships between environmental stressors and observed effects in fish from natural systems is difficult due to many intrinsic environmental variables. Presently there are no widely accepted and proven approaches for determining their relationships (Adams 2003). Adams (2003) found that field studies have advantages, in that they represent the natural environmental conditions, but they also have the limitations that causal factors co-occur, resulting in high variability.

**Glucose**

Glucose can be used as an indicator in assessments of fish health (Robertson et al. 1987). The standard methodology for measurement of glucose has been to collect blood and then run a laboratory assay to analyze the sample. Recent technology created for diabetic patients uses handheld glucometers that provide an instantaneous reading. These handheld glucometers may not capture the full range of glucose values for fish as they were created to detect mammalian ranges. A point of care system that has been utilized by veterinarians for many years on a wide range of animals including marine mammals, and reptiles is an Abbott i-STAT analyzer. An Abbott i-STAT
analyzer can analyze a suite of blood parameters. The Abbott i-STAT is sensitive to
temperature and humidity levels and therefore can be difficult to use under field
conditions. If the temperature of the unit is too hot it will give an error reading, requiring
the use of more than one cartridge per fish which becomes expensive. There have
been a few studies that have evaluated the use of handheld meters in fish (Iwama et al.
1995; Wells and Pankhurst 1999). Their ease of use, portability, and quick sample
analysis make handheld meters promising alternatives to traditional laboratory
methodologies (Venn Beecham et al. 2006). Both an i-STAT meter and Contour
glucometer will be used to test glucose concentrations according to the methods of
Venn Beecham et al. (2006) and Brown et al. (2008). The Abbott i-STAT and Contour
glucometer are not validated for the red drum species and values obtained will need to
be validated for accuracy. A laboratory glucose absorbance assay validation will
evaluate the accuracy, sensitivity, and reliability of these two field glucometers.

Objectives and Hypotheses

To date no studies exist that have incorporated a non-lethal health assessment on
a wild population of red drum, and that evaluate the sex and stress hormones of the fish
temporally. The goal of this study (Chapter 2) is to evaluate non-lethal techniques to
assess the external and internal “health” of a wild red drum population inside NASA’s
Kennedy Space Center (KSC) Reserve. We hypothesize that 1) The red drum
population inside the KSC Reserve will score in the healthy range, (above 45) of the
SERF health index, 2) there will be a significant seasonal difference in glucose values of
red drum due to foraging pattern changes that correspond to the reproductive period,
such as a hyperglycemic response during pre and post-spawning and a hypoglycemic
response during spawning, 3) there will be seasonal variation in the sex steroid profiles
of 11-ketotestosterone, and 17β-estradiol that correspond to the reproductive period, and 4) there will be seasonal variation in the concentration of cortisol that corresponds with the reproductive period.
Figure 1-1. Kennedy Space Center security zone de-facto no-take fisheries reserve is outlined in yellow.
Table 1-1. Health index scoring system developed for red drum by the Stock Enhancement Research Facility (Dukeman et al. 2006). Maximum possible scores are shown below. A score of 45 or greater is considered consistent with ‘health’. A standard condition factor equation was used for the calculation.

<table>
<thead>
<tr>
<th>Fish ID</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition factor x 5</td>
<td>10</td>
</tr>
<tr>
<td>Eyes</td>
<td>1</td>
</tr>
<tr>
<td>Mouth</td>
<td>1</td>
</tr>
<tr>
<td>Mucous</td>
<td>3</td>
</tr>
<tr>
<td>Microbial infections</td>
<td>2</td>
</tr>
<tr>
<td>Gill parasite pathogen</td>
<td>2</td>
</tr>
<tr>
<td>Gill parasite #</td>
<td>4</td>
</tr>
<tr>
<td>Gill parasite load</td>
<td>4</td>
</tr>
<tr>
<td>Gill abnormal</td>
<td>5</td>
</tr>
<tr>
<td>Skin/fin/ parasite pathogen</td>
<td>2</td>
</tr>
<tr>
<td>Skin/fin parasite #</td>
<td>4</td>
</tr>
<tr>
<td>Skin/fin parasite load</td>
<td>4</td>
</tr>
<tr>
<td>Scale loss</td>
<td>2</td>
</tr>
<tr>
<td>Wounds</td>
<td>2</td>
</tr>
<tr>
<td>Other abnormality</td>
<td>4</td>
</tr>
<tr>
<td>Health index score</td>
<td>50</td>
</tr>
</tbody>
</table>
CHAPTER 2
HEALTH ASSESSMENT OF ADULT RED DRUM

Understanding seasonal variation in plasma concentrations of hormones and other parameters is important for the assessment of health, and physiological activities including reproduction, growth, and metabolism. Although significant work in this area has been done with captive populations of various fish species, mostly those associated with fish stocking programs and aquaculture, an improved understanding of wild fish in their natural environment is critical if we are to understand the impact of human activities and environmental perturbations. This is especially critical for economically important sport fish, including red drum (*Sciaenops ocellatus*). Non-lethal health assessments have not been carried out on red drum in Florida despite the significant recreational catch and release fishery present. The impact of this fishery on the health of individual fish is poorly understood. Few studies have resulted in baseline values for blood parameters. Thus, there has been little data for comparative studies among wild populations.

Previous studies on the reproductive biology of this species are also limited primarily to age and growth studies complemented by histological assessments of maturation rates (Murphy and Taylor 1990; Murphy 2009). Stock assessments are currently conducted by Florida Fish and Wildlife Conservation Commission (FWC). These consist of monthly sampling of red drum from both coasts of Florida using a variety of fishing gear, targeting different size classes of fish. Non-lethal assessments are conducted on fish that do not have obvious gross lesions. These fish are measured and released; however minimal data is collected to assess the internal health of the fish in hand. These non-lethal samples provide insight into catch rates over time. The most
recent stock assessments were conducted in 2008 and indicated that overfishing was not occurring on either coast of Florida (Murphy 2009). Lethal exams are conducted so that gonads and otoliths can be harvested for reproduction status, age and growth analysis. It is not ideal that lethal sampling must be conducted to verify that fish are indeed healthy from an economic perspective of the fishery. Red drum stock assessments can be improved so they provide more information while decreasing the use of lethal harvest techniques. Some limits to non-lethal sampling are that age and growth determination from otoliths is not possible, along with internal organ assessments for condition indexes and sex determination. New methods such as fin clips for age determination and blood hormone analysis for sex determination are currently being investigated as alternative means of collecting this data.

The Stock Enhancement Research Facility (SERF) developed a health index for juvenile red drum which is used to assess the health status of their fingerlings. The health index can be performed without lethal harvest. SERF currently utilizes this as a lethal method since information on internal organ health is needed by the hatchery. The health index metric assesses the external condition of the fish utilizing quantitative and qualitative data based on the evaluation created by Dukeman et al. (2006). This health index ranges in a possible score of 0-50 based upon external condition. When a red drum scores a value below 45, its health is determined to be compromised, whereas a score of 45 and above is considered in the “healthy” range.

**Study Objectives**

The goal of this study was to develop non-lethal techniques for assessment of “health” of a wild red drum population inside NASA’s Kennedy Space Center (KSC) Reserve. The objectives of this project were to evaluate the potential use of the SERF
health index on a wild population of red drum inside the KSC Reserve, to determine if there are seasonal changes in blood glucose concentrations in wild red drum that change with reproductive period, to evaluate plasma concentrations of sex steroid hormones 11-ketotestosterone and 17β-estradiol in three different reproductive periods and to evaluate plasma cortisol concentrations in three different reproductive periods. We hypothesize that 1) the red drum population inside the KSC Reserve will average their score in the healthy range, (above 45) of the SERF health index, 2) there will be significant seasonal differences in glucose values of red drum due to foraging pattern changes that correspond to a higher appetite and body demand during the reproductive period, 3) there will be seasonal variation in the sex steroid profiles of 11-ketotestosterone, and 17β-estradiol that correspond to the reproductive period, and 4) there will be seasonal variation in the concentration of cortisol that corresponds with the reproductive period.

Health can be loosely defined as the level of functional or metabolic efficiency of a living organism. Many blood parameters are utilized to assess health in marine mammals and reptiles as well as fish. Given the lack of baseline blood parameters for red drum, this project concentrated on four blood parameters which were most commonly assessed in the literature. We propose that data such as these will enable researchers, and state or federal agencies interested in the well-being of fish for management or sport, a better understanding of the animal’s overall health by being able to refer to reference values for sex and stress steroids in a wild population of red drum.
Materials and Methods

Fish Capture Methods

All fish were handled according to procedures approved by the Institutional Animal Care and Use Committee (IACUC) NASA, protocol # GRD-11-077. Fish were sampled according to the guidelines of the special activity license # SAL-09-0512A-SR, issued from the Florida Fish and Wildlife Conservation Commission (FWC) and a special use permit # 2011SUP001, issued from the Merritt Island National Wildlife Refuge (MINWR). All fish were caught using a hook and line approach designed to decrease fight time. Barbs were shaved off of circle hooks to lessen any potential injury to the fish or staff and to minimize handling time. Large surf rods and 30 lb. test line were used to land fish in the boat quickly and reduce handling time.

Only fish over 650 mm standard length (SL) were used for this project. All fish were caught in the KSC Banana River reserve area and all fishing sites were visited during each of the three reproductive periods (Figure 2-1). The three reproductive periods were defined as pre-spawning (May), spawning (September and October), and post-spawning (December). Angling time and handling time were recorded for each fish using a handheld timer. The timer was started when the angler felt the fish bite the hook. Fight time was recorded and kept under five minutes. Fish were netted when close to the boat, immediately de-hooked, and then placed in lateral recumbency for the blood draw procedure.

Plasma Collection

Fish 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 gill arch 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 (Figure 2-2). The time interval from the fish being hooked to blood sample collection was recorded, and was kept under seven minutes. Several drops of blood were immediately extracted from the vacutainer for glucose analyses using a Contour® (Bayer Healthcare, Tarrytown, NY) glucose strip for every fish. A subset of ten fish from pre-spawning and seven from spawning were tested for glucose on an i-STAT® (Abbot, Princeton, NJ) analyzer. Blood was placed on ice until it was returned to the laboratory and centrifuged for a minimum of 10 minutes at 3000 g to separate the plasma and cell fractions. Plasma was then aliquoted into 1.8ml cryovials and frozen at -70 °C.

**External examination protocol**

The external exam included traditional morphometric measurements outlined by Lagler (1962). These included standard length (SL: tip of snout to the posterior end of the last vertebra), total length (TL: tip of snout to tip of the caudal fin) and weight, which were recorded to the nearest centimeter and gram, respectively. A gill biopsy and skin mucus were collected using plastic microscope slide covers. Wet mounts of these tissues were examined immediately at 100x for the presence of external parasite fauna and the number of organisms per sample was recorded. The external exam also evaluated the eyes, mouth, external mucous layer, scales, and the fins of the fish. Physical deformities were noted, along with the presence of lesions that could suggest bacterial, fungal or other infections (e.g. presence of ulcers). External parameters were recorded and scaled according to severity using the SERF health index (Dukeman et al. 2006) (Table 1-2).
Sex Identification

Field attempts to identify sex of collected fish included listening for the fish’s drumming sound and applying pressure to the abdomen to determine if milt was exuded during the fall spawning period, both indicative of a male (Figure 2-3 A). Possible females were probed in the vent area with a pipette to attempt to determine sex by locating the urinary orifice, anus, and ovipositor (Figure 2-3 B). If sex was not clearly distinguished then “undetermined” was noted on the field data sheet.

Tagging and Handling

As a means to permanently identify fish, a PIT tag (Biomark 12 mm 125 kHz) was inserted using a sterile 12 gauge needle placed subcutaneously near the cheek on the left side. Also the dorsal portion of the caudal fin was clipped as an external mechanical tag. Capture positions were recorded using global positioning system (GPS) equipment. The fish were released immediately after examination and sample collection was completed, typically < 15 minutes from hooking time.

Water Quality

Water chemistry parameters (e.g., temperature, salinity, dissolved oxygen and pH) were recorded using a Yellow Springs International water quality sonde, model 6920, at all fish capture locations during each sampling period. The temperature data collected was compared to NOAA buoy, station 41113, the Cape Canaveral Nearshore station using a t-test.

Condition Factor

Condition factor was calculated for all fish using the equation of fish weight (g) x 100 /standard length³ (Fulton 1904).
Steroid Assays

A solid-phase radioimmunoassay using a 96-well plate format (Perkin Elmer, Boston, MA, Protein A Flash Plate Plus) was used to determine concentrations for estradiol-17β (E₂) as previously described by Hamlin et al. (2011). Plasma samples were thawed on ice and extracted twice using 5 ml diethyl ether by vortexing the plasma samples vigorously with ether. The aqueous phase was removed by snap freezing in liquid nitrogen and the organic phase was decanted into a new clean tube. The diethyl ether extract was evaporated over filtered air and stored overnight at -20°C. Prior to running the assay, each sample was reconstituted with phosphate buffered saline with gelatin (1%). The samples were run in duplicate and each of the four plates contained duplicate wells for interassay variance and a blank. Sample plates were analyzed using a microplate reader (Perkin Elmer MicroBeta 2 2450 Microplate counter, Waltham, MA).

E₂ was validated in triplicate for red drum by verifying that serial dilutions of pooled plasma were parallel to the standard curve. The interassay variance samples were verified by measuring the steroid in extracts obtained from a sample of plasma pooled from three males and three females. The assay validation response was parallel to the respective RIA standard curve for E₂. Spikes were also performed and were validated. The interassay variances were less than 5% among plates. Unknown concentrations were extrapolated from standard curves plotted as the % bound versus the log₁₀ concentration. Plasma E₂ concentrations in some fish were higher than the standard curve, so the max value approach was utilized. The highest value validated on the standard curve was substituted for the values that were too high off the standard curve.
Plasma 11-ketotestosterone (11-KT) and cortisol were assayed using commercially available enzyme immunoassay kits (ELISA) (Cayman Chemical, Ann Arbor, MI, USA; cat.582751 and cat. 500360 respectively). Plasma samples for both 11-KT and cortisol ELISA’s were extracted as described for E₂. Desiccated samples were reconstituted with EIA buffer provided with the kit. Both assays were validated for red drum by verifying that serial dilutions of plasma and spiked standards were parallel to the standard curve. The samples were run in duplicate and each of the six plates contained duplicate wells for interassay variance and a blank. Both kits followed the recommended incubation time of eighteen hours at 4°C. Sample plates were analyzed at a wavelength of 412 nm using a microplate reader (Molecular Devices Versa Max tunable, Sunnyvale, CA). The interassay variances were less than 7% for all plates of the assays. Unknown concentrations were calculated from standard curves plotted as the % bound versus the log₁₀ concentration.

**Glucose Analyses**

Glucose was analyzed using several methodologies, including hand held devices potentially useful in the field and a standard laboratory-based technique. First, a drop of blood was immediately obtained following the collection of the blood sample in the field and applied to a Bayer brand Contour® glucose strip. The strip then was inserted into the Contour® glucometer and the glucose value recorded.

Plasma glucose also was measured by an absorbance assay, using an Invitrogen, Amplex® Red Glucose/ Glucose Oxidase Assay Kit (A22189). Plasma samples were thawed on ice and evaluated according to the instructions provided. The samples were diluted 20X 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).

**i-STAT Analyzer**

A sub-sample of fish during the pre-spawning (n = 10) and spawning periods (n = 7) also were tested in the field with an Abbott i-STAT® analyzer using one drop of blood per cartridge. The CG4+ and CG8+ cartridges were used. The CG4+ cartridge provided values for lactate, PCO₂, PO₂, TCO₂, HCO₃, sO₂. The CG8+ cartridge provided values for glucose, hematocrit and hemoglobin. The Abbott i-STAT analyzer was not available for use during the post-spawning reproductive period, so only glucose values for those periods were utilized to compare results with the laboratory assay and field glucometer.

**Statistical Analyses**

Individual red drum served as sampling unit replications. Treatments consisted of the three sampling periods (pre-spawning, spawning, and post-spawning) representative of three reproductive periods. To compare water quality data, cortisol data, 11-KT data and E₂ data, ANOVA and t-tests were used. Health index data did not pass the normality test, Shapiro-Wilk (p = 0.03, 0.02, 0.02) data were log transformed, and a one-way Analysis of Variance was used to test for differences among the three periods of reproduction, F (2, 123) = 36.58, p = 0.14. To compare glucose data, Kruskal-Wallis non-parametric analysis of variance followed by Dunn’s test were used. A p value was considered significant if lower than 0.05. Statistical analyses were performed with Sigma Plot (11.0, San Jose, CA).

Discriminant function analysis (DFA) was used to predict the sex of fish for which sex could not be determined in the field (Venables 2002). Known values for the
parameters cortisol, 11-KT, E₂ and lab glucose were used as explanatory variables. Predictor variables were log transformed to meet the assumption of multivariate normality. Values of 11-KT and cortisol below the detection threshold were replaced with one-half of the minimum detection value. DFA, with all explanatory variables, was used to predict the sex of undetermined fish. A step-wise DFA was used to explore reduced models that could be useful in understanding relationships between variables for use in sex determination in future field studies. Graphical methods and statistical tests were used to access how well the data met the two key assumptions of DFA multivariate normality and equality of the variance covariance matrices (McGarigal et al. 2000). Quadratic discriminant function analysis was used to verify results of the DFA for indications that failure to meet statistical assumptions influenced the results. All statistics were performed in R Development Core Team (2012). DFA was conducted using the MASS package (Venables 2002) and stepwise DFA was performed using the klaR package (Weihs et al. 2005).

**Results**

**Morphometrics & Condition Factor**

A total of 126 fish were caught during the three sampling periods. Total length of pre-spawning period fish (n=38) averaged 774 ± 96.2 S.D. mm with average weights 5.39 ± 2.72 S.D. kg and an average condition factor of 1.6 ± 0.10 S.D. in May 2011. Spawning period fish (n=45) had an average total length of 797 ± 79 S.D. mm with average weight of 6.12 ± 2.79 S.D. kg and an average condition factor of 1.72 ± 0.18 S.D. in September and October 2011. The post-spawning period fish (n=42) had an average length of 847 ± 79 S.D. mm, average weight of 7.64 ± 2.71 S.D. kg and an
average condition factor of 1.77± 0.17 S.D. in December 2011. Morphometric data and condition factor are presented in Table 2-1.

**Water Quality**

Temperature, dissolved oxygen, salinity, and pH data for each sampling site per capture day exhibited little variation among sampling periods (Table 2-2). An independent samples t-test was conducted to compare the field temperature to the NOAA buoy station 41113. There was no significant difference between the measurements for field temperature ($M= 25.53$, $SD=4.38$) and NOAA buoy temperature ($M= 24.63$, $SD=3.33$) $t (4) = 0.28$, $p = 0.79$.

**Health Index**

Using the SERF health index, the fish obtained during this study exhibited average scores above 45 during the pre-spawning and spawning periods, which is considered the threshold for “healthy” at the SERF hatchery. The average score decreased slightly to 42 in post-spawning fish (Figure 2-4). A post hoc Tukey test showed reproductive periods, spawning vs. post-spawning differed significantly at $p <0.05$, and pre-spawning vs. post-spawning differed significantly at $p < 0.05$. The spawning vs. pre-spawning periods were not significantly different $p = 0.25$.

**Parasites**

The majority of red drum (90%) sampled in this study had grossly visible external parasites. Three types of parasites were commonly identified: *Argulus* sp., *Caligus* sp. and *Ergasilus* sp. One *Argulus* sp. per fish was observed, on average, usually on the ventral surface. In contrast, *Caligus* sp. was more common, averaging 15 per fish, again, found on the ventral surface. A mean of one *Ergasilus* sp. per fish was found inside the operculum of each fish.
Plasma Glucose Concentrations

Three different methods were used to determine glucose concentrations for the Pre-spawning period and Spawning period. The Abbott i-STAT was not used in the Post-spawning period. Significant differences in glucose values were found between the glucometer and the lab assay (Table 2-2). Since only a sub-sample of glucose values were obtained with the Abbott i-STAT there were not enough data values to make statistical comparisons between the lab glucose data or the glucometer.

Glucose data for the lab absorbance assay and the glucometer for all three reproductive periods did not pass the normality test, Shapiro-Wilk ($p < 0.05$). A Kruskal-Wallis Analysis of Variance on Ranks was used to test for differences among the three periods of reproduction between the absorbance assay and the glucometer, $H (2) = 22$, $p = <0.001$. A pairwise multiple comparison procedure, Dunn's post hoc test, was performed, spawning period vs. pre-spawning differed significantly $Q (4.01)$, $p<0.05$, spawning period vs. post-spawning differed significantly $Q (4.09)$, $p<0.05$. Pre-spawning period and post-spawning were not significantly different $Q (0.03)$ $p >0.05$.

Linear regression between fish fight time and lab glucose concentration had an $R^2$ value of 0.03. Similarly a linear regression between total fish handling time from hook up and lab glucose concentration also had an extremely low $R^2$ value of 0.007.

Plasma Cortisol Concentrations

On average, plasma cortisol concentrations ranged from 0.93 ng/ml to 1.25 ng/ml for the three reproductive periods examined (Table 2-2). The EIA for cortisol reported nine fish concentrations above the maximum value on the standard curve, so a maximum concentration approach was employed. The highest value validated on the standard curve was substituted for the values that were originally higher than the
highest value validated on the standard curve. A t-test was conducted to compare cortisol among the reproductive periods. There was a significant difference between the periods ($M = 1.11$, $SD = 2.02$); $t(111) = 5.81$, $p < 0.001$ (Figure 2-5).

**Plasma 11-KT Concentrations**

On average, plasma 11-KT concentrations ranged from 0.040 - 0.058 ng/ml during the three reproductive periods examined (Table 2-2). The EIA for 11-KT reported values that were below the minimum value on the standard curve so a minimum concentration approach was employed. This approach substituted half of the lowest value from the validated standard curve for those values that were below the minimum value of the standard curve. A t-test was conducted to compare 11-KT among the reproductive periods. There was a significant difference among the periods ($M = 0.23$, $SD = 0.55$); $t(121) = 4.73$, $P < 0.001$ (Figure 2-6).

**Plasma E$_2$ Concentrations**

On average, plasma E$_2$ concentrations ranged from 0.41 ng/ml to 2.28 ng/ml during the three reproductive periods (Table 2-2). E$_2$ had some values that were higher than the standard curve, so the maximum value approach was engaged. The highest value validated on the standard curve was substituted for the values that were originally higher than the highest value validated on the standard curve. Only four fish had values that were too high and they were positively field identified as females in the field during spawning period. A t-test was conducted to compare E$_2$ among the reproductive periods. There was a significant difference between the periods ($M = 1.1$, $SD = 2.71$); $t(124) = 4.51$, $P < 0.001$. Plasma concentrations of E$_2$ were significantly different among reproductive periods according to the t-test ($M = 1.1$, $SD = 2.71$); $t(124) = 4.51$, 

53
P<0.001(Figure 2-6). However, plasma E<sub>2</sub> concentrations were significantly elevated in females during the spawning period compared to males.

**Sex Identification**

Cortisol and lab glucose met the assumptions of the DFA multivariate normality and equality when comparing the means and plots. E<sub>2</sub> and 11-KT resulted in differences of group means and plots indicating some departure from normality. The classification function derived was: 1.110972 -0.1028649log (cortisol) + 0.6881698 log (11-KT) -1.0840710 log (E<sub>2</sub>) +1.5684448log (lab glucose). The group mean for females was -1.123013 and for males was 1.314225. Among the fish of known sex used to derive the discriminant function, 22 of 25 females and 41 of 48 males were correctly classified. The quadratic DFA resulted in 24 of 25 females and 43 of 48 males correctly classified. Quadratic DFA does not assume multivariate normality, so the DFA could perform better when this assumption is not met (Venables 2002). Using this approach, we predicted that twenty-four of the unknowns were female and that eleven were likely to be male (Table 2-5).

**Recaptures**

The majority of fish (n=124, 98%) used for this study were only captured once, but two individuals were captured twice. The within-study recapture rate ([# of fish recaptured/ # of fish captured] multiplied by 100) was 1.6%. One male initially caught during the pre-spawning (May 9, 2011) period was recaptured during the post-spawning period, December 15, 2011 period. The measurements for this fish display rapid growth in length and weight gain, from 710 mm TL to 761 mm TL and 3.75 kg to 4.90 kg. The second recaptured fish, a female that was initially caught during the spawning period, October 10, 2011, at 784 mm TL and 5.00 kg and was recaptured during the post-
spawning period, December 13, 2011. She was caught at the same location and measured 784 mm and weighed 5.75 kg.

**Discussion**

This study defined parameters that can be used to assess wild red drum in the field and further defined baselines for plasma concentrations of cortisol, glucose, 17β-estradiol (E₂) and 11-ketotestosterone (11-KT) for adult red drum in the KSC Reserve during three phases of the reproductive cycle. Further, we evaluated wild caught fish using external parameters outlined in the SERF health index. This is the first study in red drum to examine the relationship between a measure of stress (e.g., plasma cortisol concentrations) and potential reproductive function status, as indicated by the plasma concentrations of various sex steroids in a de-facto no-take fisheries reserve. This is also the first study to obtain blood samples from the branchial vessels in non-anesthetized wild red drum.

**Predicted Outcomes**

I predicted that a no-take fishery reserve, such as the KSC Reserve, would have fish that score in the “healthy” range of the SERF health index. We observed such a pattern for fish caught during the pre-spawning and spawning periods. Fish caught in the post-spawning period scored slightly below this range. A factor that contributed to the depressed score during the post-spawning period was parasite load. The external parasites identified in this study were those that were visible to the naked eye. These were primarily found on the ventral surface of the fish and under the operculum. Microscopic examination of gill tissue and skin mucus did not reveal protozoans or other parasites beyond those macroscopically visible. None of the gill biopsy samples showed any abnormal signs of hyperplasia or hypertrophy.
The SERF health index was developed to assess cultured juvenile red drum. These fish are not expected to have parasites, which was the main difference in the health scores between hatchery reared fish and wild fish sampled in this study. Over 90% of wild fish sampled had parasites. This is not surprising when evaluating wild fish, and Landsberg et al. (1998) suggested that parasites on wild fish may be indicative of healthy ecosystems. However excessive parasite loads on a fish suggest a compromised immune system and may be indicative of comprised health. Evans et al. (1997) considered wild red drum from estuaries in South Carolina with fewer than three common external parasites, and no other visible lesions, as ‘healthy’. If that determination was used in this study, only 10% of the study animals would have been ‘healthy’, even when categorized as trophy sized fish according to their condition factor. All fish with three types of parasites evaluated in this study were still robust in length and weight and had excellent condition factor scores. These fish had no ulcers or other external lesions. Even while using barbless circle hooks, we were able to catch trophy-size red drum with ease suggesting that barbed hooks may be unnecessary for catching these fish. Barbed hooks may cause more harm if swallowed during catch and release procedures.

Fish collected during the post-spawning sampling period actually had the highest average condition factor suggesting that there was minimal spawning condition deterioration in this population. The reason for the slightly lower health index score in this group was due to higher parasite numbers on these fish. Since fish collected in all three of the sampling periods averaged in the “excellent” category that would give the impression that the sampled population of red drum in the reserve were thriving. One
The greatest drawback to the hook and line technique employed for this study was that it did not catch fish that did not bite the hook. Fish that were caught during this study were hungry or interested in feeding since they attempted to feed on our bait / tackle. Using the technique of netting/encircling fish would have eliminated that concern and could have resulted in a different outcome. Previous research has demonstrated that sportfish within the KSC Reserve achieve a larger mean size and exist in higher densities than those in adjacent public areas (Johnson and Funicelli 1991).

My second hypothesis stated that there would be a significant difference in seasonal blood glucose concentrations, potentially indicative of metabolic disturbance caused by changes in fish feeding habits or environmental conditions. The detectable range for the Contour® glucometer for this study was 10-600 mg / dL. Glucose values obtained using the Contour® glucometer had a broad range of values during each reproductive period. There were no seasonal trends in glucose values. A population of fish having large ranges for glucose is not uncommon. However to pinpoint a reason for this is more difficult without stomach analysis to determine what the diet consisted of or when the fish last ate. The capture stress and handling also can affect glucose values, typically inciting a hyperglycemic response. Since the fish captured in this study were all brought to the boat in less than seven minutes and a blood sample was collected in under eight and a half minutes, the time necessary for the glucose to reflect any changes from the animal’s baseline value may not have been long enough. The values collected may then reflect actual baseline values of the animal.

Handheld glucometers may not capture the full range of glucose values for fish since they were created to detect mammalian ranges. A study by Iwama et al. (1995)
investigated the use of glucometers for field use on cultured Atlantic salmon by holding the fish in a dip net out of the water for thirty seconds and then collecting a blood sample at 2, 4, 6, 8, and 24 hours afterwards from the caudal vein. They found that glucose values obtained by the glucometer were two times higher than the laboratory assay and the reason for the difference was unknown. Venn Beecham et al. (2006) compared a glucose meter to a laboratory assay for channel catfish. The study was performed by putting some ‘control’ fish into a container with MS-222 and then some ‘fatigued’ fish were chased with a dip net for ten minutes and then also placed in a container with MS-222. Both groups were sampled from the caudal vasculature for a blood sample. They found that the Accu-Chek Advantage meter was consistently lower than the values obtained from the laboratory reference method for both groups of fish, although there was low variability between replicates for the meter. Glucose meters can be useful tools for measuring plasma glucose values in the field; however they should only be utilized when relative measurements are appropriate.

The laboratory glucose absorbance assay had average values of 36 mg/dL during the pre-spawning period, 38 ml/dL for spawning and 29 ml/dL for post-spawning. These values are similar to the basal values reported for Siberian sturgeon. Hamlin (2007) reported basal values for plasma glucose for captive Siberian sturgeon of 36.3 mg/dL with a peak during capture and handling stress of 84 mg/dL using the same commercial glucose oxidase kit. Some higher glucose values were obtained for red drum that were similar to those values for Siberian sturgeon that endured handling stress, however there were only weak correlations between the times from fish hook-up to landing, and from hook-up to the blood sampling. It is possible that the element of time on the hook
or time to blood sample collection was not highly correlated with glucose concentration but rather the individual response of the red drum is highly variable.

There was a significant difference between the absorbance assay and glucometer for the spawning period vs. pre-spawning period and spawning vs. post-spawning period. These differences are difficult to isolate to a specific cause, however it is noted that there were no significant differences between pre-spawning and post-spawning glucose concentrations, suggesting that the spawning period may be affecting glucose concentrations. Reproductively active periods are overall taxing on fish as much of their energy reserves are utilized for production of eggs and sperm, and their feeding behavior may be altered to put more effort into spawning, which may be a factor for these observed differences. A lessened appetite may cause a reduction in glucose values if food was not eaten recently in conjunction to the sampling time.

The third hypothesis examined seasonal variation in the sex steroid profiles of 11-KT and E$_2$ during the three reproductive periods examined. The sex of individual fish had to be determined during field collections. Unfortunately, red drum have no external characteristics that allow easy identification of the sex of an individual, unless drumming or exuding milt during spawning were observed which would confirm that the fish was male. Thirty-five fish were not identified to sex during field collection. A discriminant function analysis was employed using the hormone data from the fish of known sex from this study. A predicted sex was assigned to the fish based on this data from this study. I was able to establish baseline values for the sex steroid hormones 11-KT and E$_2$, which is the first time sex steroid profiles for wild red drum have been reported. Both 11-KT and E$_2$ had significant differences among reproductive periods.
Both males and females had significantly elevated 11-KT during the spawning period. Higher 11-KT concentrations during spawning for males are likely due to final maturation of the sperm. Webb et al. (2002) found that plasma concentrations of 11-KT in mature males were higher than that found in mature females for wild white sturgeon. Kucherka et al. (2006) measured plasma 11-KT concentrations that were significantly higher in males, 0.8 ng/ml, than females, 0.5 ng/ml, during early stages of gonadal growth in both cultured and captive red drum.

This study found no significant difference between sexes or reproductive period when measuring E₂ concentrations. This data precludes E₂ as an indicator for sex identification; however future research should be performed to compare these values to other populations of red drum. One reason for these low values could be that some fish sampled were not adults, since some of the males sampled were below the 50% sexually mature length cut off. The E₂ concentrations of red drum sampled by Kucherka et al. (2006) were lower in captive males and females before gonadal recrudescence and then increased significantly with the progression of vitellogenesis in females. Males used in this study were all over the 50% maturity length of 511 mm (FL) since the sampling cutoff was set at 650 mm (SL), however Atlantic coast females have a 50% maturity length of 900 mm (FL), and only 11% of the female sampled in this study were over 900 (TL). The E₂ values for all females on average for spawning and post-spawning reproductive periods were higher than males. Only four fish had values that were higher than the standard curve and they were positively field identified as females during the spawning period. Webb et al. (2002) measured levels of E₂ for mature wild white sturgeon females to be higher than those of mature males.
Kucherka et al. (2006) was able to establish a 2 ng/ml threshold of E$_2$ for females when attempting to determine unknown sex; however that threshold is not reflected in this study. All the values obtained for red drum in this study are an order of magnitude lower than previously published studies. This may be a factor of newer RIA test kits which have decreased the cross reactivity between the hormones in the blood sample from 15% to less than 0.01% in this study.

Cultured red drum are typically kept between 23.7 - 28.4 °C and kept at 26°C when maintaining spawning condition (Adams et al. 2010). The temperatures recorded in the field during the spawning period averaged the same as the NOAA buoy temperature data which was 28°C. This temperature falls within the spawning temperature range that is utilized at hatcheries. This information may be relevant to understanding why some red drum in east-central Florida are spawning in the estuary and not inlets or the ocean during a spawning period. Reyier (2011) found that acoustically tagged and monitored red drum in the IRL, Florida, exhibited strong site fidelity from winter through early summer. Movement increased within its range during the fall spawning months; however the majority of tagged fish remained within the lagoon year-round.

Lastly the fourth hypothesis was that there would be variation in cortisol concentrations during different reproductive periods. This hypothesis was accepted since there was a significant difference in cortisol concentration among periods. The recorded levels of cortisol for red drum in this study, some below detectable limits of the assay and up to 1.88 ng/ml were low compared to other fishes in the literature. I believe that these low values were partly due to improvements in analytical techniques and that the collection techniques used for this study were very fast.
Fish were not anesthetized during our study. Many studies have used MS-222 anesthesia for cortisol studies in fish. MS-222 is recognized as a powerful anesthetic for fish, and its use may affect cortisol data, especially when comparing fish sampled without anesthesia. This difference is because unbuffered MS-222 is a powerful stressing agent for fish and previous studies resulted in higher cortisol values (Blaxhall 1972). Anesthetics may lessen the effects of stress and reduce the cortisol response in fish (Crosby et al. 2006). Another factor that may affect cortisol values is the time of day the sample was collected. Cortisol concentrations in green sturgeon, white sturgeon and Florida gar have been shown to be highly sensitive to diurnal variation and it is necessary to specify the sampling time period to reduce the possibility of time as a confounding variable (Hamlin et al. 2007). All fish in this study were collected between 9:00 AM and 2:00 PM to minimize effects of potential daily fluctuations in hormone concentrations. However many other parameters may affect cortisol values in fish such as handling treatments, handling time, and salinity. Barton and Iwama (1991), reported ‘prestress’ cortisol values for *Sciaenops ocellatus* as <5 ng/ml and a ‘post-stress’ range from 14 – 250 ng/ml based on studies that had differing procedures for handling treatments, handling times and salinities.

**Stress Response**

When drawing general conclusions from available literature on stress responses in fish, only a very small fraction of the teleost species have been investigated (Bonga 1997). The basal levels of cortisol reported for Siberian sturgeon were 5 ng/ml with peak levels during stress studies reaching 75 ng/ml (Hamlin et al. 2007). In a previous study, cultured juvenile red drum were challenged with a 2-minute transfer stressor. This consisted of transferring fish by dip net and exposing them to air for two minutes,
after which a blood sample was collected by cardiac puncture. The results from this study by Thomas and Robertson (1991) noted rapid elevations in plasma cortisol and glucose concentrations after fifteen minutes. Concentrations reached >100 ng/ml for cortisol and hyperglycemia >100mg/100ml for glucose at fifteen minutes post stressor and persisted for 120 minutes. However when red drum were challenged with a five second exposure to air during transfer, there was no plasma cortisol stress response, and only a slight hyperglycemic response (Thomas and Robertson 1991).

Bonga (1997) stated that for most stress studies, fish from domesticated stocks were used and such fish may have a blunted stress response when compared with wild-type strains of the same species. The adult red drum used in this study were challenged with an air exposure time ranging from between two to seven minutes, and all blood samples were collected between two and a half minutes and eight and half minutes. This rapid collection of the blood sample is a main point to consider when comparing the low cortisol values of this study to others in the literature. Cortisol values were below 2ng/ml which is well below the basal levels reported for sturgeon and equal to the basal levels reported for cultured red drum (Kucherka et al. 2006; Hamlin et al. 2007). Perhaps the intensity of the hook and line rapid capture was below the threshold required to induce corticosteroid responses in adult red drum.

**Water Quality**

During sampling, the water quality parameters did not change markedly among each of the three periods. Adams and Tremain (2000) noted that seasonal movements by juvenile red drum in similar estuarine systems are likely related to changes in water temperature, salinity or prey availability. Gonadal steroidogenesis is regulated by gonadotrophins, and gametogenesis in red drum can be initiated by environmental cues
such as water temperature and day length, similarly to common snook (Roberts et al. 1999). Also Johnson and Funicelli (1991) suggested that stable estuarine salinity provides suitable conditions for egg and larval survival mitigating the need for ontogenetically-cued estuarine shelf migrations. Salinity measurements during the post-spawning period averaged 30 ppt which is within the optimal range for red drum egg survival (Holt et al. 1981). 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 temperate winters compared to other estuaries of the south U.S. Atlantic and Gulf of Mexico.

**Further Research**

One effective way to have an understanding of the impacts of human activities on natural systems is to have reference areas with minimal human impact (Bohnsack 1993). Reference areas can help resource managers detect changes and attempt to distinguish if these changes are natural or caused by human actions. The KSC Reserve is a great example of a reference area with minimal impacts due to its protected status. The next step toward a better understanding of red drum physiology and building baseline stress and sex hormone data base would be to compare the KSC Reserve red drum population to other nearby heavily fished waters such as Mosquito Lagoon which is part of the IRL system, but open fishing to the public year-round. Thomas and Robertson (1991) noted that fish that had been repeatedly exposed to similar shallow-water stressors during routine aquaria maintenance had reduced subsequent biochemical stress responses to the adverse stimulus. Barton (2002) also
found that repeated exposures to mild stressors can desensitize fish and attenuate the neuroendocrine and metabolic responses to subsequent exposure to stressors.

Results obtained from fish used in this study may be compared to other sub-populations of red drum in adjacent areas that experience intense angling pressure, and more urbanization of habitat. Collaboration with FWC or fishing tournaments for red drum could aid in providing fish to be sampled according to the health assessment outlined by this project. Field logistics, sample volumes, personnel training and availability of sample storage are issues to be considered when developing collaborative studies (Kucklick 2010b).

Current assumptions within the sportfish industry are that catch and release fishing returns fish back to their environment and they survive the process without detrimental effects. However underestimating the discard mortality rate of a fishery was associated with the collapse of an entire red snapper fishery (Diamond and Campbell 2009). Sportfish in general, and red drum specifically, are known to tolerate repeated catch and release events from recreational fishing, but not much is known about how these repeated events may affect the overall health and reproductive potential of the animal. Adult red drum in Mosquito Lagoon, which is adjacent to this study site, were part of an acoustic telemetry study and had a conservative 41% estimated angler recapture rate, which far exceeds most other mark-recapture studies (Reyier et al. 2011). Anglers have discovered that good fishing, and the largest fish, are likely to be caught near reserves (Bohnsack 1993). Bohnsack (2011) reported on the coastal protected areas in Florida and their effects on recreational world records and found that the highest concentration of records was near the KSC Reserve. Red drum specifically had 55% of records near
the KSC Reserve, which indicates that the reserve’s fishery restrictions allowed
recreational anglers to achieve more records than if the area had been regulated only
by statewide regulations (Bohnsack 2011).

High fishing pressure on an estuarine-dependent spawning red drum population
may have significant negative impacts on the breeding population. Further research is
needed to determine if detrimental effects from angling pressure, including catch and
release, can be determined by utilizing a similar health assessment protocol. Since the
area of east-central Florida seems to contain a population of estuarine-dependent
spawning adults within the confines of a reserve and relatively small scale management
actions could result in rapid enhancement to local populations adjacent to areas
managed by MINWR and Canaveral National Seashore. Reyier et al. (2011) suggested
seasonal closures, limited access areas, or catch and release only zones. Growing
scientific evidence indicates that marine reserves are effective and benefit both fishery
and non-fishery activities (Bohnsack 1993). Before more management actions are
employed, additional quantitative health assessment data would benefit the decision-
making process.

This study provides much needed baseline data for stress and sex hormones on
adult red drum in a reserve area safe from angling pressure and many other
anthropogenic stresses. However for reserves to be successful, public education and
awareness about their functions and importance is needed. Also as resources within
reserves increase, adequate surveillance and enforcement is necessary (Bohnsack
1993). The continental shelf on the east coast of Florida has had relatively minimal
research on the abundance and distribution of adult red drum so it remains unclear if
the estuarine spawning fish constitute a large portion of the spawning biomass (Reyier et al. 2011). Data from this study support that year-round residency as well as spawning is occurring in the KSC Reserve area of the IRL complex. The red drum data may also be comparable to other physiologically-related estuarine sportfish including such as the black drum, (*Pogonias cromis*) in this region.

The new insights of this non-lethal health assessment approach for wild red drum could positively impact fishery management by decreasing the need to cull fish for collection of data needed to manage the fishery. There is already a large database in Florida on length-weight relationships relative to age of red drum as well as gonadosomatic index data. Blood plasma data could fill in sex data and age could be extrapolated based on fish lengths. Also fin clips could be utilized for age determination and compared to test the accuracy of the methodology. There is an increasing concern about how stable the red drum stock status is in Florida, although the bag limit was increased in 2012 for certain areas. State managers are still considering a red drum stocking program. A complete understanding of the wild stock’s health is necessary before starting a stocking project. The health parameters presented here provide the baseline data necessary to monitor the health of red drum in the future. More plasma parameters such as hematocrit, hemoglobin, erythrocyte counts, leukocyte counts, and comparing the structure of the blood cells may need to be evaluated in the future to attempt to determine health status differently if endocrine disrupting patterns or other deteriorations in health are observed. An important concept to move forward is to implement more non-lethal methods for red drum health assessments which would be a win-win for fisheries management and recreational anglers.
Figure 2-1. Kennedy Space Center security zone de-facto no-take fisheries reserve. Study area of the KSC Reserve is outlined in yellow.
Figure 2-2. Collecting a 4ml blood sample from the branchial vessels in the gill of a wild caught red drum. A 1 ½ inch needle and lithium heparin vacutainer ® (BD, Franklin, NJ) were used. Photo courtesy of Carla Garreau.

Figure 2-3. Sex determination of wild caught red drum in the field. A) Soft pressure applied to the abdomen of a red drum during spawning period induced free flowing milt indicating it was a male. B) Examination of a red drum that was not heard drumming was considered to be a possible female. The fish was probed in the vent area with a pipette to determine sex and if the urinary orifice, anus, and ovipositor were found it was a female. Photos courtesy of Carla Garreau.
Figure 2-4. Health index score of wild caught red drum (± S.D.) presented by sampling period. Pre-Spawning, May n=38, Spawning September – October n=46 and Post-Spawning December 2011 n=42.

Figure 2-5. Plasma cortisol concentrations of wild caught red drum (± 1 S.E.) presented by reproductive period and by sex.
Figure 2-6. Plasma 11-KT concentrations of wild caught red drum (± S.D.) presented by reproductive period and sex.

Figure 2-7. Plasma E₂ concentrations of wild caught red drum (± S.D.) presented by reproductive period and sex.
Table 2-1. Morphometric data and condition factor for each reproductive period

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>n</th>
<th>Condition factor (k) mean ± S.D.</th>
<th>Weight (kg) mean ± S.D.</th>
<th>Total length (mm) mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-spawning</td>
<td>38</td>
<td>1.60 ± 0.10</td>
<td>5.39 ± 2.72</td>
<td>774 ± 96.2</td>
</tr>
<tr>
<td>Spawning</td>
<td>45</td>
<td>1.72 ± 0.18</td>
<td>6.12 ± 2.79</td>
<td>797 ± 79</td>
</tr>
<tr>
<td>Post-spawning</td>
<td>42</td>
<td>1.77 ± 0.17</td>
<td>7.64 ± 2.71</td>
<td>847 ± 79</td>
</tr>
</tbody>
</table>

Table 2-2. Plasma values for each reproductive period

<table>
<thead>
<tr>
<th>Plasma parameter</th>
<th>Pre-spawning</th>
<th>Spawning</th>
<th>Post-spawning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± S.D.</td>
<td>n</td>
</tr>
<tr>
<td>Glucometer glucose (mg/dL)</td>
<td>38</td>
<td>19.0 ± 5.00</td>
<td>46</td>
</tr>
<tr>
<td>Abbott i-STAT glucose (mg/dL)</td>
<td>10</td>
<td>34.2 ± 4.24</td>
<td>7</td>
</tr>
<tr>
<td>Lab assay glucose (mg/dL)</td>
<td>38</td>
<td>36.0 ± 6.83</td>
<td>46</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>33</td>
<td>0.93 ± 1.53</td>
<td>41</td>
</tr>
<tr>
<td>11-KT (ng/ml)</td>
<td>37</td>
<td>0.05 ± 0.04</td>
<td>46</td>
</tr>
<tr>
<td>E2 (ng/ml)</td>
<td>37</td>
<td>0.40 ± 0.35</td>
<td>46</td>
</tr>
</tbody>
</table>
### Table 2-3. Water quality in the KSC Reserve during each sampling period

<table>
<thead>
<tr>
<th></th>
<th>Pre-spawning</th>
<th></th>
<th>Spawning</th>
<th></th>
<th>Post-spawning</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>N</td>
<td>Mean</td>
<td>S.D.</td>
<td>N</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>27.61</td>
<td>1.64</td>
<td>10</td>
<td>28.57</td>
<td>2.22</td>
<td>21</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>7.9</td>
<td>1.42</td>
<td>10</td>
<td>6.78</td>
<td>2.57</td>
<td>21</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>35.63</td>
<td>1.35</td>
<td>10</td>
<td>37.78</td>
<td>1.89</td>
<td>21</td>
</tr>
<tr>
<td>pH</td>
<td>8.39</td>
<td>0.15</td>
<td>10</td>
<td>8.24</td>
<td>0.50</td>
<td>21</td>
</tr>
</tbody>
</table>

### Table 2-4. Predicted sex for undetermined red drum caught in the KSC Reserve

<table>
<thead>
<tr>
<th>Mean female probability ± S.D.</th>
<th>Mean male probability ± S.D.</th>
<th>Predicted sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9 ± 0.11</td>
<td>0.10 ± 0.11</td>
<td>female</td>
</tr>
<tr>
<td>0.32 ± 0.12</td>
<td>0.68 ± 0.12</td>
<td>male</td>
</tr>
</tbody>
</table>
LIST OF REFERENCES


Maita, M. 2007. Fish Health Assessment. Dietary Supplements for the Health and Quality of Cultured Fish. Tokyo, Tokyo University of Marine Science and Technology.


BIOGRAPHICAL SKETCH

Carla Marie Garreau was born in Winfield, Illinois. She grew up in the suburbs of Chicago, Illinois graduating from Trinity High School in 2002. During that time she spent many summers in northern Wisconsin at her family summer home on a lake which encouraged her love of fishing as well as going on family vacations to Mexico and the Caribbean. She earned dual Bachelor of Science degrees in marine biology and aquaculture from Florida Institute of Technology (FIT) in 2006. She also holds her Dive Master certification from the Professional Association of Diving Instructors, and has completed over 600 dives around the world.

Before she graduated from FIT, she worked as an Environmental Sciences intern for the St. John’s River Water Management District and then also began working part time for Dynamac Corp. at NASA’s KSC as an Aquatic Biologist. She has worked with many threatened and endangered species at KSC, which is managed by Merritt Island National Wildlife Refuge, such as sea turtles, gopher tortoises, scrub jays and the Southeastern beach mouse. She currently works for Inomedic Health Applications at KSC under the medical and environmental services contract.

Upon completion of her master's degree program Carla will continue her thirst for knowledge by looking to become a part-time teacher at Brevard Community College in addition to her career at NASA. Carla has become an avid sprint triathlon athlete and has recently completed her first ½ marathon and hopes to continue working and playing outdoors in Florida.