PROCEEDINGS FROM THE ATLANTIC BILLFISH RESEARCH PROGRAM SYMPOSIUM

Gulf States Marine Fisheries Commission Spring Meeting
Galveston, Texas
March 11, 2008

September 2008 **GSMFC No. 158**

Gulf States Marine Fisheries Commission

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ACKNOWLEDGEMENTS

The Gulf States Marine Fisheries Commission and the National Marine Fisheries Service extends our gratitude to all of the institutions and organizations that worked on these important projects. We appreciate all of the hard work that was conducted and critical information that was collected. This information will assist in the management and conservation of billfish.

INTRODUCTION

The Gulf States Marine Fisheries Commission, through a partnership with the National Marine Fisheries Service, is pleased to announce grant awards totaling $1.8 million through the Atlantic Billfish Research Program (ABRP). These ABRP grant awards will support research and data collection on billfish that will enhance billfish conservation, management, and rebuilding efforts, and provide updated information for stock assessments. This program will provide financial assistance for research and development projects that will assist in the accomplishment of: (1) ecological and biological research; (2) fishery and socio-economic research; and (3) the development of innovative analytical methods and research tools.

The competitive proposal review and evaluation process, conducted in early December 2004, resulted in eleven (11) projects selected for funding over the next two years, beginning January 2005. The projects include:

- A Comprehensive Statistical Modeling Effort to use Historic Tagging Data to Elucidate Growth Characteristics of Atlantic Billfish - *University of Miami, Rosenstiel School of Marine and Atmospheric Science*

- Age and Growth, Reproduction and Genetics of Billfish in Gulf of Mexico Waters off Texas - *Texas Parks and Wildlife Department*

- Development of a Portable, Universal Assay for Determination of Gender and Reproductive Status in Istiophorid Billfish - *Virginia Institute of Marine Science*

- Use of Pop-up Satellite Archival Tags to Estimate Post-Release Survival and Habitat Preferences of Sailfish, *Istiophorus platypterus,* from Commercial Pelagic Longline Gear in the Southern Gulf of Mexico - *Virginia Institute of Marine Science*

- Analysis of the Effect of Offset Circle Hooks on Post-Release Survival and an Estimation of the Relative Hooking Efficiency of Circle Hooks and Standard J-Hooks in the Recreational Fishery for White Marlin - *Virginia Institute of Marine Science*

- White Marlin Essential Fish Habitat and Possible Resident Populations in the Desoto Canyon Area of the Northern Gulf of Mexico - Assessment of Residence, Movements, and Migrations Using Satellite Pop-up Archival Tagging and Oceanographic Remote Sensing - *University of South Florida, College of Marine Science*

- Constituent Tag/Recapture and Fishing Effort Monitoring Enhancement Program - *The Billfish Foundation*

- An Atlantic-wide Study of Age and Growth in Atlantic Marlins - *University of Miami, Rosenstiel School of Marine and Atmospheric Science*

- Survey of U.S. Virgin Islands Recreational Fishing Boats that Target Billfish and Other Pelagic *Species - U.S. Virgin Islands Department of Planning and Natural Resources, Division of Fish and Wildlife*

- Evaluating the Contribution of Spawning and Nursery Habitats within the Straits of Florida to the EFH of Atlantic Billfish. Billfish - *University of Miami, Rosenstiel School of Marine and Atmospheric Science*

- Reproductive Biology, Potential Spawning and Nursery Areas, and Larval Identification of Blue Marlin*, Makaira nigricans,* in the North-central Gulf of Mexico - *University of Southern Mississippi, Gulf Coast Research Laboratory*

In 2008, the Gulf States Marine Fisheries Commission held an Atlantic Billfish Research Program symposium in conjunction with the Commission's Spring meeting. The purpose of the workshop is to provide an overview of the work that was conducted and present some of the preliminary findings from a subset of the funded projects. This document provides a summary of the presentations given at the symposium.

DEVELOPMENT OF A PORTABLE, UNIVERSAL ASSAY FOR DETERMINATION OF GENDER AND REPRODUCTIVE STATUS OF ISTIOPHORID BILLFISH

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Abstract

We developed a universal assay for vitellogenin (Vtg) detection and quantification in fish blood and mucus (Van Veld et al. 2005). The assay is based on the high molecular weight and high phosphoserine content of all known Vtgs. The assay was validated in serum of vitellogenic common dolphinfish *(Coryphaena hippurus).* The female-specific phosphoprotein was excised from polyacrylamide gels and analyzed by tandem mass spectrometry (MALDI-TOF-MS/MS) in order determine if amino acid sequence of tryptic peptides matched teleost Vtg sequences in the NCBI data base. Significant ion scores and high confidence interval percentages for a number of teleost Vtgs confirmed that the protein of interest was a Vtg. No Vtg was present in the mucus of live *C. hippurus.* Further, no Vtg was detected in any tournament or live billfish sampled during the course of this project. We nonetheless attempted to develop a portable version of the universal assay that could be for determination of gender and estimation of reproductive status of istiophorid billfish or other species. Preliminary trials with vitellogenic mummichog *(Fundulus heteroclitus)* indicated that a portable test using gel filtration or cold filtration to isolate Vtg and phosphoprotein staining for detection would be a feasible approach for a portable assay. However, efforts to fully develop the portable assay were hampered by our inability to detect Vtg in billfish or to obtain sufficient quantities of vitellogeic mucus from billfish or other pelagic species for continued assay development. In related studies two dimensional gel electrophoresis (2DE) was performed to determine if alternative, non-vitellogenin sex-specific proteins could be identified in mucus of king mackerel *(Scomberomorus cavalla).* However, large differences in protein expression between individual females and between individual males prevented us from performing female-male comparisons.

Purpose

The objectives of this project were: a) to develop a universal assay for Vtg that can be used in all fish species b) to develop and optimize a portable field kit for Vtg detection in billfish c) to identify additional sex-specific proteins in billfish mucus using two dimensional electrophoresis and *de novo* sequencing and d) to develop a procedure for assay of protein(s) identified in (c).

Data acquired from istiophorid billfish tagging studies are limited by low tag return rates and our inability to determine the sex of tagged fish. As a result, important information on sex-specific migration, sex-specific growth trajectories and age-specific migration is absent from most data sets (Ortiz et al. 2003). In order to maximize the amount of information gained from tagging studies, a need exists for identification of sex-specific markers and development of portable test kits to detect these markers in the field.

The family Istiophoridae is made up of three genera of billfish: Maikara including blue marlin (*Makaira nigricans*) and black marlin (*Makaira indica);* Tetrapturus including white marlin *(Tetrapterus albidus)*, striped marlin (*Tetrapturus audax)* and longbill spearfish (*Tetrapturus*

pfluegeri) and Istiophorus sailfish *Istiophorus platypterus)*. Compared with other large pelagic species (e.g. tunas, sharks), relatively little is known about billfish biology (Holland 2003). Most species appear to be batch spawners with spawning occurring in tropical and subtropical waters (Nakamura 1985). Billfish are capable of long-range migration and dispersal (Graves and McDowell 2003; Ortiz et al. 2003). However, data acquired from tagging studies are limited by low return rates (1-2%) and by difficulties in determining the sex of tagged fish. Though females of some species (e.g. blue marlin) attain larger sizes than males, this difference is not apparent in other species and no species exhibit sexual dimorphism in morphological features or color pattern. Thus, information related to sex-specific or age-specific migration as it relates to spawning and other activities is unavailable. Specific information on temporal and geographic aspects of spawning is also lacking. While we know that billfish grow extremely fast, little information exists on differences in growth trajectories between sexes.

In order to maximize the amount of information gained from tag returns, noninvasive diagnostic tools are needed to determine the sex of tagged fish. A simple, low-cost, portable kit would be particularly useful because it would eliminate logistical impracticalities associated with the preservation and shipment of samples by vessel personnel to analytical facilities. Numerous disposable tests have been developed for rapid diagnosis of pathogen and drug exposure and early pregnancy in humans. The majority of these tests (dipstick and agglutination tests) are antibody-based and detect the presence of specific antigens in human blood or urine. Collection of blood (or urine) from a vessel at sea while the fish is overboard is extremely difficult and time consuming. In addition, antibodies to protein markers are species-specific and normally cannot be relied upon to cross-react with other species.

A test for determination of sex and reproductive status of tagged fish requires a marker that can be obtained by rapid and noninvasive methods and assayed by a universal method amenable to all species. In the present study we were interested primarily in the egg yolk protein precursor protein vitellogenin (Vtg), present under normal circumstances only in female fish (Specker and Sullivan 1994; Mommsen and Walsh 1988).

Vitellogenesis is the process by which female egg-laying vertebrates produce and store nutrients for developing eggs and embryos. In response to environmental stimuli, gonadotropin - releasing hormone (GRH) is secreted by the hypothalamus of the brain. GRH stimulates the pituitary to release gonadotropic hormones (GTH) that stimulates ovarian follicle cells to produce and secrete estradiol-17β (E_2) the major female hormone of vertebrates (Specker and Sullivan 1994; Mommsen and Walsh 1988). Increasing blood titers of E_2 induce the liver to produce vitellogenin (VTG). VTGs are lipid transport and storage proteins that serve as ligands for the delivery of nutrients to the egg yolk. VTG and associated lipids secreted by the liver are transported via the blood to the ovary where proteins of the low density lipoprotein (LDL) - receptor superfamily mediate entry of the complex into developing oocytes (Byrne et al. 1989; Mann et al. 1999). Within the oocytes, VTG is cleaved into smaller yolk proteins (phosvitin, lipovitellin, beta- component) that accumulate in yolk (Mommsen and Walsh, 1988). The phosvitin domain of Vtg is the most phosphorylated amino acid sequence in nature ranging from 30 probable phosphoserines in rainbow trout *(Oncorhynchus mykiss)* (Mouchel et al. 1996) to 124 phosphoserines in tilapia *(Oreochromis aureus)* (Lim et al. 2001).

Several studies indicate that circulating levels of Vtg cmight be used to discriminate between male and female fish and to indicate sexual maturation in females (Idler et al. 1981; LeBail and Breton, 1981; Heppell and Sullivan 1999; Takemura et al. 1999). Vtg is readily detectable in plasma or serum of vitellogenic female fish but is normally absent or non-detectable in male fish and in immature female fish. Several investigators have suggested that Vtg could be used not only as an indicator of sex but also as an indicator of reproductive status of female fish. Heppell and Sullivan (1999) demonstrated a strong relationship between circulating Vtg and oocyte maturation in gag grouper *(Mycteroperca microlepis).* Circulating Vtg was not detected in immature female gag and was low or non-detectable in fish during primary oocyte growth. Gag Vtg was elevated only in females whose oocytes revealed histological evidence of Vtg uptake. Vtg was present in female greater amberjack (*Seriola dumerili*) during a four month spawning season but was undetectable or near detection limits for the remainder of the year (Takemura et al. 1999). In greenback flounder *(Rhombosolea tapirina),* circulating Vtg was nondetectable in the summer, rose steadily during oocyte maturation and became strongly correlated with oocyte diameter during later stages of oocyte development (Sun and Pankhurst 2004). Vtg was not detected in serum of red porgy *(Pagrus pagrus)* during reproductively inactive periods but was elevated during oocyte maturation (Kokokiris et al. 2001). In English sole *(Parophrys vetulus),* Vtg was detectable in regressed and previtellogenic females, became elevated during vitellogenesis and remained elevated in spawned-out females (Johnson et al. 1991). Vtg was present throughout the reproductive cycle in fathead minnow *(Pimelphales promelas)* but levels did not vary significantly relative to stage of oocyte development (Jensen et al. 2001).

While studies described above involved measurements of circulating Vtg, numerous investigators have reported high levels of Vtg in mucus of reproductively active female fish (Gordon et al. 1984; Kishida et al. 1992; Kishida and Specter 1994; Takemura 1994; Takemura et al. 1999). Takemura et al. (1999) found that Vtg was present in skin mucus of mature female greater amberjack (*Seriola dumerii*) several months prior to spawning. A close relationship existed between Vtg levels in serum and mucus of that species. These authors concluded that in addition to being used for gender identification, changes in skin mucus Vtg can be used to estimate gonadal maturation. In coho salmon (*Oncorhynchus kisutch*), high levels of Vtg levels were observed during the initiation of ovarian development (Gordon et al. 1984). In striped bass (*Morone saxatilis*), mucosal Vtg was low before spawning and remained relatively high immediately after spawning (Kishida et al. 1992). As in studies with serum, existing data on mucus Vtg indicates that there are some species-specific differences in the timing of Vtg appearance relative to stage of oocyte development and spawning.

Relative to that in other species, little is known on the appearance or presence of Vtg in mucus of istiophorid billfish. Tan et al. 2006 extracted proteins from ovaries of gravid *M. nigricans* and generated polyclonal antibodies against these vitellogenin-derived yolk proteins (VDYP). The identity of the proteins recognized by the antibodies was not determined. However, using these antibodies the investigators subsequently reported elevated levels of VDYPs in mucus of female *I. platypterus* relative to that observed in males of that species.

Approach

Objective 1: Develop and optimize a universal assay for VTG that can be used in billfish and other fish species.

For development of a universal Vtg assay we planned to take advantage of two features of Vtg in all species: a) high molecular weight of Vtg allowing quick separation of this protein from lower molecular weight phosphoproteins and b) high degree of Vtg phosphorylation allowing detection of VTG with a commercially available dye that recognizes phosphoproteins.

Small fish collection. Our preliminary work involved testing and development with a variety of small freshwater and estuarine fish including Eastern mosquitofish *(Gambusia Holbrooki)*, fathead minnow *(Pimelphales promelas)* and mummichog *(Fundulus heteroclitus). F. heteroclitus* were collected from King Creek, Gloucester County, Virginia, USA, and maintained in seawater aquaria. Male *P. promelas* were purchased from Chesapeake Cultures (Gloucester, VA, USA) and maintained in well water. *G. holbrooki*, an ovoviviparous species, were collected from a spring in York County, Virginia, and maintained in aquaria with well water. Female *F. heteroclitus* and *G. holbrooki* were vitellogenic at the time of capture.

Tournament sampling. In June 2005, we obtained mucus, blood and gonads from four female *M. nigricans* at the weigh station of the Big Rock Blue Marlin Tournament in Morehead City, NC. Gonads were inspected and oocytes appeared to be regressed or recrudescent. In August 2006, we collected mucus samples from three female *M. nigricans*, and from three female and two male *T. albidus* at the White Marlin Invitational Tournament in Ocean City, Maryland.

Pelagic sampling. In February 2006, we collected mucus from two live *I. platypterus* while fishing aboard a private vessel (Heldter Skeldter) working out of Tropic Star Lodge in Panama. During July-August 2006 we collected mucus from four live *T. albidus* and from several live male and female dolphinfish *(C. hippurus)* aboard the sportfishing vessel Runaway, Hatteras Village NC. Dave Kerstetter (co-PI) obtained samples of mucus, blood and gonads from two species of billfish *(T. albidus, I. platypterus)* and from several live swordfish *(Xiphias gladius)* and several live *C. hippurus* during several 2005-2006 long lining trips to the South Atlantic Bight. In November-December 2006, we collected mucus from live king mackerel *S. cavalla* by angling out of Hatteras Village NC.

Mucus collection. For collection of mucus from *G. holbooki* (0.3– 0.6 g) and *P. promelas* (1.5–2.0 g), fish were euthanized by overdose with tricaine methane sulfonate (MS-222, 300 mg/L) and transferred to individual 5-ml disposable glass tubes containing 250 ml of Tris-buffered saline (TBS). After three, 5 second bursts with a vortex mixer, TBS and mucus were transferred to 1-ml cryovials precoated with protease inhibitor (aprotonin) at 0.132 trypsin-inhibiting units per milliliter (Parks et al. 1999) and frozen in liquid nitrogen. For collection of mucus from *F. heteroclitus*, fish (6–12 g) were anesthetized, and a sample of skin mucus was obtained from each with a stainless steel weighing spatula, transferred to aprotonin- treated tubes containing 250 ml of TBS and frozen in liquid nitrogen. For collection of mucus from dead tournament fish at weigh stations, skin was wetted with deionized water repeatedly until fresh mucus appeared. Mucus samples were scraped into cryogenic vials, amended with an equal volume of 2X Tris-buffered saline (TBS), amended with aprotonin and frozen in liquid nitrogen. For collection of mucus from pelagic species, (billfish, dolphinfish), the surface mucus was scraped either with a stainless steel spatula or with the lip of a 50 ml Falcon tube. Mucus samples were amended with an equal volume of 2X TBS, mixed in aprotonin-treated tubes and frozen in liquid nitrogen

Blood collection. A single method was used to obtain blood from *G. holbrooki*, *F. heteroclitus* and *P. pimephales*. Blood was drawn from incised caudal veins of anesthetized fish into heparin-coated capillary tubes (Becton Dickinson, Franklin, NJ, USA). Following centrifugation, plasma was transferred to aprotonin-treated tubes. Mucus and plasma were stored in liquid nitrogen until use. For dead tournament billfish, blood was collected from the gill cavity from each fish with an 18 ½ gauge needle, transferred to aprotonin-treated microfuge tubes and placed on ice. Within five hours of collection, blood was centrifuged and serum was transferred to cryogenic vials and frozen in liquid nitrogen. For collection of blood from live *(C. hippurus)*, gill arches were severed and blood directed into a 50 ml Falcon tubes. Blood was immediately transferred to aprotonin-treated microfuge tubes and allowed to clot on ice. Within five hours of collection, serum was separated from cells by centrifugation, serum was transferred to a new aprotonin-treated tube and frozen in liquid nitrogen.

Induction of Vtg. Vtg was induced in male *P. promelas* by intraperitoneal injection with estradiol (E_2) as described previously (Parkes et al 1999). Fish were injected with E_2 (30 ug E_2/g fish) in a corn oil carrier. Control fish received corn oil only. Fish were reinjected a second time 72 h later. Mucus and blood were collected 48 h after the second injection.

Electrophoresis, phosphoprotein staining, and detection. Plasma, serum and mucosal proteins (1–5 ug) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in discontinuous, pre-electrophoresed, 8% polyacrylamide gels in a Mini-Protean II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to manufacturer's instructions. Gels were stained with Pro-Q Diamond phosphoprotein gel stain (Molecular Probes, Eugene, OR, USA) according to manufacturer's instructions with minor modifications. All staining steps were performed at room temperature, in the dark, with gentle agitation. Gels were fixed overnight in 50% methanol and 10% acetic acid in ultrapure water (Barnstead International, Dubuque, IA, USA). Gels were washed three times with ultrapure water for a total of 30 min, stained with Pro-Q Diamond stain for 90 min and destained twice for a total of 2 h with Pro-Q Diamond phosphoprotein gel destaining solution (Molecular Probes, Eugene, OR, USA). Fluorescent images were collected and quantified with a Fluorchem SP Imaging System (Alpha Innotech, San Leandro, CA, USA), with excitation at 365 nm, or with a Kodak Spectroline Model TR-302 302-nm ultraviolet (UV) transilluminator (Kodak, Rochester, NY, USA).

Protein standards. *F. heteroclitus* Vtg and *P. promelas* Vtg standards from the plasma of E_2-injected male fish were purified by, and purchased from, the University of Florida, Protein Chemistry Core Facility (Gainesville, FL, USA). *F. heteroclitus* Vtg (250kD) and *P. promelas* Vtg (156 kD) span the molecular weight range of most teleost Vtg and serve a extremely useful markers for locating Vtg polyacrylamide gels stained with Pro-Q Diamond. Bio-Rad Precision Plus Protein Dual Color molecular weight standards (Biorad Laboratories, Hercules CA) contains a synthetic 75 kD peptide that also fluoresces under *uv* light following staining with Pro-Q Diamond stain.

Immunodetection. Immunodetection of *F. heteroclitus* Vtg was performed with the use of monoclonal antibody Mab FV10-9 to *F. heteroclitus* Vtg (Maclatchey et al. 2003). Mucosal proteins were separated on 8% polyacrylamide gels as described above and transferred to 0.20-µm nitrocellulose sheets. After a blocking step in TBS with 5% nonfat dry milk, the

nitrocellulose was incubated with primary antibody, followed by alkaline phosphatase–linked goat anti-rabbit secondary antibody.

Validation of universal Vtg assay by MALDI-TOF-MS/MS. Protein bands corresponding to putative Vtg from three gravid female *C. hippurus* individuals were excised from polyacrylamide gels and sent to the Microchemical and Proteomics Facility at Emory University (Atlanta GA). Proteins were subjected to trypsin digestion and mass spectrometric analysis (MALDI-TOF-MS/MS) as described previously (Medzihradszky et al. 2000;Venkataraman et al.2005). Mass spectra were analyzed using GPS Explorer 2.0 software (Applied Biosystems, Foster City CA) and the Mascot search engine (www.matrixscience.com). Peptides were searched against the National Center for Biotechnology Information (NCBI) database for identification.

Objective 2: Developments and Optimization of a portable field kit for Vtg detection in billfish

Development of a portable universal assay for Vtg in billfish mucus was undertaken with three assumptions a) Vtg is present in the mucus of all gravid teleost fish b) mucosal Vtg can be separated from lower molecular weight mucosal phosphoproteins by gel filtration and c) Vtg is heavily phosphorylated and can be detected in gel filtration fractions by commercially available phosphoprotein detection kits.

Gel filtration. For initial development of a portable universal asaay, mucus was obtained from 12 female *F.heteroclitus*, diluted with 1X TBS and gently homogenized with several up-and down strokes using plastic pestles (Kontes Glass Co., Vineland NJ) designed specifically to fit the aprotonin-treated tubes described above. Mucus was gently centrifuged (1000*xg*) for one minute to pellet scales.and debris. Mucus was applied to XK 1.6 x 70 cm column (Amersham Biosciences Inc., Piscataway NJ) packed with S-300 Sephacryl media (Amersham Biosciences Inc., Piscataway NJ) that had been previously calibrated against the following molecular weight markers: blue dextran (2,000 kD), bovine serum albumin (66 kD), cytochrome c (12.4 kD) and cyanaocobalamin 1.4 kD. The fractions containing blue dextran represents the void volume. We hypothesized that Vtg would elute in the void volume and that smaller mucosal phosphoproteins would elute in later fractions. Fractions were collected using a fraction collector model 2110 (BioRad Laboratories, Hercules CA) and electrophoresed on SDS-PAGE gels. Gels were stained with Pro-Q Diamond phosphoprotein stain to evaluate the effectiveness of gel filtration in separating Vtg from other phosphoproteins.

Cold filtration. In addition to gel filtration, we tested an alternate and much simply method to separate mucosal Vtg from other phosphoproteins. We will hereafter refer to this technique as cold filtration. Briefly, samples of plasma were diluted 1:1 with 2X TBS. The sample was added to a five ml glass Luer Loc syringe equipped with a stainless steel filter housing containing a 0.45µ glass fiber filter. The assembly was chilled to ice-cold temperature and the samples were passed through the filter. The filters were removed from the assembly, homogenized in 1X TBS and filter debris was separated from filter extracts by centrifugation. Samples of filter extracts and filtrate (fraction that passed through the filter) were electrophoresed on SDS-PAGE gels and gels were stained with Pro-Q Diamond as described above.

Phosphoprotein Phosphate Estimation Kit. The final step in the portable assay was to be phosphoprotein detection we using the Phosphoprotein Phosphate Estimation Assay Kit (Pierce Biotechnology Inc., Rockford IL). We tested this kit on selected samples according to manufacturer's specifications. Briefly, samples were added to glass test tubes. Samples received 0.2 ml of 2.0 N NaOH. Tubes were covered with parafilm, vortexed and incubated at 65 ^{0}C for 30 minutes in a water bath. Samples received 0.2 ml of 4.7 N HCl followed by 0.2 ml of phosphate reagent. Samples were vortexed and incubated at room temperature for 30 minutes. Optical density was measured at 620nm and compared with optical density of purified phosvitin standards supplied by the manufacturer.

3) Identify additional, non-Vtg, sex-specific proteins in billfish mucus using two dimensional electrophoresis and de novo sequencing.

Two dimensional gel electrophoresis (2DE) - 2DE was performed to determine if non-vitellogenin, sex-specific proteins could be identified in mucus. In November-December 2006, we captured three female and three male king mackerel *(Scomberomorus cavalla*) by angling out of Hatteras Village NC. Aliquots of mucus from male and female individuals were gently scraped from each fish, diluted with an equal volume of 1X TBS and stored in liquid nitrogen. Visible examination of gonads allowed us to distinguish between male and female individuals. Oocytes were poorly developed or recrudescent. Electrophoresis followed by Pro-Q Diamond phosphoprotein staining indicated that no Vtg was present in *S. cavalla* mucus. Aliquots of mucus from male and female individuals were stored in liquid nitrogen for 2DE.

Mucosal proteins from each of three male and three female *S. cavalla* were sent to the Proteomics Resource Facility (University of Georgia). Individuals were labeled with one of two fluorescent CY dyes (Amersham Biosciences) as described previously (Tonge et al. 2001). Differentially labeled proteins from two individual *S. cavalla* were then electrophoresed on the same large format gels. In order to evaluate the utility of 2DE to identify sex-specific proteins we first performed male-male and female-female comparisons. It was important that no significant differences be present between males or between females before we could perform male-female comparisons. Proteins from two (male-male or female-female) differentially labeled individuals were separated by denaturing isoelectric focusing (1st dimension) using 24 cm Immoboline (Amersham Biosciences Inc. Piscataway NJ) dry strips (pH 3-7) with an IPGphore (Amersham Biosciences Inc). For second dimension separation, the strips were transferred to a large format (Ettan Dalt II Large Vertical System, Amersham Biosciences Inc) and proteins were through gradient (8-15%) SDS-polyacrylamide gels. Images were collected and analyzed with a Typhoon 9400 imaging system (Amersham Biosciences Inc) and DeCyder software (Amersham Biosciences Inc). Gender-specific proteins, if present, were to be picked and subjected to *de novo* sequencing at the Proteomics Resource Facility at the University of Georgia.

Findings

Objective 1: Development and optimization of a universal assay for VTG that can be used in all billfish and other fish species.

Preliminary work with small estuarine and freshwater fishes. In preliminary work with *P. promelas, G. holbrooki* and *F. heteroclutus*, Pro-Q Diamond phosphoprotein staining allowed sensitive in-gel detection of Vtg in plasma or mucus of females or E2-treated males in all species tested (Van Veld et al 2005). Specifically, Vtg was highly induced and appeared as a high–molecular weight (156 kDa) phosphoprotein in plasma and mucus of individual E_2-injected male *P. promelas* and was not detected in control individuals. Vtg was also present in mucus of vitellogenic *G. holbrooki* individuals, but not in mucus of male individuals of this species (Figure 1). Vtg was observed in blood and mucus of vitellogenic female *F. heteroclitus.* To evaluate quantitative features of the assay, we compared Pro-Q Diamond phosphoprotein detection with antibody-based (Western Blot) detection of *F. heteroclitus* Vtg standards. In our hands, phosphoprotein staining exhibited sensitivity similar to that of immunodetection and allowed quantitative analysis of plasma and mucosal Vtg (Van Veld et al. 2005).

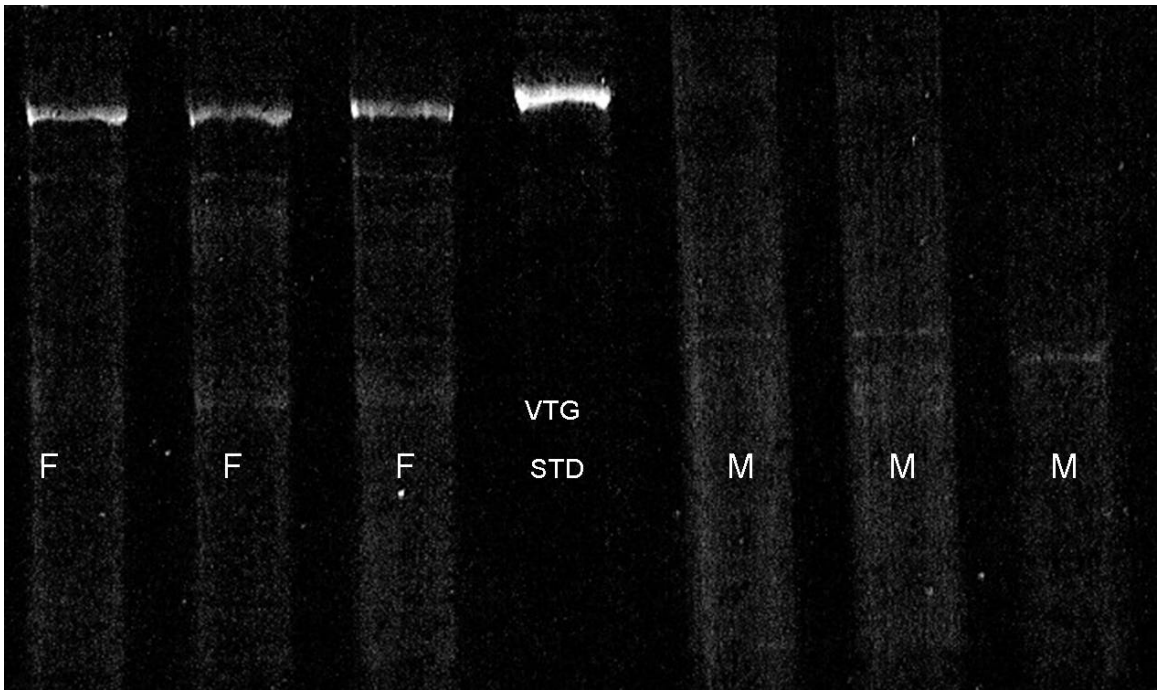

Figure 1. In-gel phosphoprotein staining of vitellogenin (Vtg) in mucus of individual female *Gambusia holbrooki* (left three lanes). No Vtg was present in mucus of male individuals (right three lanes). Purified *Fundulus heteroclitus* Vtg (250kD) is in middle lane. Normal image collected with Alpha Innotech Fluorchem SP Imaging system. Figure adapted from Van Veld et al. (2005).

Tournament sampling and testing. Samples of mucus were collected from moribund tournament fish in order to obtain information on the presence and stability Vtg in tournament fish. If Vtg was present, we would use this mucus for continued portable assay development. However, no evidence for mucosal Vtg was observed in any of the tournament billfish sampled. In June 2005, we obtained mucus, blood and gonads from four female *M. nigricans* from the Big Rock Blue Marlin Tournament in Morehead City, NC. Serum and mucosal proteins were subjected to gel electrophoresis and phosphoprotein staining (Figure 2) as described in Methods. A (~ 205 kD) protein band falling within the molecular weight range of known Vtg was observed in plasma of one specimen (Figure 2; lane 3). This band was absent in mucus of all individuals tested (Figure

1; lanes 6-9). In mucus, we consistently observed a high molecular weight (~ 240 KD) protein band hereafter referred to as Unidentified 240 (U_{240}). In August 2006, we collected mucus samples from three female *M. nigicans* , three female *T. albidus* and two male *T. albidus* at the White Marlin Invitational Tournament in Ocean City, Maryland. U_{240} was present in all mucus samples collected. Although U_{240} was initially a protein of interest (high molecular weight phosphoprotein), the presence of this protein in all female and male mucus samples analyzed suggests that this protein is not Vtg. Thus from tournament sampling we observed one likely Vtg positive individual serum sample (Figure 1; lane 3) but no Vtg in any mucus sample. No attempt was made to obtain amino acid sequence information on the putative *M. nigricans* Vtg observed in plasma.

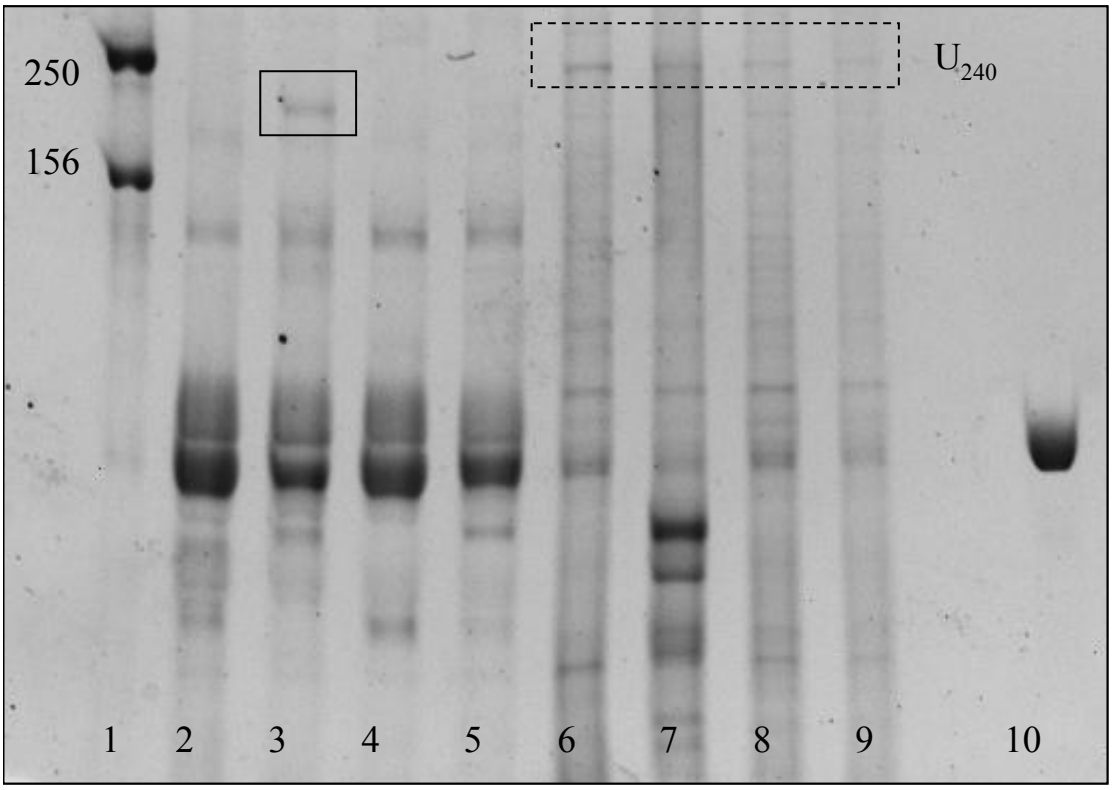

Figure 2. In-gel phosphoprotein staining of female *M. nigicans* serum (lanes 2-5) and mucus (lanes 6-9) collected at the Big Rock Blue Marlin tournament (Morehead City, NC, June 2005). Highlighted solid square (lane 3) represents possible Vtg protein of interest. All mucus samples contained unidentified protein U_{240} outlined by dashed rectangle. Lane 1 contains *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards. Lane 10 contains a 75kD standard (Bio-Rad Precision Plus Protein Dual Color molecular weight standard kit). Reverse image collected with Alpha Innotech Fluorchem SP Imaging system.

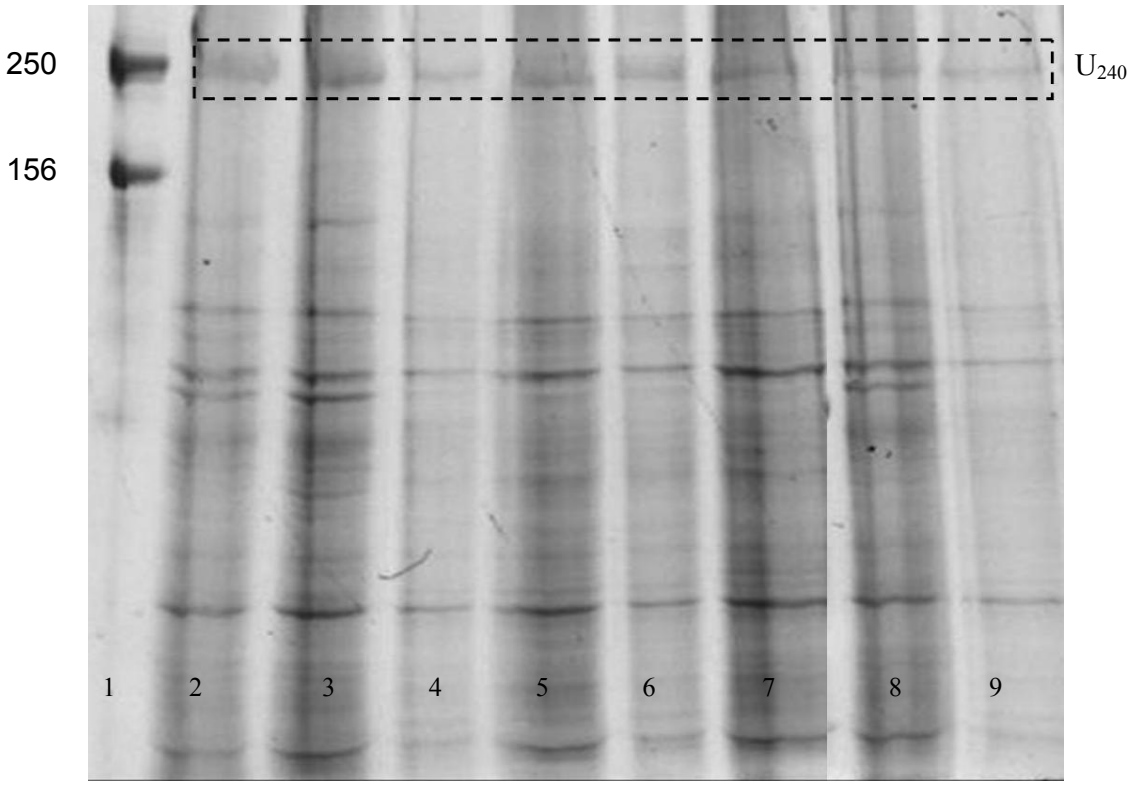

Figure 3. In gel phosphoprotein staining of mucus from female *M. nigicans* (lanes 2-4), female *T. albidus* (lanes 5-7) and male *T. albidus* (lanes 8,9) collected at the White Marlin Invitational Tournament in Ocean City, Maryland in August 2006. All mucus samples contained unidentified protein U_{240} outlined by dashed rectangle. Lane 1 contains *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards. Reverse image collected with Alpha Innotech Fluorchem SP Imaging system.

Pelagic sampling and testing. Samples of mucus were obtained from live billfish and live dolphinfish *(C. hippurus)* in order evaluate presence of Vtg in pelagic species and to obtain sufficient vitellogeic mucus to allow development of the portable, universal assay. In February 2006 we collected mucus from two live *I. platypterus*, eight live female dolphinfish *(C. hippurus)* and three live male *C. hippurus* from Panama U_{240} was relatively uniformly expressed in both sailfish and in all female and male *C. hippurus* (Figure 4). We also observed relatively uniform expression of U_{240} in three live female *C.hippurus*, two live male *C.hippurus* and four live *T. albidus* sampled out of Hatteras North Carolina in July 2006 (Figure 5). In July 2005, D. Kerstetter collected serum and mucus from live *Xiphias gladius* from the South Atlantic Bight. One *X. gladius* was a confirmed female with clearly visible oocytes. In that female a 210kD Vtg-like band was observed in the serum (Figure 6; panel a; lane 2) but not in mucus (Figure 6; panel b). U_{240} was the dominant high molecular weight phosphoprotein band in the mucus of all *X. gladius* in that study (Figure 6; panel b). Because of consistent expression of U_{240} in all mucus samples including mucus from ive male individuals we concluded that U_{240} was likely not a Vtg and not a protein of interest.

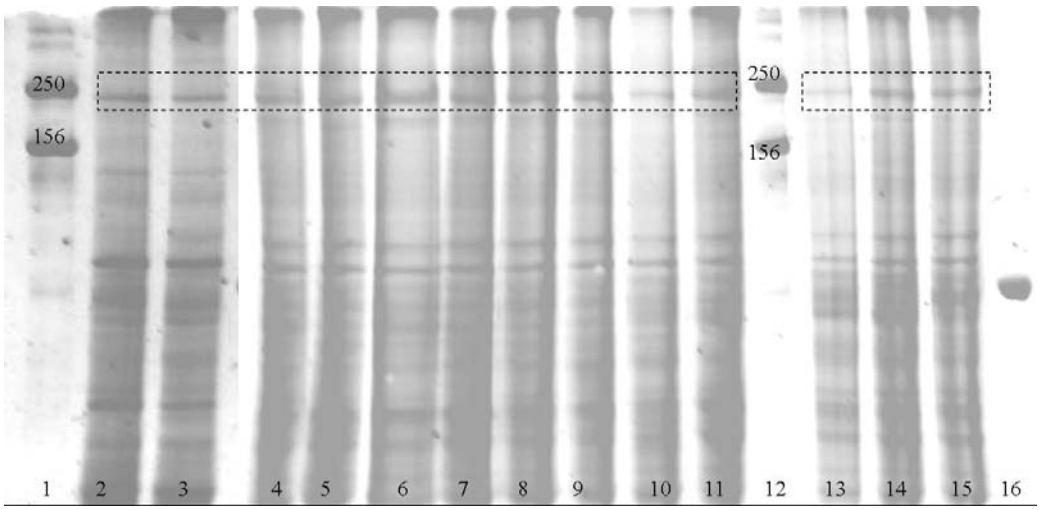

Figure 4. In-gel Phosphoprotein staining of mucosal proteins from *I. platypterus* (lanes 2,3), female *C. hippurus* (lanes 4-11) and male *C. hippurus* (lanes 13-15) from fish collected off Pacific Coast of Panama in February 2005. All samples contained Unidentified protein U_{240} outlined by dashed rectangle. Lanes 1 and 12 contain *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards. Lane 10 contains a 75kD standard (Bio-Rad Precision Plus Protein Dual Color molecular weight standard kit). Reverse image collected with Alpha Innotech Fluorchem SP Imaging system.

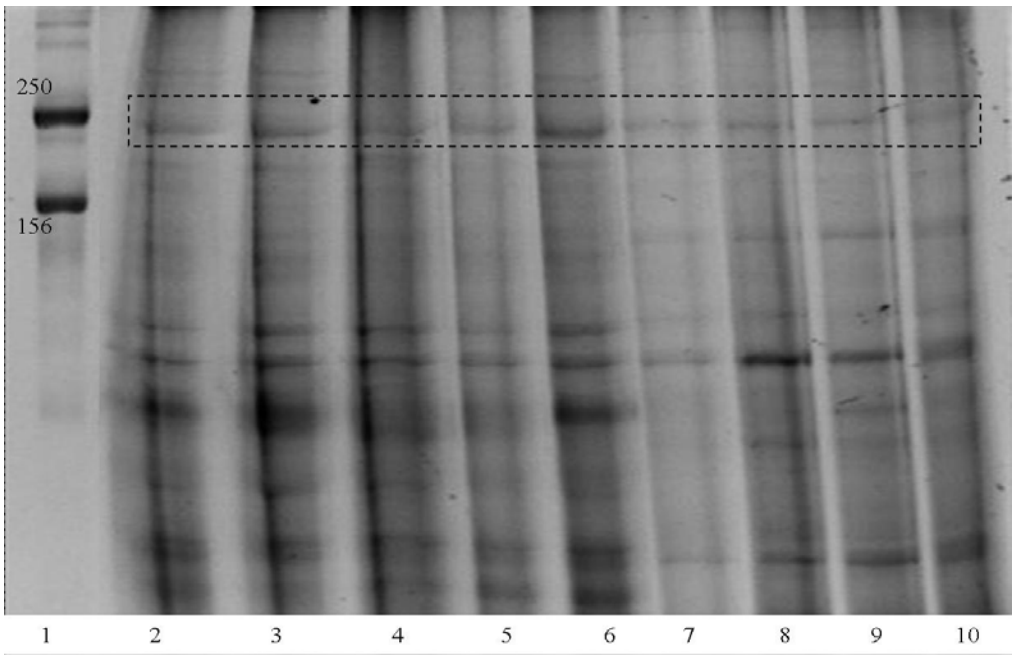

Figure 5. In-gel phosphoprotein staining of mucosal proteins from female *C. hippurus* (lanes 2-4), male *C. hippurus* (lanes 5,6) and *T. albidus* (7-10) from live fish collected off of Cape Hatteras NC in July 2006. U_{240} outlined in dashed rectangle was present in all mucus samples. Lane 1 contains *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards. Reverse Image collected with an Alpha Innotech Fluorchem SP Imaging system.

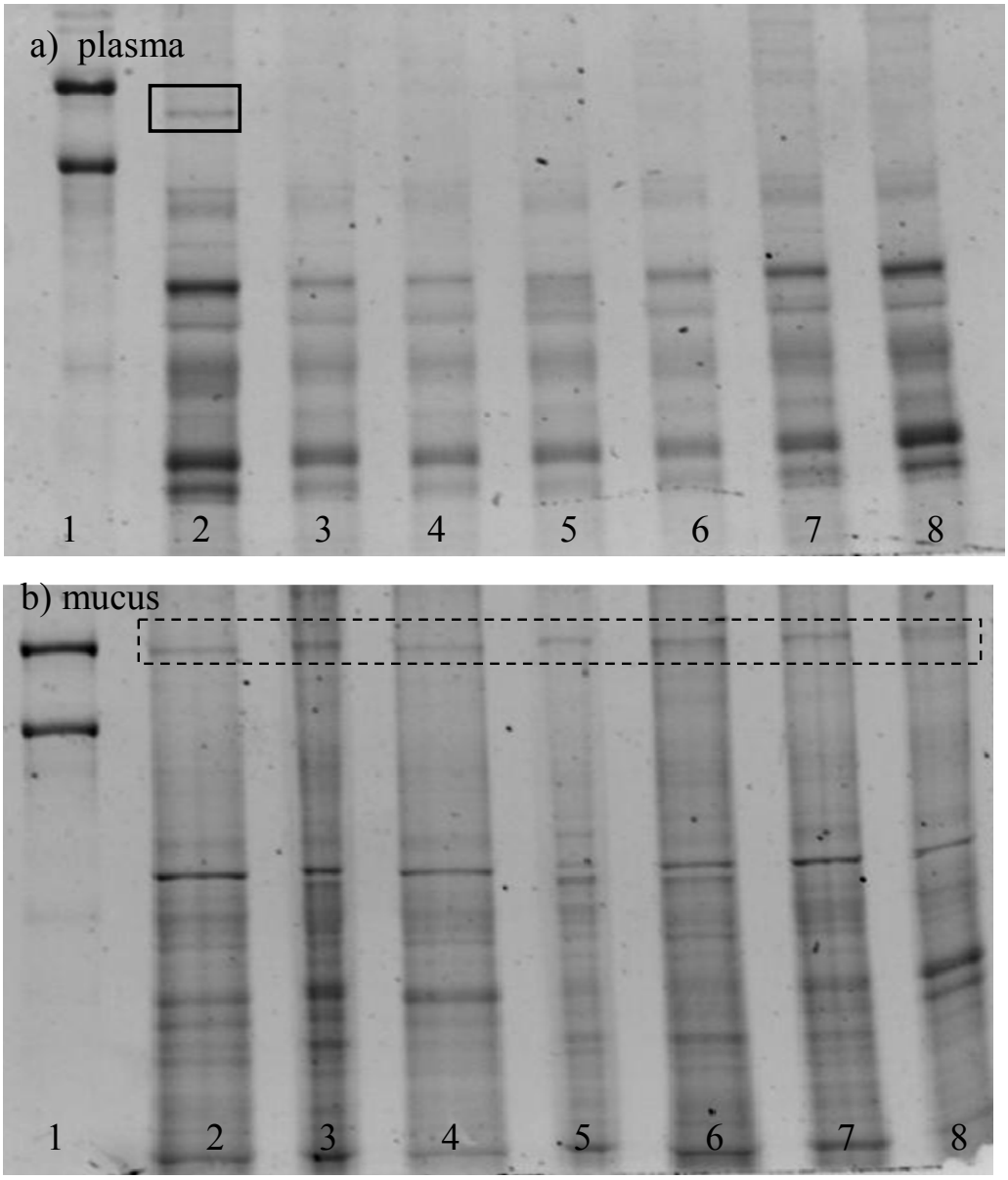

Figure 6. In-gel phosphoprotein staining of *X. gladius* serum (panel a) and mucus (panel b). Lanes 2a (plasma) and 2b (mucus) were sampled from a live female specimen with clearly visible oocytes. A ~210 phosphoprotein (likely Vtg) outlined in solid rectangle was visible in serum of vitellogenic female (panel a; lane 2) but not mucus. Lanes 3-5 were sampled from likely females with poorly developed (recrudescent) oocytes and lanes 6-8 were males. U_{240} was present in mucus of all individuals tested (panel b). Lane 1 contains *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards. Reverse Image collected with an Alpha Innotech Fluorchem SP Imaging system.

Several factors could contribute to the absence of Vtg or our inability to detect Vtg in mucus samples from tournament billfish, live billfish and other pelagic species sampled during this

project: a) the apparent absence of Vtg in mucus of tournament fish could be the result of protein proteolytic degradation. Proteolysis of diverse proteins in moribund individuals is always a concern in biochemical studies. Billfish sampled at weigh stations had been dead for undetermined lengths of time and were transported on decks of vessels. In all cases skin was dry and required wetting to allow sampling b) Vtg is cleaved into smaller products that lack the phosvitin domain before it appears in mucus of billfish making detection by phosphoprotein staining impossible c) The absence of mucosal Vtg could be related to the stage of oocyte development in individuals sampled or to factors related to the timing of appearance of this protein in billfish species tested. The biological role of mucosal Vtg and the mechanisms responsible for the appearance of Vtg in mucus are unknown. It is possible that the times that we sampled did not fall within the stages of the vitellogenesis where Vtg may be present in mucus of billfish d) It is possible that all live billfish that we sampled were male individuals. However this was clearly not the case with *C. hippurus* where males and vitellogenic females were sampled e) Our universal assay may lack the sensitivity or specificity to detect Vtg in those species tested. This possibility is unlikely in that the assay is based on high molecular weight and high phosphoserine content of all known species. In addition we have used the assay to detect Vtg in plasma and mucus of diverse freshwater and estuarine fish and have found that it is just as sensitive as antibody-based methods (Van Veld et al. 2005). f) Billfish and other pelagic species tested do not express Vtg in mucus at all or at least not during the times that we sampled. However Schultz et al. reported elevated levels of vitellogenin-derived yolk proteins (VDYPs) in mucus of female *I. platypterus* relative to that observed in males of that species.

Validation of the universal assay with C. hippurus. Following repeated attempts to detect Vtg in mucus of billfish and other pelagic species, we shifted focus and conducted a study to validate the universal assay in gravid *C. hippurus*. Male and female *C. hippurus* (6-30 kg) were captured approximately 15 miles southeast of Hatteras Inlet, North Carolina in July 2006 by angling. Blood was collected from each fish by severing a gill arch and allowing blood to flow into 15 ml polycarbonate tubes. Samples were placed on ice and within 8-12 hours of collection, serum was separated from cells using a hand-driven centrifuge (Lab Depot, Dawsonville,GA). Serum samples were immediately frozen in liquid nitrogen for transport to the laboratory.

Phosphoprotein staining of serum proteins revealed the presence of a high molecular weight (~203 kDa) phosphoprotein in serum of all vitellogenic female *C.hippurus* (Figure 7). This female specific protein appeared as the largest phosphoprotein in all female fish and was absent or non-detectable in males. These results are similar to previous studies in which Vtg is the dominant female-specific, high molecular weight (150-250 kDa) phosphoprotein in teleost blood (Van Veld et al 2005).

The female-specific phosphoprotein was excised and analyzed by tandem mass spectrometry (MALDI-TOF-MS/MS) in order determine if the amino acid sequences of tryptic peptides matched teleost Vtg sequences existing in the NCBI database. Highly significant ion scores and confidence interval percentages (C.I. %) for a number of teleost Vtgs in the database confirmed that the protein of interest was a Vtg (Table 1). Highest scores were obtained with red seabream *Pagrus major* (Table 1) where 18 *C. hippurus* peptides obtained exhibited 100% amino acid sequence homology with that species (Table 2).

Preparation of a standard curve using purified *P. promelas* Vtg standard allowed quantitative analysis of *C. hippurus* Vtg in seven female individuals used in this study (Figure 8) where Vtg content was estimated at 27 ± 3 ug Vtg/ µl serum. Because the phosphoserine content of Vtg is varies between species it may be appropriate to express values as *P. promelas* Vtg 'equivalents' as described previously (Van Veld et al 2005). This convention could be avoided by using purified Vtg from the species of interest in preparation of the standard curve.

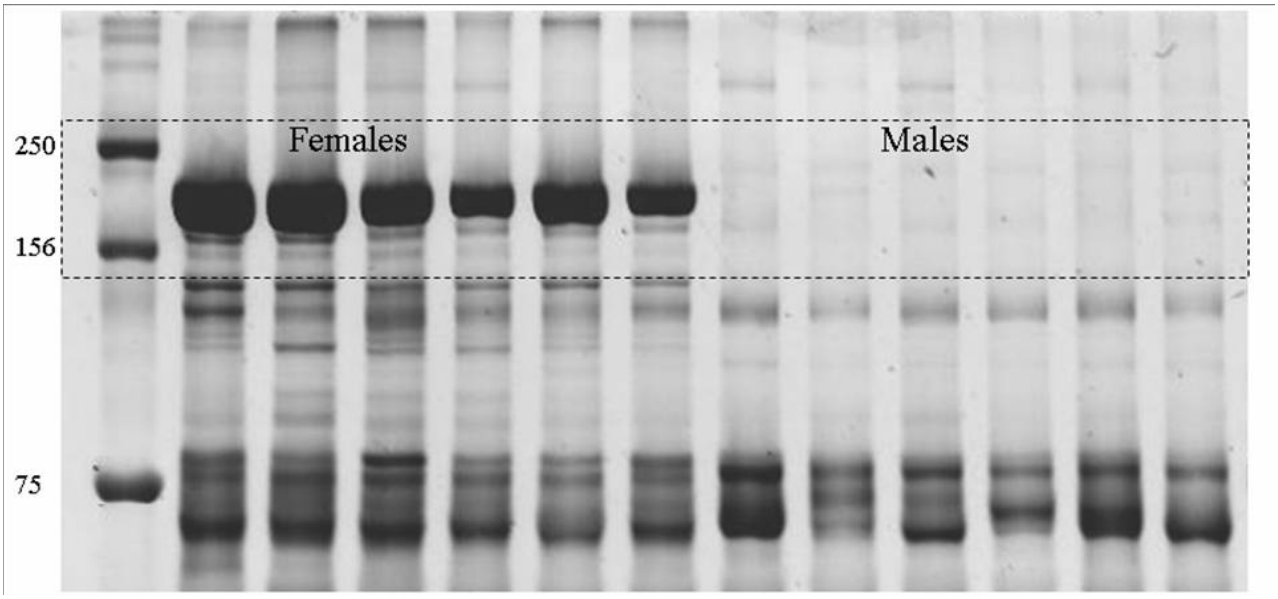

Figure 7. Phosphoprotein staining of plasma proteins in *C. hippurus*. All females (lanes 2-7) contained a 205 kD phosphoprotein subsequently confirmed as Vtg. Vtg was absent on all males. Lane 1 contains *F.heteroclitus* (250kD) and *P. promelas* (156kD) Vtg standards and a 75 kD standard .

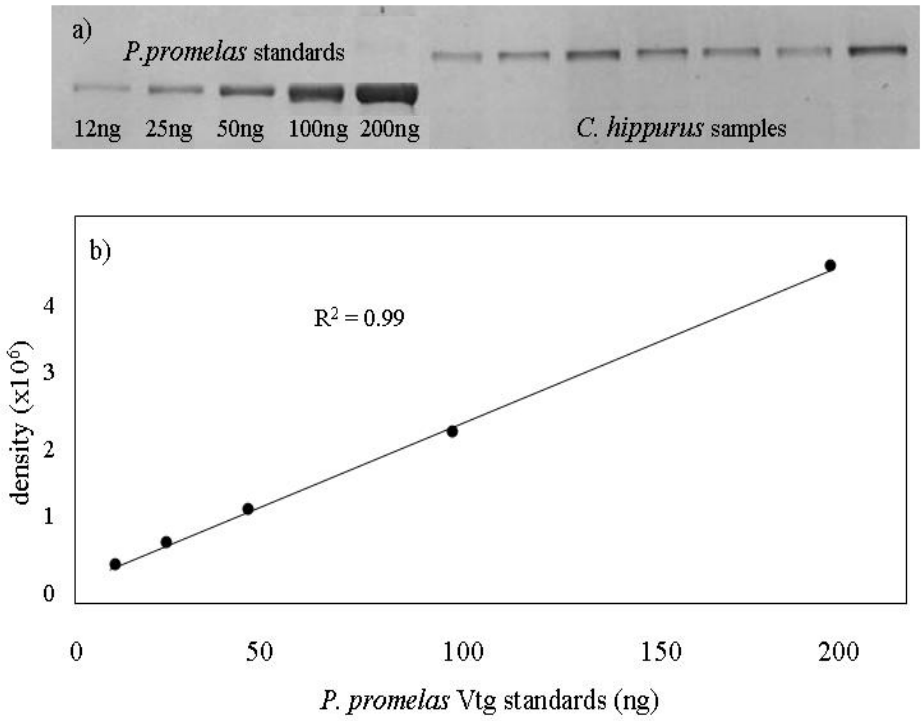

Figure 8. Quantitative analysis of *C. hippurus* vitellogenin (Vtg). Panel a) Purified *P. promelas* Vtg (12, 25, 50, 100 and 200ng) and female *C. hippurus* serum from seven individuals were electrophoresed on the same gel and stained with Pro-Q Diamond phosphoprotein stain. Serum samples were diluted 400-fold with 1X Tris-buffered saline (TBS) prior to electrophoresis. Gel image is cropped so that Vtg is the only *C. hippurus* phosphoprotein visible. The image was obtained with an AlphaInnotech Fluorchem Imaging System. Panel b) Standard curve prepared from *P. promelas* Vtg standards used to quantify *C. hippurus* samples.

Table 1. Teleost vitellogenins recognized by MALDI – TOF MS/MS analysis of *Coryphaena hippurus* vitellogenin. GenBank accession numbers are given in parentheses.

Species (Accession No.)	Peptide count [a]	Total ion score [b]	Total ion C.I.% [c]
Pagrus major (BAE43871)	18	295	100
Verasper moseri (BAD93696)	11	173	100
Epinephelus coioides (AAW29031)	9	73	99.999
Pleuronectes platessa (CAC94863)	4	73	99.999
Zoarces viviparus (CAC94861)	3	73	99.999
Fundulus heteroclitus (Q98893)	10	69	99.997
Gambusia affinis (BAD93698)	11	69	99.997
Rivulus marmoratus (AAQ16635)	8	43	98.841
Acanthogobius flavimanus (BAC06191)	8	42	99.481

[a] The number of peptides used for each identification

[b] When ion scores are ≥ 30, the protein match is considered statistically non-random at the 95% confidence interval (Venkataraman et al. 2005).

[c] Confidence index percentage calculated from the total ion score. The closer the C.I.% value is to 100, the more likely the protein is correctly identified (Stenbrg et al. 2005).

Table 2. Peptides obtained by MALDI – TOF MS/MS analysis of *Coryphaena hippurus* vitellogenin that exhibit 100% sequence homology with red seabream *(Pagrus major)* vitellogenin (Sawaguchi et al 2006; Genbank Accession Number BAE43871). These 18 *C hippurus* peptides covered 14% of the *P. major v*itellogenin amino acid sequence.

peptide	*P. major* start-end sequence
YEALLMGGLPEEGLAR	34-49
DLNHCQERIVK	184-194
FIELIQLLR	331-341
LAEGLAMHHK	415-424
VLGNAGHPASLKPIMK	483-498
VHIDTVLALR	524-523
MVQDIAVQLFMDK	532-544
TYLAGAYADVLEFGVR	668-683
ARVHTIVPAK	871-880
MEARIDMIK	881-889
MCAVIETFGIKACTEIESR	988-1006
IEIEIQVGDKAAEK	1040-1043
VINMSEEEEILEDKNVLMK	1057-1075
FTKNHIHQHAVSTAR	1209-1223
TNSKSSAYSFEAIYNK	1224-1239
YLANTISPAVTILIR	1242- 1256
LMAKLAWGIECK	1307-1318
VVDWMR	1573-1578

Objective 2: Optimization and testing a portable field kit for VTG detection in billfish.

Gel filtration. For development of a portable, universal Vtg that would be amenable to all species including billfish, we planned to separate high molecular weight Vtg from other mucosal phosphoproteins using small gravity-fed gel filtration columns that could be used at-sea. Initial tests involved separation of mucosal Vtg from other mucosal phosphoproteins in mucus from vitellogenic female *F. heteroclitus* using large gel filtration columns. Mucosal proteins from 12 female *F. heteroclitus* were passed through a 1.5 x 70 cm Sephacryl S-300 column as described above. The column had previously been calibrated with known molecular weight markers including blue dextran that eluted in the void volume (Figure 9a). Fractions of mucosal proteins were collected and absorbance at 280nm was recorded as an indicator of total protein in each fraction. Some separation of mucosal proteins was achieved as indicated by two major protein peaks eluting from the column (Figure 9b). Fractions comprising the void volume were electrophoresed as decribed above and stained with Pro-Q Diamond phosphoprotein stain. Although Vtg eluted in the void volume as anticipated, other non-Vtg phosphoproteins co-eluted with Vtg in the void fraction as well. These results could be related to association of mucosal

proteins with complexes of mucopolysaccarides preventing efficient separation by size. It is possible that a more thorough homogenization using a motor driven rotor/stator type tissue homogenizer would be sufficient to completely homogenize, disperse, and emulsify mucus samples allowing more efficient separation. During this period of laboratory investigation, it became apparent that we needed a good supply of Vtg positive billfish mucus in order to refine a simple method for Vtg separation. Therefore increased efforts were put into pelagic sampling to find a source of vitellogenin-positive billfish.

Cold filtration, an alternative, simple method for separation of Vtg from other phosphoproteins. Our supply of Vtg-postive F. heteroclitus was depleted and we tested this method on *F. heteroclitus* plasma (Figure 10). Qualitative evaluation of this method indicated that enrichment of Vtg was achieved as filters appeared enriched in Vtg and contained lesser amounts of non-Vtg phosphoproteins than did corresponding filtrates (Figure 10). While we are uncertain of the mechanism responsible for the partial success of cold filtration it is likely that lipid aggregation at cold temperatures may play a role. For example, triacylglyceride rich lipoproteins (chylomicrons, very low density lipoproteins) can be partially separated from ice-cold plasma or cytosol using coarse filtration. At low temperatures, the lipid portion of these lipoproteins assume a solid state and aggregation occurs. Vtg is a very high density lipoprotein containing about 20% phospholipid (Mann et al. 1999). At cold temperatures, it may is likely that Vtg-associated phospholipid also assumes a solid state an contributes to formation of "fat-pad" on the surface of the filter. Before refinemnt of the cold filtration method we needed to obtain a reliable source of Vtg-positive mucus from billfish or other pelagic species.

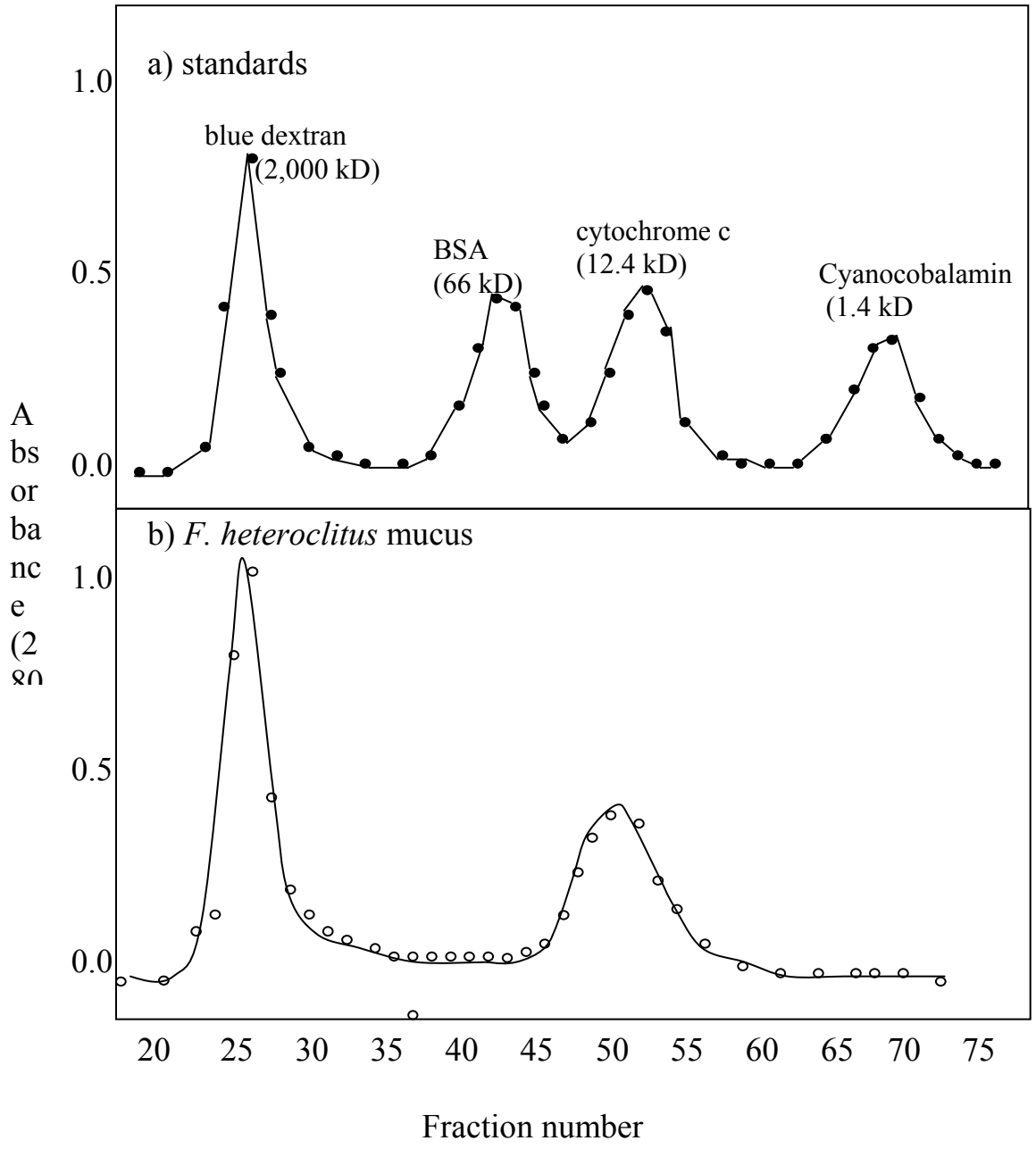

Figure 9. Gel filtration of *F. heteroclitus* mucus. Panel a – Calibration of Sephacryl S-300 column with molecular weight markers. Panel B - Mucus from 12 vitellogenic *F. heteroclitus* was passed through the column, fractions (two ml) were collected and protein content was determined by absorbance at 280 nm. The first large peak in (fractions 22-2) corresponds to the void volume in panels a and b.

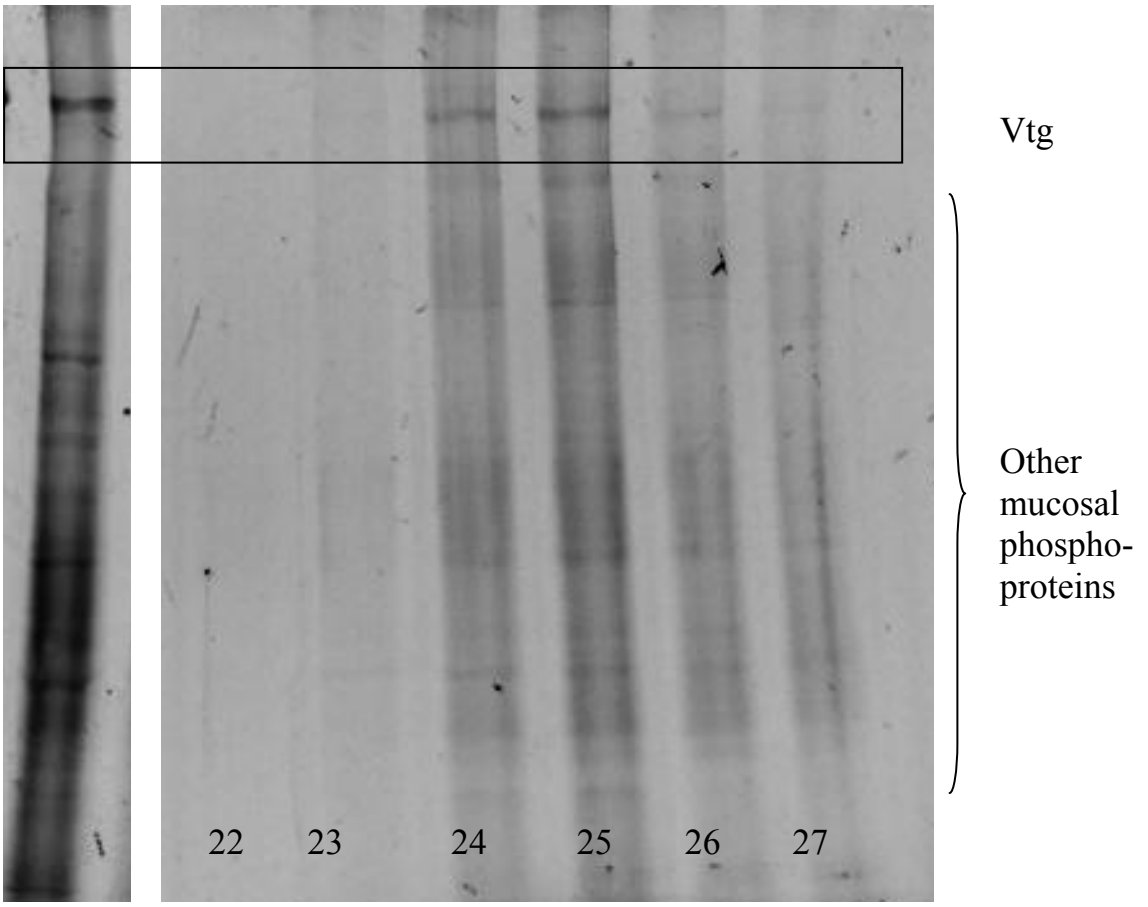

Figure 10. Qualitative analysis of the effectiveness of gel filtration in isolation mucosal Vtg from lower molecular weight phosphoproteins. The far left lane contained sample of *F. hetroclitus* mucus as applied to the column (note abundant low molecular weight phosphoproteins). Void volume fractions (fractions 24-27) were obtained from a Sephacryl S-300 gel filtration column and stained with Pro-Q Diamond phosphoprotein stain. Vtg eluted from column in void fractions as anticipated. However, void fractions contained other lower molecular weight phosphoproteins as well. It is likely that more rigorous mucus homogenization would have resulted in better separation and lesser amounts of non-Vtg phosphoprotein contamination in void fractions.

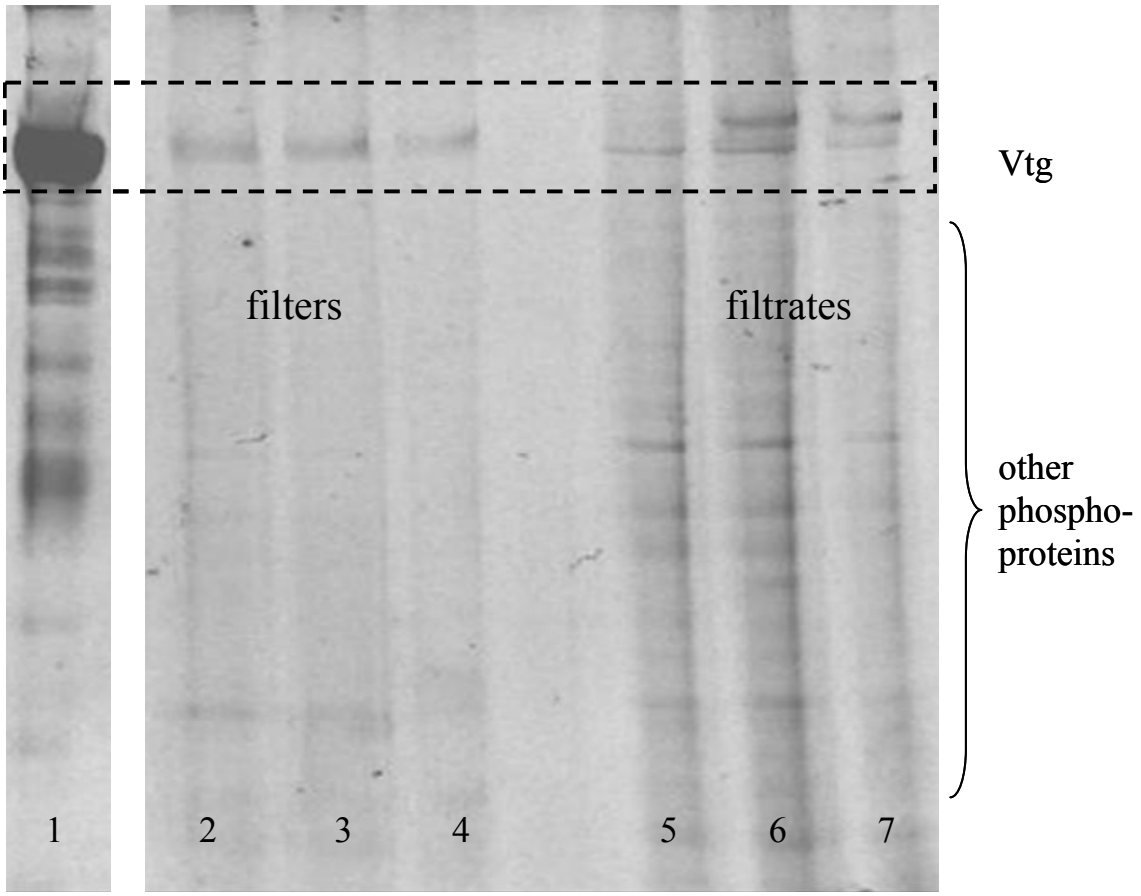

Figure 10. Qualitative analysis of the effectiveness of cold filtration method in isolating Vtg from other phosphoproteins. Samples of plasma from vitellogeic *F. heteroclitus* were drawn into an ice-cold glass Luer Loc syringe equipped with an ice-cold stainless steel Filter housing and a 0.45µ glass fiber filter. Filters were homogenized and centrifuged to remove filter debris. Samples of filter extract and filtrate were electrophoresed and stained with Pro-Q Diamond phosphoprotein stain. Some enrichment of Vtg appears evident from visualization of phosphoprotein staining of filters and filtrates.

Our proposal called for modification of the Pierce Phosphoprotein Phosphate Estimation kit for use in a ship-board assay for Vtg. We tested this kit in the laboratory on numerous occasions to evaluate the potential for use in a portable assay. For example, when serum from three vitellogenic female *C. hippurus* and three male *C. hippurus* was assayed in side-by-side (female-male) comparisons, all three samples of female mucus contained higher levels of phosphoprotein phosphate than that of males (Figure 11). Higher levels of phosphoprotein phosphate in females is likely the result of the contribution of Vtg to total levels of phosphoprotein in females (Figure 11). Though our portable assay was never sufficiently refined enough to include a shipboard version of the phosphoprotein detection system,we believe that this kit could easily be modified for use at-sea.

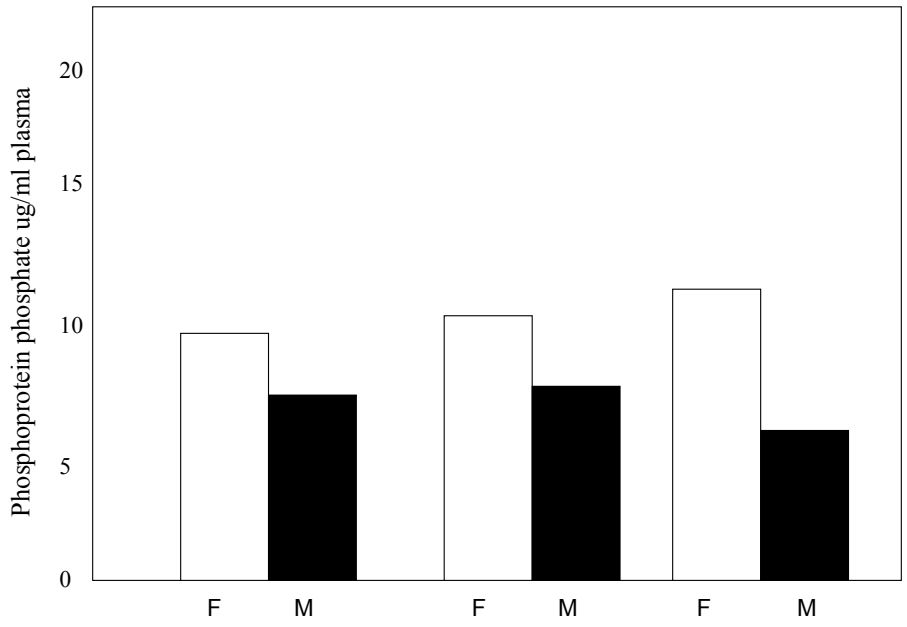

Figure 11. Evaluation of the use of Phosphoprotein Phosphate Estimation Kit (Pierce) for use in portable universal assay. Three female-male side by side comparisons were made between samples of plasma from vitellogenic female *C, hippurus* and male *C, hippurus*.

Objective 3 to identify additional sex-specific proteins in billfish mucus using two dimensional electrophoresis and de novo sequencing.

Two dimensional gel electrophoresis (2DE) - Two dimensional gel electrophoresis (2DE) was performed to determine if non vitellogenin sex-specific proteins could be identified in mucus of *S. cavalla.* However, when we compared protein expression between female-female or between male-male *S. cavalla*, we found that large differences in protein expression occurred between individuals regardless of sex. Sex-specific differences in protein expression, if present, was masked by large differences in protein expression between individuals of the same sex. As a result we were unable to select candidate sex-specific protein for identification by de novo sequencing.

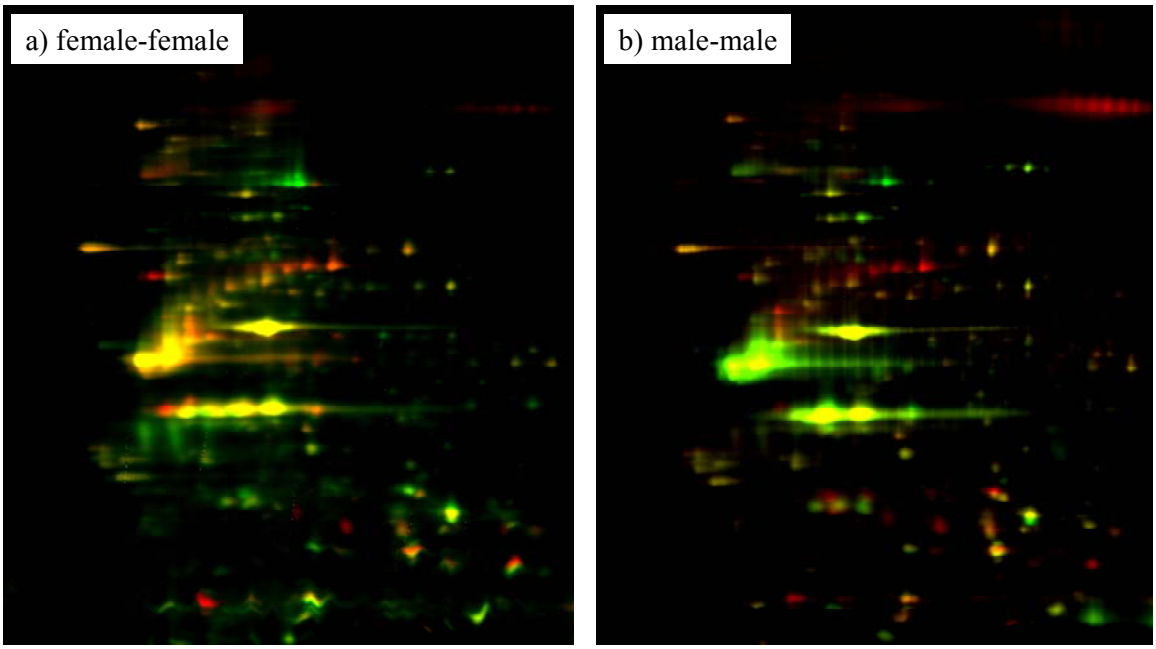

Figure 12. Two dimensional electrophoresis of fluorescent-tagged mucosal proteins from king mackerel *(S. cavalla)*. Panel a) female-female comparison and panel b) male-male comparison. Using different fluorescent labels for proteins in each treatment, large differences in protein expression appear as intense red or green spots in gel overlays. No differences appear as yellow spots (Tonge et al. 2001). In this figure, large differences in expression of individual proteins (red and green spots) were observed in female-female comparisons (panel a) and in male-male comparisons (panel b).

Objective 4. To identify additional sex-specific proteins in billfish mucus using two dimensional electrophoresis and *de novo* sequencing. No sex-specific proteins were identified in mucus by two dimensional electrophoresis. Therefore no activity in the area of assay development occurred.

Conclusions

The most significant problem that we encountered during the course of this study was an apparent absence of Vtg in the mucus of billfish and other pelagic species that we sampled. These included known female blue marlin and white marlin sampled at tournaments and several live white marlin and sailfish. Vtg was apparently absent in mucus of a female *X. gladius* where a Vtg-like protein was present in the serum of the same fish. Vtg was absent in the mucus of vitellogenic female *C. hippurus* whose plasma tested positive for Vtg. Because of our initial success in detecting Vtg is several small estuarine and freshwater fish, these results were unexpected. We know that the universal Vtg assay using phosphoprotein detection is at least as sensitive as antibody based detection. We believe that if Vtg was present in mucus of individuals sampled during this study that we would have detected it.

During early stages in development of the universal portable assay for Vtg detection, we started to become concerned about the increasing number of Vtg-negative samples that we had collected

from pelagic species. We felt an urgent need to intensify mucus sampling in pelagic species both to address the basic assumption that Vtg is present in mucus of female individuals and to secure a supply of Vtg positive mucus for continued portable assay development.

Questions related to the biological role of mucosal Vtg, the mechanisms by which this large protein arrives at mucus, the timing of the appearance of the protein in mucus as it relates to oocyte development and species specificity of Vtg appearance in mucus remain unresolved. These questions need to be addressed before mucosal Vtg can be used as a reliable indicator of gender in species of interest. We are particularly interested in differences in results observed in our study compared to that of (Schultz et al 2006). Using our universal assay it does not appear that mucosal Vtg will be a reliable indicator of sex in pelagic species tested. Shultz et al. 2006 reported evidence vitellogenin-derived yolk proteins (VDYPs) in surface mucus of *I. platypterus.* The antibody used was generated against proteins associated with developing *M. nigricans* oocytes. Further studies are needed to more clearly identify the protein detected by that antibody. It is possible that billfish and other species excrete relatively small Vtg-derived proteins into the mucus rather than intact Vtg. The smaller ppeptides or proteins may or may not retain the phosvitin domain. A combination of small size relative to intact Vtg and loss of all or part of the phoisvitin domain could contribute to the negative results we obtained with billfish and *C. hippurus*.

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ANALYSIS OF THE EFFECT OF OFFSET CIRCLE HOOKS ON POST-RELEASE SURVIVAL AND AN ESTIMATION OF THE RELATIVE HOOKING EFFICIENCY OF CIRCLE HOOKS AND STANDARD J-HOOKS IN THE RECREATIONAL FISHERY FOR WHITE MARLIN

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Abstract
The fate of 60 white marlin caught on three different models of circle hooks varying in shape and degree of offset was followed with pop-up satellite archival tags programmed to release from the animals 10 days after release. Only one mortality was inferred from the PSAT data and no significant differences in post-release survival were noted among fish caught on the different hook models. In contrast, relative to white marlin caught on straight shank ("J") hooks under similar conditions in a previous study, the three models of circle hooks combined demonstrated highly significant reductions in deep hooking, fish trauma, and post-release mortality. Analysis of hooking location for circle hooks and "J" hooks on larger sample sizes of white marlin, sailfish and blue marlin that were not tagged with PSATs, demonstrated significant differences in internal and external hooking locations and the presence and absence of bleeding (hook induced trauma) between the two hook types. These data indicate that the use of circle hooks encompassing a range of shapes and minor offsets can provide a significant conservation benefit in the offshore recreational fishery for white marlin and other istiophorids.

Purpose
The main objective of this study was to estimate the post-release survival of white marlin caught on three models of commonly used circle hooks that differ in shape and offset by using pop-up satellite archival tags (PSATs). Secondary goals were to evaluate differences in hooking location and fish condition of a much larger sample size of white marlin caught on circle hooks and standard "J" hooks, and to determine if there were significant differences in hooking efficiency between the two hook types.

The U.S. Atlantic offshore recreational fishery interacts with a multispecies assemblage of epipelagic fishes that includes several istiophorid billfishes, swordfish *Xiphias gladius*, a variety of tunas *Thunnus* spp. and smaller scombrids, as well as the dolphinfish *Coryphaena hippurus*. The white marlin *Kajikia albida* (revised by Collette et al. (2006) from *Tetrapturus albidus*), is an important component of this assemblage and is targeted by several tournaments along the U.S. mid-Atlantic coast. The apparent abundance of white marlin has been greatly reduced over the past fifty years. At the most recent full stock assessment of white marlin in 2002, the Standing Committee for Research and Statistics (SCRS) of the International Commission for the Conservation of Atlantic Tunas (ICCAT) estimated that the Atlantic-wide stock of white marlin is overfished and that overfishing is occurring (ICCAT 2003). Several simulations undertaken by the SCRS during the assessment resulted in estimates of current biomass that were less than 20% of that necessary for maximum sustainable yield. In response to declining stock abundance, the U.S. National Marine Fisheries Service (NMFS) was petitioned in 2001 to list white marlin as threatened or endangered under the U.S. Endangered Species Act. Although NMFS

concluded that there was not sufficient evidence to list white marlin as threatened or endangered at that time, it was noted by the White Marlin Status Review Team (2002) that international cooperation would be necessary to significantly reduce fishing mortality on this overfished species.

White marlin occur as an incidental bycatch species in commercial fisheries and are targeted by directed recreational fisheries throughout their range in the Atlantic Ocean. The majority of fishing mortality on Atlantic white marlin results from the pelagic longline fishery for tunas and swordfish (ICCAT 2001). In an effort to reduce fishing mortality on white marlin by the pelagic longline fishery and maintain current effort for target species whose stocks were in better condition, ICCAT adopted a binding management measure in 2000 that requires the release of all white marlin and blue marlin *Makaira nigricans* alive at the time that pelagic longline gear is retrieved (hauled back) (ICCAT Recommendation 00-13). For almost 20 years, U.S. domestic regulations have prohibited pelagic longline fishermen from possessing Atlantic istiophorid billfishes, regardless of disposition. Logbook records and data from the U.S. observer program indicate that the majority of white marlin are alive at the time of haulback (Cramer 2000), and a recent study demonstrated that most white marlin released alive from pelagic longline gear survive (Kerstetter and Graves 2006).

The magnitude of Atlantic-wide recreational fishing mortality on white marlin is considerably smaller than that of the pelagic longline fishery, but it is by no means negligible (ICCAT 2001). In the USA, Goodyear and Prince (2003) estimated that the annual recreational catch of white marlin along the Atlantic and Gulf coasts ranges from 4,000 to 8,000 individuals. Domestic recreational landings of white marlin are currently regulated by a minimum size of 168 cm (66 in.) lower jaw fork length (LJFL) and bag limit of one white marlin per boat per day. In addition, since 2000 the U.S. Atlantic recreational fishery has been limited to an annual landings quota of 250 blue marlin and white marlin combined (ICCAT Recommendation 00-13). As a result, it is estimated that up to 99% of white marlin caught in the U.S recreational fishery are now released alive (Goodyear and Prince 2003).

A variety of terminal tackle is used in the offshore recreational fishery for white marlin, including artificial lures, natural baits, and bait-and-lure combinations. Most anglers targeting this species slowly troll dead ballyhoo *Hemiramphus brasiliensis* at the surface (Jesien et al. 2006). As white marlin approach trolled baits from behind, many anglers will "drop-back", manually decreasing the tension on the line (drag) for several seconds to allow billfishes time to ingest rigged baits without feeling resistance (Mather et al. 1975; Jolley 1975; Prince et al. 2007). Hooks are set by applying tension several seconds after the fish has overtaken and ingested the bait, often resulting in deep hooking and hook-induced injuries to vital internal organs (Horodysky and Graves 2005; Prince et al. 2007). Both separately and especially when they co-occur, suboptimal hooking locations and hook-induced trauma greatly reduce post-release survival of white marlin (Horodysky and Graves 2005).

Previous studies have demonstrated that the use of circle hooks in recreational fisheries targeting piscivorous species can greatly reduce the incidence of deep hooking, hook-induced tissue trauma, and/or mortality relative to straight shank ("J") hooks (Muoneke and Childress 1994; Cooke and Suski 2004). For white marlin, Horodysky and Graves (2005) reported that circle

hooks significantly reduced the incidence of deep hooking and bleeding relative to "J" hooks. Furthermore, they noted a highly significant difference in post-release survival of white marlin caught on "J" hooks and circle hooks (65% and 100%, respectively). Based on the depleted condition of the white marlin resource and the significant difference in post-release survival of white marlin caught on circle and "J" hooks, the Final Consolidated Highly Migratory Species Fisheries Management Plan the NMFS implemented a regulation on 1 January 2007 requiring the use of circle hooks when fishing natural baits in all Atlantic billfish tournaments (71 FR 58058: October 2, 2006).

A wide array of circle hook models encompassing a range of sizes, shapes, and degree of offset between the planes of the point of the hook and the shank is available to recreational offshore anglers (Cooke and Suski 2004). Prince et al. (2002) demonstrated that frequency of deep hooking in sailfish *Istiophorus platypterus* increased with the magnitude of circle hook offset; hooks with severe offsets of 15º had deep hooking percentages similar to "J" hooks. In some cases, similar outcomes can also result from the use of non-offset or mildly-offset circle hook models in combination with specific fishing practices. For example, Prince et al. (2007) noted that extending drop-back times with a circle hook bearing a more traditional "J" hook shape results in a higher incidence of deep hooking than experienced with similar procedures using a more rounded circle hook, although the trend was not significant. While the effects of different circle hook models on the post-release survival of white marlin are unknown, it is clear that these data are essential for the development of a meaningful and enforceable management regulation.

Approach

Three circle hook models commonly used with natural baits in the recreational fishery for white marlin were selected for this study: 1) non-offset Eagle Claw Circle Sea (model L2004EL) sizes 7/0 – 9/0, which has moderately elongated circular bend; 2) non-offset Owner SSW In-Line Circle Hook (model 5379-161) size 6/0, which has a bend region shaped more like a "J" hook; and 3) 5º offset Mustad Demon Fine Wire (model C39952BL) size 7/0, which has a circular bend (Figure 1). Two of these circle hook models (Eagle Claw and Mustad) were employed by Horodysky and Graves (2005) in their comparison of post-release survival of white marlin caught on J hooks and circle hooks. The results from those 20 fish caught on circle hooks (11 on Eagle Claw and 9 on Mustad) are incorporated into this analysis. Numerous rigging techniques were used to attach the circle hooks to the ballyhoo bait (wire harness attached to a barrel swivel, plain wire harness, rigging floss harness), but all methods left the circle exposed on the top of the head of the bait (Figure 1). Typically we fished one model of circle hook per vessel per day.

White marlin were caught, tagged, and released in the waters offshore of Cape May, NJ, Virginia Beach, VA, and Manteo, NC along U.S. mid-coast; Isla Mujeres, Mexico; and La Guaira, Venezuela between November 2002 and November 2006. As white marlin attacked a trolled ballyhoo, the bait was dropped-back for 4 – 10 sec, a typical practice in the mid-Atlantic white marlin fishery (Jesien et al. 2006). Fish were caught on 20 – 30 lb class sportfishing tackle using 60 to 80 lb test leaders 1 – 2 m in length. Fight times were consistent for the fishery, usually 5 to 30 minutes. In some instances a fight was prolonged to ensure that the white marlin was sufficiently calm before it was brought next to the boat, a practice that facilitated accurate tag placement. A cursory examination of each white marlin was undertaken prior to tagging to note the condition of the animal and hook location. We used the binary hooking location designations

of Horodysky and Graves (2005) in this study: fish hooked in the jaw, bill, or foul-hooked on the body were grouped into an "externally visible" category, while fish hooked in the palate, gills, esophagus, or viscera were grouped into a "deep/not externally visible category. We tagged the first 60 fish available to us; no fish was rejected for tagging due to condition.

Fish were tagged with a Microwave Telemetry PTT 100 HR pop-up satellite archival tag programmed for release (pop-up) after 10 days. This tag model records temperature, pressure (depth) and light levels approximately every 90 - 120 s and upon surfacing, transmits the unprocessed data to satellites of the Argos system. The rigging and deployment of satellite tags followed the protocols presented in Graves et al. (2002) and Horodysky and Graves (2005), respectively.

Survival of released white marlin was inferred from temperature and depth profiles as well as net movement from the point of release to the location of the first transmission of the surfaced tag following the protocols provided in Horodysky and Graves (2005). Cochran-Mantel-Haenszel (CMH) tests were used to address the effect of different models of circle hooks on survival, hooking location, and the degree of hook-induced trauma. A Yates correction for small sample size was applied when expected cell values were less than 5 (Agresti 1990). All statistical analyses were conducted using SAS (version 9.1, SAS Institute, Cary, NC). Bootstrapping simulations were performed to determine effect of sample size on the 95% confidence intervals of the release mortality estimates using software developed by Goodyear (2002). Distributions of estimates were based on 10,000 simulations with an underlying release mortality equivalent to that observed for the three circle hook models and no sources of error (e.g. no premature release of tags, no tagging-induced mortality, and no natural mortality).

In addition to noting the hook location in the 60 white marlin tagged with PSATs, observations were made of the hooking location on all other istiophorids caught on our tagging trips. To obtain larger samples sizes for a comparison of hooking location among circle hooks and J hooks, three charter captains were contracted to collect hook location information for the istiophorids caught on their charters. The captains were provided with data sheets to log information on hook location and fish condition. Hook location categories consisted of corner of jaw, lower jaw, top of head, bill, inside mouth, deep, and foul (list location), bleeding was noted as present or absent, and hook models were designated as either circle or J. Cochran-Mantel-Haenszel (CMH) tests were used to assess whether hooking location and the degree of hook-induced trauma varied between circle and straight shank J hooks for these data. Yates' corrections for small sample size were applied when expected cell values were less than 5 (Agresti 1990). All statistical analyses were conducted using SAS (version 9.1, SAS Institute, Cary, NC).

Findings

Twenty white marlin were caught on each of the three circle hook models, tagged with Microwave Telemetry PSATs, and released. Nineteen white marlin were released off the U.S. mid-Atlantic coast, six off Isla Mujeres, Mexico, and 35 off La Guaira, Venezuela. The estimated size of released fish ranged from 16 kg to 41 kg, with an average size of 22.5 kg. Fight times (including the tag application procedure) ranged from 4 – 40 minutes, with the average fight and tag deployment lasting just over 13 minutes.

Of the 60 white marlin caught on circle hooks in this study, 59 (98.3%) were hooked in externally visible locations, 55 (91.7%) of which had the hook in the jaw. All three models of circle hooks preferentially hooked fish in the corner of the jaw (Eagle Claw 100%; Owner 90%; Mustad 70%). Two white marlin were hooked under the bill, one on an Owner hook (VZ 05-05) and one on a Mustad hook (VZ 06-02). Two fish were foul hooked, one in the branchiostegal membrane with an Owner hook (VZ 05-04) and the other in the caudal fin with a Mustad hook (MX 06-01). The only white marlin hooked in a deep/not externally visible location was caught on an Owner circle hook that lodged in the palate (MA 06-12). None of the sixty fish exhibited major hook-induced trauma. The lone white marlin observed bleeding in this study (VZ 03-04) was caught on a 9/0 Eagle Claw hook that punctured the orbit but not the eye. One additional fish appeared to suffer recent trauma not induced by a hook: white marlin VZ 05-06 had a fresh circular wound on its flank that was most likely the result of a bite from a cookie cutter shark *Isistius brasiliensis*. Both white marlin MA 06-12 and VZ 05-06 survived for the full ten day tag deployment duration.

All 60 Microwave Telemetry PSATs transmitted data to satellites of the Argos system upon releasing from study animals. Three tags detached from their hosts prior to the ten day programmed release: one after one day (VZ 06-09), and two after five days (VZ 05-04 and VZ 06-02). Five days has been considered a sufficient time period for the expression of relevant sources of post-release mortality (Graves et al. 2002), therefore we decided to include the data from the two tags that remained attached for five days in subsequent analyses. Tag VZ 06-09 released prematurely after approximately one day, with the first signal location 310 km from the point of release. Despite the large net displacement, we felt that one day of information following release was not sufficient to determine survival and this fish was eliminated from subsequent analyses. Consequently, our sample size for the Mustad hook was only 19 individuals. Pop-up satellite archival tags typically do not successfully transmit 100% of the stored data for a variety of reasons including limited battery life, sea state, biological interactions, etc. In this study transmission of the archived data from each tag ranged from 24% to 79%, with a mean of 62%.

Temperature/depth profiles and net displacement data suggested that 58 of 59 tagged white marlin (98.3 %) survived recreational release. Surviving white marlin made repeated daily vertical excursions of up to 200 m depth throughout the tag deployment duration. Furthermore, the net movement of these fish from the point of release to the location of the first tag transmission was consistent with that expected for actively swimming animals (Horodysky et al. 2007). The single inferred mortality in this study (MA 05-02) was a white marlin that was caught on an Owner circle hook that lodged in the center of the fish's lower jaw. The white marlin had a fight time of 14 minutes and was considered to be in excellent condition (coloration, movement, lack of visible injuries) at the time of release. Like many white marlin released off the U.S. mid-Atlantic coast, this animal spent the first few days following release in the upper 30 m of the water column. However, during the fourth day following release, the tag (and presumably the animal, as the tag is positively buoyant) sank to the ocean bottom at 1160 m and remained there for four days until it released from the fish and floated to the surface.

No statistically significant differences in hooking location, hook- induced trauma, or mortality were evident among the different circle hooks due to the limited number of negative impacts on white marlin caught on the three types of circle hooks used in this study (one mortality, one non-external hooking location, one bleeding fish). Since the null hypothesis of no difference between hook types could not be rejected, data from all three models of circle hooks were thus pooled to generate an overall post-release mortality rate of 1.7% for this terminal gear type. Using the model developed by Goodyear (2002), the results of 10,000 simulated experiments at an underlying true mortality rate of 1.7% indicate that more than 80 PSATs would have to be deployed to reduce the confidence intervals to ± 5%. In our study, in which 59 tags were deployed on white marlin caught on non-offset or minor offset circle hooks, the approximate confidence intervals for mortality estimates range from 0% to 6% in the absence of any confounding factors.

While we observed no major differences in hooking location or post-release mortality among white marlin caught on the three models of circle hooks used in this study, the pooled circle hook data exhibited a significant decrease in the incidence of deep (internal) hooking (Yates' corrected CHM $\chi^2 = 26.4$, $p < 0.0001$), hook-induced trauma (Yates' corrected CHM $\chi^2 = 22.6$, $p < 0.0001$) and post-release mortality (Yates' corrected CHM $\chi^2 = 15.5$, $p < 0.0001$) relative to white marlin caught on "J" hooks under similar conditions (Horodysky and Graves 2005). Odds ratios revealed that "J" hooks were 38 times more likely to hook fish deeply, 31 times more likely to induce trauma, and 20 times more likely to cause mortality than circle hooks.

The same trends were evident in our expanded analysis of hooking location and fish condition of the 60 white marlin tagged with PSATS as well as additional observations made by the authors and recreational captains of the catch of 212 white marlin, 132 sailfish, and 97 blue marlin on circle hooks and "J" hooks. These data collected by demonstrated that white marlin exhibited a significant decrease in the incidence of deep (internal) hooking (Yates' corrected CHM $\chi^2 = 50.5$, $p < 0.0001$), hook-induced trauma (Yates' corrected CHM $\chi^2 = 36.3$, $p < 0.0001$) relative to white marlin caught on "J" hooks under similar conditions. Sailfish caught on circle hooks also experienced a significant decrease in the incidence of deep (internal) hooking (CHM $\chi^2 = 24.1$, $p < 0.0001$), hook-induced trauma (Yates' corrected CHM $\chi^2 = 17.5$, $p < 0.0001$). Odds ratios revealed that "J" hooks were 10.6 times more likely to hook fish deeply, 11.9 times more likely to induce trauma. No significant differences in hooking location or degree of trauma induced between circle and straight shank J hooks were evident in the blue marlin data collected by recreational captains, presumably due to low sample sizes. However, odds ratios suggest that trends in the blue marlin data are similar to conclusions reached for white marlin and sailfish: circle hooks were 7 times more likely to hook blue marlin in the jaw and 6.8 times less likely to cause hook induced trauma than straight shank J hooks.

Conclusions

All 60 satellite tags deployed in this study released from the white marlin and transmitted to satellites of the Argos system. Most studies applying satellite tags to istiophorid billfishes and other large pelagic fishes have experienced one or more instances where tags failed to report, and reporting rates tend to decrease with tag deployment duration (Graves et al. 2002; Domeier et al. 2003; Kerstetter et al. 2004; Horodysky et al. 2007; Kerstetter and Graves 2006). Non-reporting tags can result from mechanical failure, biological interactions, or fishery gear interactions

(Graves et al. 2002; Hoolihan 2004; Kerstetter et al. 2004). The interpretation of such non-reporting tags can be problematic for studies of post-release survival: rates of post-release survival are biased upwards by eliminating non-reporting tags from analyses, and biased downwards by assuming that all non-reporting tags represent mortalities (Goodyear 2002; Kerstetter and Graves 2006). In this study we used short deployment durations to minimize the incidence of non-reporting tags and the ambiguities associated with their interpretation. The tagging duration was selected to account for most sources of mortality associated with the capture event, while minimizing other potential sources of natural and additional fishing (e.g. recapture) mortalities that could confound our results (Goodyear 2002; Graves et al. 2002; Kerstetter et al. 2004).

All but three of the 60 PSATs deployed in this study remained attached to the white marlin for the full ten day deployment duration (95%). As previously stated, tag VZ 06-09 was attached for approximately 24 hours and was eliminated from this study. The two remaining tags (VZ 05-04 and VZ 06-02) prematurely released on the fifth day following deployment. These two white marlin exhibited a pattern of several deep dives during each of the five days at liberty, similar to other surviving white marlin in that area. The majority of white marlin mortalities noted from the recreational and pelagic longline fisheries have occurred shortly after release, and in only a few instances have mortalities occurred after 48 hours (Horodysky and Graves 2005; Kerstetter and Graves 2006). Based on these data, we assumed that these two individuals survived their encounters with recreational fishing gear.

We did not detect a significant difference in hooking location of white marlin between non-offset (Eagle Claw, Owner) and minor (5 º) offset (Mustad) circle hooks. Only one of the 59 white marlin considered in this study was hooked in a deep location, and this fish was caught on a non-offset Owner circle hook. In a study of 75 Atlantic sailfish caught on live baits rigged with non-offset, slightly offset (4º), or severely offset circle hooks (15º), Prince et al. (2002) demonstrated that circle hooks possessing minor or no offset were highly associated with external hooking locations and little trauma while circle hooks with severe offsets were highly associated with deep hooking and bleeding. Our results are consistent with the observation that both non-offset circle hooks and circle hooks with minor offsets ($\leq$5º) have a very low incidence of deep hooking.

The differing degree of offset used in this study likewise did not significantly affect hook-induced trauma. The single white marlin to suffer hook-induced trauma resulting in bleeding (VZ 03-04) was caught on a 9/0 non-offset Eagle Claw hook that punctured the orbit but did not damage the eye. Ocular trauma has been reported for a variety of fishes caught on circle hooks including bluegill *Lepomis macrochirus*, Chinook salmon *Oncorhynchus tshawytsch*, Atlantic bluefin tuna *Thunnus thynnus*, striped marlin *Kajikia audax* (formerly *Tetrapturus audax*), and white marlin (Grover et al. 2002; Skomal et al. 2002; Cooke et al. 2003; Domeier et al. 2003; Kerstetter and Graves 2006). The size of circle hook relative to the fish may be an important consideration for optimum performance and conservation benefit of circle hooks (Cooke et al. 2003). Hook-induced eye trauma may be more likely with the use of larger circle hooks for the target quarry (Skomal et al. 2002), as was the case in our study. The distance between the hook eye and bend of the 9/0 Eagle Claw hook approximates the same distance to that between the corner of the mouth and the eye socket of most white marlin, and we did not observe eye trauma

with smaller hook sizes used in this study (6/0 - 8/0). Larger circle hooks (16/0 and 18/0) employed in the pelagic longline fishery also cause ocular trauma. Kerstetter and Graves (2006) observed eight of 19 white marlin (42.1%) to be hooked near the eye; six in the eye socket and two in the eye proper. Seven of the eight PSATs attached to these fish reported after 10 days and the data from all seven were consistent with survival over the tag deployment duration. The conservation benefits of circle hooks may be mitigated if eye damage resulting from the hook causes bleeding or impaired vision, which could increase the likelihood of delayed mortality due to reduced foraging capacity, increased risk of predation, and/or disease (Cooke and Suski 2004).

The lone mortality inferred in this study (MA 05-02) occurred on the fifth day after release. Most post-release mortalities in billfishes have occurred within the first 24 – 48 hours after release (Domeier et al. 2003; Horodysky and Graves 2005; Kerstetter and Graves 2006); however, delayed mortalities have been observed with PSATs. Reasons for longer term mortalities are poorly understood but may include physiological stress associated with catch and release, infection, predation and natural mortality (Wood et al. 1983; Bourke et al. 1987; Kerstetter et al. 2004). White marlin MA05-02 was caught on an Owner circle hook that lodged in the center of the lower jaw, presumably a non-lethal location. Circle hooks are defined as having the point perpendicular to the shaft (Cooke and Suski 2004), and by this definition the Owner hook is a circle hook (Figure 1). As has been suggested for "J" hooks, it is possible that the Owner circle hook may have damaged vital soft tissues before lodging in the lower jaw (Prince et al. 2002; Horodysky and Graves 2005). This same model of hook was also responsible for the single incidence of deep hooking observed in a white marlin (MA 06-12 was hooked in the palate). Prince et al. (2007) employed the same models of Owner and Eagle Claw hooks used in this study to evaluate the incidence of deep hooking in sailfish caught on live bait off the Florida east coast. They compared the performance of these hooks at four different drop-back treatments (0-5, 6-10, 11-15, and >15 sec) and reported a higher proportion of hooking in "undesirable locations" for the Owner hook at all four drop-back intervals, although the differences were not statistically significant. Combined, the trends noted in Prince et al. (2007) and the present study suggest that the shape of a circle hook may be more important for the maximum conservation benefit than previously appreciated.

There was no apparent difference in post-release mortality observed between white marlin caught on non-offset and slightly offset circle hooks. This finding is consistent with those of Domeier et al. (2003) who reported no significant difference in the rate of post-release survival of striped marlin caught on live baits with offset and slightly offset (5º) circle hooks. While not able to investigate post-release survival, Prince et al. (2002) noted a significant increase in the proportion of deep hooking events in sailfish caught on circle hooks with severe (15º) offsets versus those caught on circle hooks bearing no or slight offsets.

We observed no major differences in hooking location or post-release mortality among white marlin caught on the three models of circle hooks used in this study; however, the pooled circle hook data exhibited a significant decrease in the incidence of deep (internal) hooking, hook-induced trauma and post-release mortality relative to white marlin caught on "J" hooks under similar conditions as reported by Horodysky and Graves (2005). The same trends were evident in our expanded analysis of hooking location and fish condition of the 60 PSAT-tagged white marlin and additional observations made by the authors and recreational captains of 212 white

marlin, 132 sailfish, and 62 blue marlin. White marlin and sailfish both exhibited a significant decrease in the incidence of deep (internal) hooking, hook-induced trauma relative to conspecific caught on "J" hooks under similar conditions. No significant differences in hooking location or degree of trauma induced between circle and straight shank J hooks were evident in the blue marlin data collected by recreational captains, presumably due to low sample sizes. However, there was a definite trend in the blue marlin data for "J" hooks to result in deep hooking and hook induced trauma relative to circle hooks.

The results of this study are consistent with those of Prince et al. (2002) who reported that Pacific sailfish caught on dead natural baits rigged with circle hooks had significantly lower rates of deep hooking and bleeding relative to fish caught on baits rigged with "J" hooks. Similarly, Domeier et al. (2003) noted a trend for decreased rates of bleeding and post-release survival of striped marlin caught on live natural baits rigged with circle hooks relative to those caught on "J" hooks, although the differences were not statistically significant.

The difference in post-release mortality resulting from the use of circle hooks or "J" hooks in the white marlin recreational fishery can have a considerable impact on the overfished stock. We observed one mortality among the 59 white marlin caught on the three models of circle hooks combined (1.7% post-release mortality) in this study, versus the seven mortalities among the 20 white marlin caught on "J" hooks (35% post-release mortality) reported by Horodysky and Graves (2005). It is estimated that recreational anglers along the U.S. Atlantic and Gulf coasts catch between 4,000 and 8,000 white marlin each year (Goodyear and Prince, 2003), the vast majority of which are caught on natural baits with "J" hooks and released. If all white marlin taken in the U.S. recreational fishery were caught using only "J" hooks, a post-release mortality range of 1400 – 2800 individuals would result (Figure 3). Alternately, if this fishery were to use circle hooks exclusively, only 68 – 136 white marlin post-release mortalities would be expected.

On 1 January 2007 the U.S. National Marine Fisheries Service (NMFS) implemented a domestic management measure requiring the use of non-offset circle hooks for all participants using natural baits in Atlantic billfish tournaments (71 FR 58058; October 2, 2006). Between 1999 and 2004, the number of white marlin released each year by U.S. Atlantic billfish tournaments ranged from 614 to 2,207 individuals (NMFS Recreational Billfish Survey data cited in 71 FR 58058 October 2, 2006). If all white marlin caught in these tournaments were taken on "J" hooks that result in 35% post-release mortality (Horodysky and Graves 2005), one would expect 215 – 772 post-release mortalities (Figure 3). Alternately, if all tournament-released white marlin were caught on circle hooks that result in a post-release mortality of 1.7% (this study), one would expect 10 – 38 post-release mortalities. In other words, perfect compliance with the NMFS management measure during those years could have decreased white marlin post-release mortality in U.S. billfish tournaments by 205 to 734 individuals. It is likely that the conservation benefit accruing from the regulatory action would be enhanced by effort outside of registered billfish tournaments, as many offshore anglers that target white marlin and fish in billfish tournaments will likely use circle hooks when fishing in non-tournament conditions in order to improve their angling techniques.

Had NMFS elected to mandate the use of circle hooks in natural baits for all U.S. recreational offshore anglers rather than only in registered billfish tournaments, the reduction in white marlin

post-release fishing mortality resulting from the U.S. recreational fishery alone would range from 1,332 to 2,664 individuals. Large recreational fisheries for white marlin that release a significant fraction of the catch also exist in several other locations throughout the Atlantic (Venezuela, Dominican Republic, Brazil, Azores, and Morocco), and the use of circle hooks in these fisheries could result in a dramatic reduction of Atlantic-wide recreational fishing mortality on this overfished stock.

Not all U.S. offshore recreational anglers that catch white marlin target that species. Comments in response to a NMFS Issues and Options Document that included a circle hook requirement for all offshore anglers as an option, clearly demonstrated the concern of some anglers that implementation of a requirement to use circle hook with natural baits in areas where white marlin occur could reduce catches of potential target species such as tunas and dolphinfish. Data elucidating the performance of circle and "J" hooks in these fisheries are clearly needed to evaluate these anecdotal concerns. However, delays in the implementation of circle hook use by either voluntary or required measures to evaluate such claims will lead to continued use of "J" hooks in the U.S. recreational fishery and thus will perpetuate high levels of post-release mortality of white marlin.

It is our impression that enforcement of a domestic management measure requiring the use of circle hooks, whether in billfish tournaments or in all offshore recreational fisheries, will be difficult. As noted by Ditton (2002), the potential reduction in billfish recreational fishing mortality resulting from the use of circle hooks will only be realized if there is general acceptance of the conservation benefits of circle hooks by the recreational fishing community. Consequently, circle hook use may be more easily and effectively achieved through angler education and outreach programs rather than federal mandate.

The results of this study suggest that there are no major differences in hooking location, hook-induced trauma, or post-release survival among white marlin caught on three models of circle hooks that differ in shape and limited degree of offset and are commonly used in the white marlin recreational fishery. However, relative to white marlin caught on "J" hooks, these circle hook models result in a significant reduction of post-release mortality of white marlin caught in the recreational fishery. Current domestic management measures requiring the use of circle hooks with natural baits in Atlantic billfish tournaments will substantially reduce U.S. recreational fishing mortality of white marlin. However, the magnitude of this conservation benefit is but a fraction of what could be gained if circle hooks were used in all recreational fisheries that target this highly overfished resource throughout the Atlantic Ocean. Obtaining international consensus on such a measure through ICCAT is a possibility, although simply having member nations report their recreational catch and effort statistics has been a challenge for this regional fishery management organization. Nonetheless, precedent exists for some nations to proactively implement circle hook regulations; the governments of Guatemala and Costa Rica both require the mandatory use of circle hooks with natural baits in recreational fisheries targeting Pacific sailfish (Cooke and Suski 2004). Management measures aside, white marlin recreational fishing mortality may be most effectively reduced by convincing recreational anglers, who have already demonstrated a strong conservation ethic for white marlin by voluntarily promoting catch and release, to employ a terminal gear that maximizes survival of the released animal.

Acknowledgments
This study greatly benefited from the insights and efforts of Dr. Guy Harvey. We thank Dick Weber, Ken Neill, Bill Hudgins and Bob DeGabrielle for providing us time on their vessels at reduced or no cost. Captains Mike Adkins, Ken Neill, Bill Hudgins, Bubba Carter, Jimmy Grant, and O.B. O'Bryan not only put us on to fish, but expertly maneuvered their boats to facilitate deployment of the satellite tags. A special thanks to Luis Angel Rincon and the crew at Mango Marina for logistic support in Venezuela. We acknowledge the Eagle Claw Corporation for their donation of hooks. In addition to funding from the Gulf States Marine Fisheries Commission **(**Billfish-2005-11), this project was supported by funding from NMFS (NA04NMF4540411) and the NMFS Highly Migratory Species Management Division (NA05NMF4721107.

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Table 1. Summary of white marlin pop-up satellite archival tag (PSAT) deployment dates and locations.

Location	Dates of tagging	Number deployed
Mid-Atlantic Coast, USA (MA)	2005: September	7
	2006: July - September	12
Isla Mujeres, Mexico (MX)	2003: June	3
	2006: May - June	3
La Guaira, Venezuela (VZ)	2002: November	6
	2003: September - October	11
		7
	2005: November	11
	2006: September	

Table 2. Summary information for tagged white marlin released from recreational fishing gear in the western North Atlantic Ocean. Fight time includes the tagging process and resuscitation, where applicable. Individuals that required resuscitation before release are denoted with the symbol "^{R}". The hook location "branch" refers to the branchiostegal membrane. The first twenty white marlin in the table (VZ02, MX03, and VZ03) were previously reported in Horodysky and Graves (2005).

Fish ID	Est. wt (kg)	Fight time (min)	Hook Type	Hook Location	Bleed (Y/N)	Fate (L/D)	Movement (km / nmi)
VZ02-01	27	9	Mustad	jaw corner	N	L	219 / 118
VZ02-02	23	13	Mustad	lower jaw	N	L	148 / 80
VZ02-03^{R}	20	6	Mustad	jaw corner	N	L	128 / 69
VZ02-04	18	10	Mustad	lower jaw	N	L	117 / 63
VZ02-05	20	8	Mustad	lower jaw	N	L	124 / 67
VZ02-06	23	10	Mustad	lower jaw	N	L	181 / 98
MX03-01	27	16	Mustad	jaw corner	N	L	319 / 172
MX03-02	18	15	Eagle Claw	jaw corner	N	L	782 / 422
MX03-03^{R}	23	26	Eagle Claw	jaw corner	N	L	391 / 211
VZ03-01	20	4	Eagle Claw	jaw corner	N	L	157 / 85
VZ03-02	30	7	Eagle Claw	jaw corner	N	L	235 / 127
VZ03-03	23	13	Eagle Claw	jaw corner	N	L	30 / 16
VZ03-04	27	11	Eagle Claw	jaw corner	Y	L	211 / 114

VZ03-05	34	24	Eagle Claw	jaw corner	N	L	74 / 40
VZ03-06	23	10	Eagle Claw	jaw corner	N	L	91 / 49
VZ03-07	23	16	Eagle Claw	jaw corner	N	L	43 / 23
VZ03-08	23	8	Eagle Claw	jaw corner	N	L	72 / 39
VZ03-09	23	11	Eagle Claw	jaw corner	N	L	235 / 127
VZ03-14	20	15	Mustad	jaw corner	N	L	243 / 131
VZ03-15	20	9	Mustad	jaw corner	N	L	237 / 128
MA05-01	23	11	Owner	jaw corner	N	L	269 / 145
MA05-02	20	14	Owner	jaw corner	N	D	280 / 151
MA05-03	25	18	Owner	jaw corner	N	L	801 / 433
MA05-04	30	12	Owner	jaw corner	N	L	329 / 178
MA05-05	25	12	Owner	jaw corner	N	L	239 / 129
MA05-06	27	16	Owner	jaw corner	N	L	633 / 342
MA 05-07	23	5	Owner	jaw corner	N	L	123 / 82
VZ05-01	16	15	Owner	jaw corner	N	L	224 / 121
VZ05-02	20	40	Owner	jaw corner	N	L	102 / 55
VZ05-03	18	18	Owner	jaw corner	N	L	296 / 160
VZ05-04	20	26	Owner	branch	N	L	298 / 161
VZ05-05	18	5	Owner	jaw corner	N	L	172 / 93
VZ05-06	23	5	Eagle Claw	jaw corner	N	L	83 / 45

VZ05-07	20	8	Eagle Claw	jaw corner	N	L	233 / 126
MX06-01	23	20	Mustad	caudal fin	N	L	725 / 392
MX06-02	18	15	Mustad	jaw corner	N	L	239 / 129
MX06-03	25	21	Mustad	jaw corner	N	L	398 / 215
MA06-01	23	20	Eagle Claw	jaw corner	N	L	487 / 263
MA06-02	18	5	Eagle Claw	jaw corner	N	L	864 / 467
MA06-03	18	14	Mustad	jaw corner	N	L	408 / 272
MA06-04	20	15	Mustad	jaw corner	N	L	81 / 44
MA06-05	23	15	Owner	jaw corner	N	L	131 / 71
MA06-06	20	12	Owner	jaw corner	N	L	357 / 193
MA06-07	20	15	Mustad	jaw corner	N	L	93 / 50
MA06-08	23	15	Owner	jaw corner	N	L	152 / 82
MA06-09	20	40	Owner	jaw corner	N	L	754 / 408
MA06-10	23	15	Owner	jaw corner	N	L	131 / 71
MA06-11	23	12	Owner	jaw corner	N	L	592 / 320
MA06-12	25	10	Owner	deep	N	L	144 / 78
VZ06-01	23	9	Mustad	jaw corner	N	L	30 / 16
VZ06-02	23	14	Mustad	bill	N	L	122 / 66
VZ06-03	18	5	Mustad	jaw corner	N	L	180 / 97

VZ06-04	41	17	Eagle Claw	jaw corner	N	L	68 / 37
VZ06-05	20	7	Eagle Claw	jaw corner	N	L	109 / 59
VZ06-06	27	12	Eagle Claw	jaw corner	N	L	72 / 39
VZ06-07	23	5	Eagle Claw	jaw corner	N	L	22 / 12
VZ06-08	20	8	Mustad	jaw corner	N	L	157 / 85
VZ06-09	25	13	Mustad	jaw corner	N	-	310 / 168
VZ06-10	18	15	Mustad	jaw corner	N	L	276 / 149
VZ06-11	20	6	Owner	jaw corner	N	L	305 / 165

Table 3. Hooking location data (internal, external) and fish condition (bleeding, not bleeding) for observed catches of white marlin (n = 272), sailfish (n = 132), and blue marlin (n = 62) on "J" and circle hooks. Data include the 60 white marlin tagged with PSATs as well as other istiophorid catches witnessed by the authors or three cooperating recreational charter captains.

Species	Hook Type	Internal	External	Bleed	No Bleed
White marlin	Circle	4	196	2	198
	"J"	32	40	24	48
Sailfish	Circle	5	76	2	79
	"J"	21	30	17	44
Blue marlin	Circle	0	25	0	25
	"J"	5	32	4	33

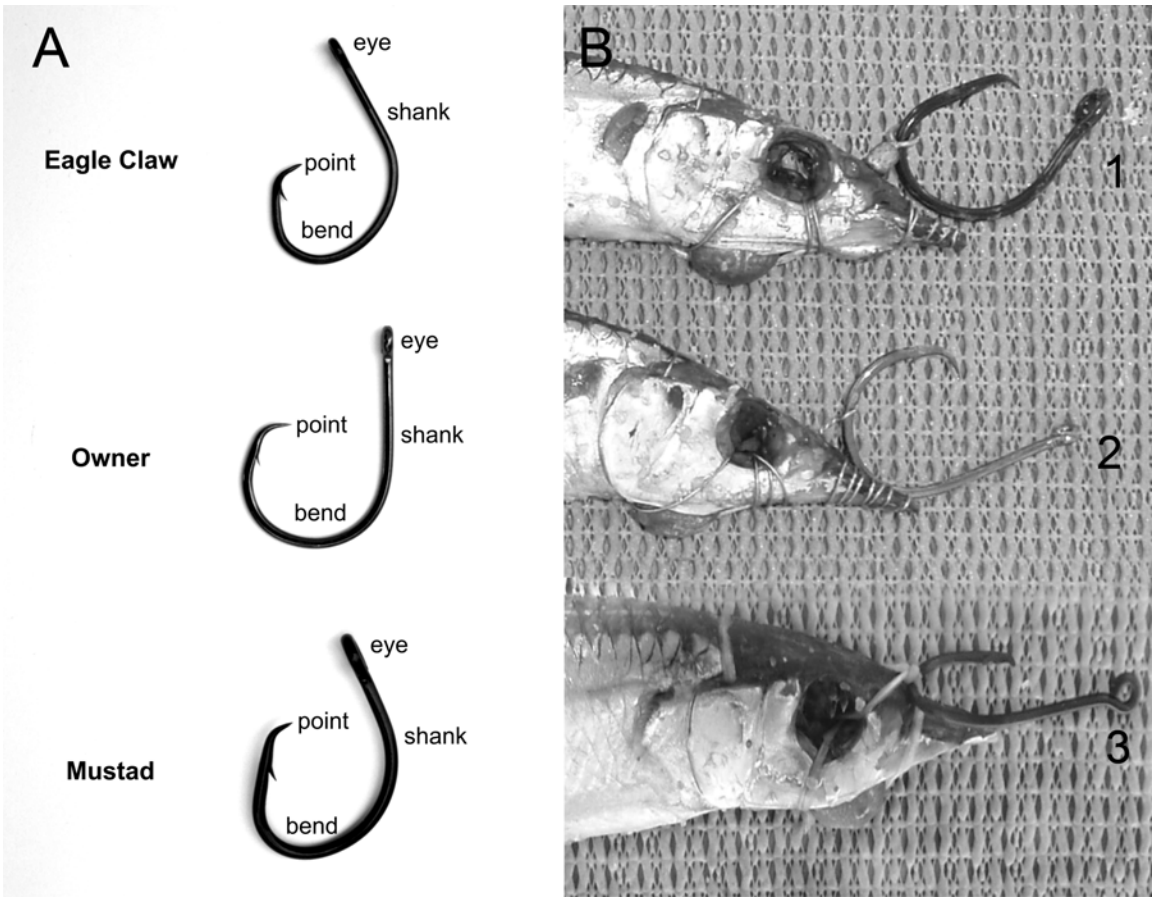

Figure 1. The models of circle hooks and types of rigging used in this study. A. Three models of circle hooks commonly used in the white marlin recreational fishery: Eagle Claw (model L2004EL, sizes 7/0 – 9/0), non-offset with a moderately elongated, circular bend; Owner (model 5379-161, size 6/0), non-offset with bend region similar to traditional straight-shank ("J") hooks; and Mustad (model C39952BL, size 7/0), 5º offset with a circular bend. B. Three methods of rigging commonly used to attach any of the three models of circle hooks to ballyhoo baits: (1) wire harness with a barrel swivel; (2) plain wire harness; and (3) a rigging floss harness.

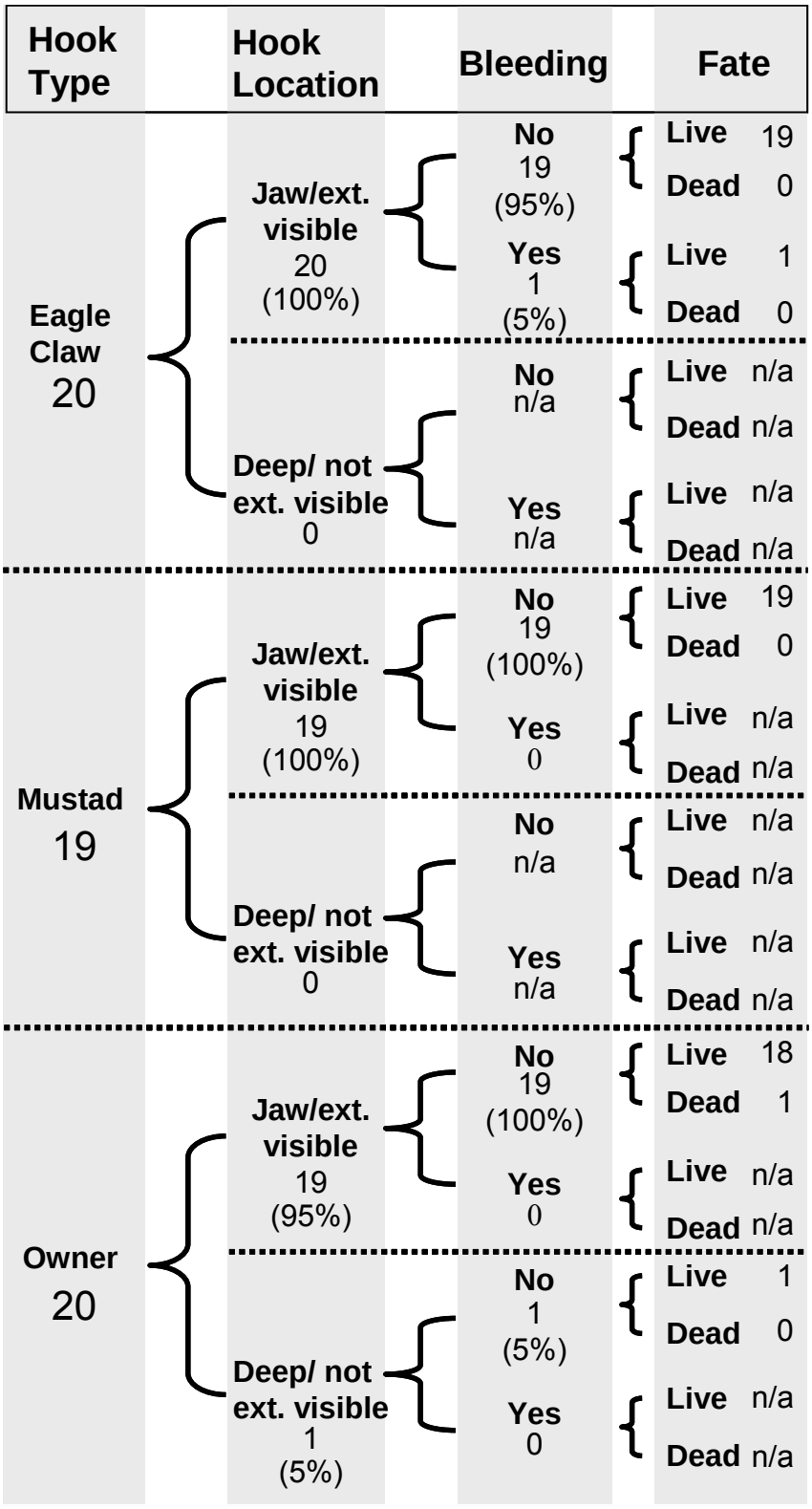

Figure 2. Effects of the three models of circle hooks on hooking location, bleeding, and fate of white marlin caught on three models of circle hooks. "N/a" refers to "not applicable".

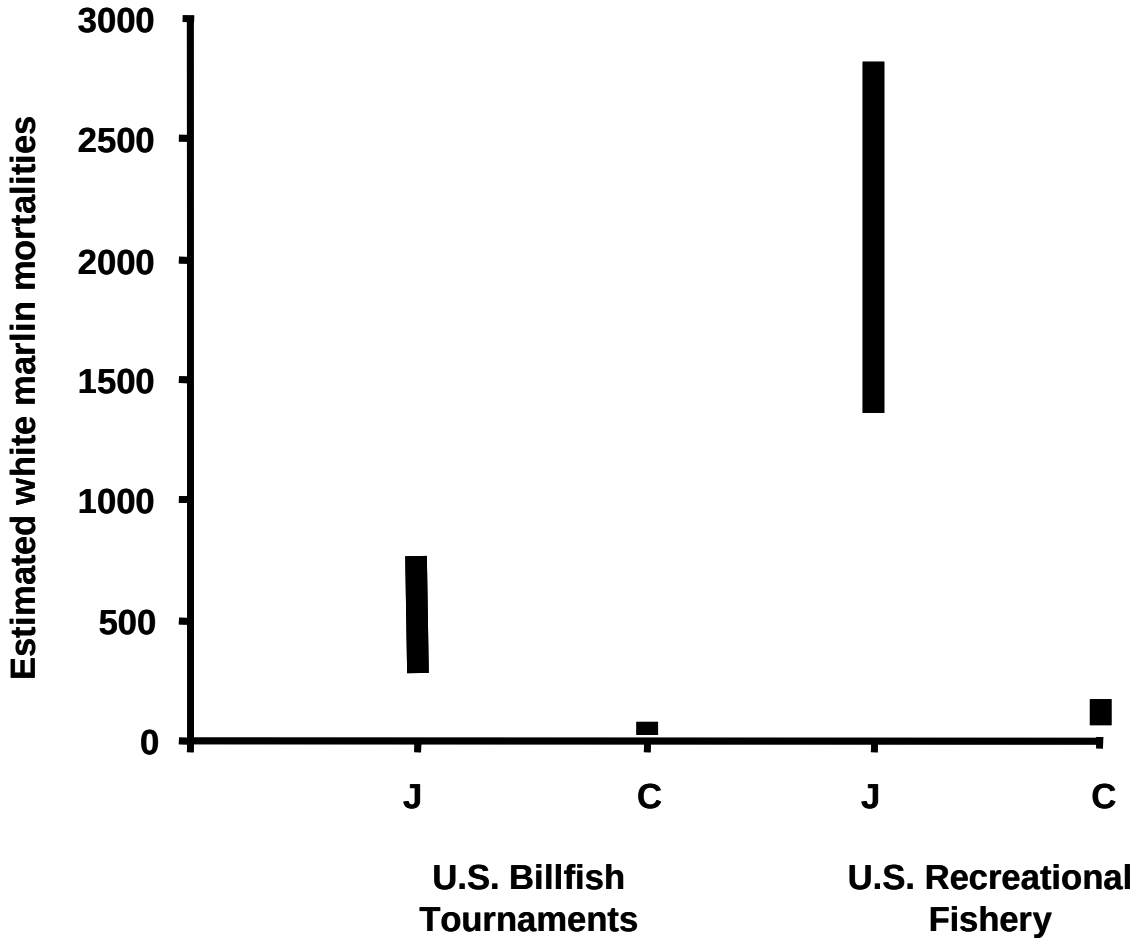

Figure 3. Ranges of estimated post-release mortalities of white marlin due to the use of "J" hooks and circle hooks in U.S. billfish tournaments and the overall U.S. recreational fishery. Estimated mortalities were calculated by applying the post-release mortality estimates for "J" hooks (35%: Horodysky and Graves 2005) and circle hooks (1.7%: this study) to the annual release estimates for U.S. billfish tournaments (614 – 2207 white marlin: NMFS Recreational Billfish Survey data) and for the overall U.S. recreational fishery (4000-8000 white marlin: Goodyear and Prince 2003).

AGE AND GROWTH, REPRODUCTION AND GENETICS OF BILLFISH IN GULF OF MEXICO WATERS OFF TEXAS

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Abstract
Samples from 61 billfish were collected for genetic analysis and samples from 23 landed billfish were available for age and reproductive biology analysis. Eight blue marlin had 2-5 growth rings, 11 sailfish had 2-3 growth rings and 1 white marlin had 2 growth rings. Examination of ovarian samples indicated 7 of 11 sailfish exhibited hydrated ova with relative fecundity estimates of 7 to 65 eggs/g gonad-free body weight. No hydrated blue or white marlin were found, but 7 of 10 blue marlin had vitellogenic ova. Sailfish with 2 growth rings were reproductively mature at 65 inches lower jaw fork length (LJFL) and blue marlin with 3 growth rings were mature at 100 inches LJFL. DNA was successfully obtained from 21 blue marlin, 13 white marlin and 19 sailfish. Twenty-five unique mtDNA haplotypes were examined for the three species, haplotype diversity was 0.71 for blue marlin and 0.89 for white marlin and sailfish.

Purpose
The objectives of the project were to Investigate the age, growth, reproductive status and genetic structure of istiophorid billfish caught and landed by fishing clubs and private anglers in Gulf of Mexico waters off Texas.

Atlantic billfish provide a unique sport fishery for U. S. recreational anglers. Recreational billfish anglers spend more than 2 billion dollars annually in the U. S. No alternate fishery can replace the high profile role of billfish in the recreational angling community, and anglers release 90 % of billfish caught. Recreational billfishing supports a range of marine industries including offshore sporting vessel construction and the manufacturing of fishing equipment, electronics and other accessories to outfit those vessels.

Age and growth estimates of Atlantic billfish are sparse and those that exist have relatively small sample sizes and would benefit from additional validation. Analysis of growth increments on hard parts is the most reliable of age estimation techniques used to age billfish. Age estimates are needed to accurately describe the age structure of existing billfish stocks. Male Atlantic billfish reach sexual maturity at smaller sizes than females, requiring separate age and growth estimates. Information on age at sexual maturity is also lacking and estimates of the proportion of mature females in the population are needed for effective management. Current genetic information indicates there is a single Atlantic population of blue (*Makaira nigricanus*) and white (*Tetrapturus albidus*) marlin, and east and west Atlantic populations of sailfish (*Istiophorus platypterus*).

Priority areas for research, as defined by the Gulf States Fishery Commission, addressed by this project are: age and growth studies, gender and maturation determination, and identification of a possible resident billfish population in the Gulf of Mexico.

Approach

Sample collection

The Texas Parks and Wildlife Department (TPWD) hired seasonal employees familiar with the billfishing community to contact fishing tournament directors, billfishing clubs and private billfish anglers to solicit their cooperation in obtaining hard parts (otoliths and spines), gonad samples and tissue samples from landed billfish and tissue samples from captured and released billfish. Tissue collection kits were distributed to volunteer anglers for the collection and preservation of fin tissue from released billfish.

Dorsal spines, heads, gonads and fin clips were collected from landed billfish with the permission of the fishermen. Billfish heads were transported to the Perry R. Bass Marine Fisheries Research Station (PRB) for removal of otoliths. The small size of billfish otoliths precluded efficient removal in the field as a dissecting microscope was necessary to identify otoliths and remove them from the inner ear tissue. Dorsal spines were sectioned and examined for growth rings. Gonads were weighed and ova examined to determine maturity stage, egg counts were made for gonads containing hydrated ova. Fin clips were used as a DNA source for DNA isolation and amplification prior to genetic analysis using microsatellites.

Tissue samples were collected from 59 billfish over the study and tissue samples were available from 2 additional fish collected in 2004 (Table 1). DNA was successfully isolated and analyzed from 53 of the tissue samples; 21 blue marlin, 13 white marlin and 19 sailfish (Table 2). Twenty-three billfish were landed, dorsal spines were collected from 20 fish and gonads were collected from 22 fish. Ten blue marlin, 12 sailfish and 1 white marlin were landed. All landed fish were females.

Age, Growth and Reproduction materials and methods

Second dorsal spines were removed from the billfish dorsal fin, and any adhering tissue was removed. Spines were placed in paper coin envelopes labeled with the sample number and dried at 36^{o} C for 24 hours to prevent decay of any residual tissue. Spines were serially sectioned from the base to a point about one third of the distance from the base to the tip of the spine using a high speed sectioning saw equipped with a 100mm diameter diamond blade. Spine sections were glued to a glass slide and examined with a dissecting microscope. Growth rings were counted and recorded for each spine and marginal increments from the last growth ring to the spine margin were measured (Table 3) using optical imaging software (Optimas 1996).

Billfish brain cases were removed from the skull and bisected longitudinally using a meat saw to expose the membranous labyrinth containing the otoliths. The membranous labyrinth was removed from the otic capsule and examined using a dissecting microscope to identify the otoliths. Otoliths were removed from the surrounding tissue with fine tipped forceps and stored dry in a plastic tube. Otoliths were examined for growth rings using both dissecting and compound light microscopes.

Gonads were weighed and an ovarian sample was placed in clearing solution (Brown-Peterson et al. 1988) and examined microscopically to determine oocyte maturity stage. Oocytes were assigned a maturity code from 1 to 5 based on the level of oocyte maturation with 1 representing primary oocytes and 5 assigned to hydrated ova. Cortical alveoli, vitellogenic and advanced

vitellogenic stages were assigned codes 2-4 respectively. Egg counts were made from all gonads that contained hydrated ova. A subsample was removed from the gonad, weighted to the nearest 0.01 g and shaken vigorously in a small glass tube containing water until individual ova were free from the gonadal tissue. The sample was then diluted to 1 liter with water and 10 5-ml subsamples were placed in a plankton counting wheel and enumerated. The total number of eggs in the gonad and the relative fecundity (eggs/gram of gonad free body weight) for each fish was then calculated.

Genetic analysis materials and methods

Clips of tissue were excised from the dorsal fin of each billfish specimen using short-angled utility shears, and stored in 70% ethanol. DNA was extracted from 20 mg of each fin clip using a Puregene® miniprep kit (Gentra Systems, Minneapolis, MN) following the manufacturer's instructions. Each sample was resuspended in a volume of 75 µl of DNA rehydration solution (provided with kit) and stored at 4 ^{o}C.

The cytochrome b gene region (cytb) of mitochondrial DNA (mtDNA) was chosen for examination, for two reasons. First, mtDNA evolves more quickly than nuclear DNA (Brown et al., 1982), and in particular cytb evolves quickly at 3rd (silent) codon positions (Irwin et al., 1991) in vertebrates, resulting in more genetic signal in comparison to other genomic regions. Second, this gene region is well characterized in blue marlin (Finnerty and Block, 1992) and has been assayed in other billfish (Finnerty and Block, 1995), such that results from this study may be compared to previous work. Polymerase chain reaction (PCR) was carried out to generate a 984 base pair (bp) target DNA fragment for sequencing, using the novel primers Billfish1f (5'-GAGAGCTTTGTTCTCTAGTC -3') and Billfish1r (5'- TTCCTCGCTATACACTACACCTC -3'), which were created after examining conserved regions in an alignment of billfish sequences downloaded from the Genbank[1] database.

Reaction conditions were as follows: an initial denaturation period of 4 min at 95 ^{o}C; followed by 5 cycles of denature (45 sec at 95 ^{o}C), anneal (1 min at 56 ^{o}C) and extension (2 min at 65 ^{o}C); followed by 30 cycles of denature (1 min at 95 ^{o}C), anneal (1 min at 52 ^{o}C) and extension (2 min at 72 ^{o}C); followed by a final extension of 10 min at 72 ^{o}C. Reactions were carried out using Ready-To-Go® PCR beads (Amersham Biosciences, Uppsala, Sweden); each reaction consisted of 1 µl template DNA (50 ng/µl), 1 Ready-To-Go® bead, and 24 µl of forward and reverse primer cocktail (0.4 µM standard primer concentration of each primer), for a total of 25 µl. PCR products were purified using QIAquick® PCR purification kits (Qiagen Inc., Valencia, California) and eluted in 30 µl of buffer EB (provided in kit).

DNA fragment concentration was estimated by running purified PCR products out on a 1.5% agarose gel with concentration standards. The PCR product concentration for each sample was adjusted to 50 ng for sequencing reactions, and the sequencing regime was 30 cycles of denature (96 ^{o}C for 20 seconds), annealing (50 ^{o}C for 20 seconds) and extension (60 ^{o}C for 4 minutes). Sequencing reactions were carried out in 10 µl volumes using DTCS Quick Start Master Mix

[1] National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov

[Last accessed on 11/16/2006]

(Beckman Coulter Inc., Fullerton, California), following the manufacturer's instructions, and using the novel primers Billfish2f (5'-CGACRGGCATGCCRCCGATTCAGG-3') and Billfish2r (5'- ACAACAGCCTTCACATCCGTAGC-3'). Sequencing samples were precipitated with 1/20 volume sodium acetate/EDTA and glycogen, and 2 volumes of 95% ethanol (per manufacturer's instructions), and spun at 3700 rpm for 30 minutes to form pellets. Pellets were rinsed twice with 70% ethanol, dried and rehydrated using sample loading solution (SLS, Beckman Coulter Inc.). Finally, sequences were electrophoresed and analyzed on a Beckman CEQ8000® capillary sequencer (Beckman Coulter Inc.) using default module parameters. Raw sequences were trimmed and edited manually, and forward and reverse traces for each sequence were aligned using the software package Sequencher v4.2 (Gene Codes Corp., Ann Arbor, Michigan).

Because of the small samples sizes for each species, genetic samples from both years of the study were pooled for analysis. Multiple alignments of sequence sets for each species were performed using Sequencher v4.2 (Gene Codes Corp.), and these alignments were then converted to NEXUS format (Maddison et al., 1997) for downstream analyses. For each species, haplotype diversity (the probability that any two individuals chosen at random will have different haplotypes, h) and nucleotide diversity (the probability that a specific base position in any two randomly chosen individuals will differ, π), was examined using the program DnaSP (Rozas et al., 2003). Subsequently, reduced median haplotype networks (Bandelt et al., 1995) for each species were constructed using the program Network[2] to diagram haplotype relatedness, and to examine the possibility of unsampled haplotypes.

Findings

Age, Growth and Reproduction results and discussion

Examination of dorsal spine sections revealed that spines exhibited a high amount of vascularization in the center of the spine. Vascularization obstructed attempts to make measurements of the growth rings, as a central area from which to initiate measurements could not be established consistently. As all billfish were landed in a relatively narrow seasonal window (June through August) we were unable to calculate time of growth ring formation based on marginal increment values. None of the billfish spines examined were in the process of forming growth rings and all spines exhibited a substantial marginal increment, indicating that growth ring formation is likely to occur after August for billfish in the Gulf of Mexico. Chiang et al. (2004) estimated Pacific Ocean sailfish formed an annulus from September to November using marginal increment ratio analysis.

All billfish landed had five or less growth rings, indicating billfish have a high growth rate and a relatively short life span. Chiang et al. (2003) found a maximum age of 8 years for Pacific Ocean sailfish and observed that sailfish reached one half of their estimated maximum size within the first year. Sailfish had 2 or 3 growth rings, 7 sailfish had 2 growth rings and 4 sailfish had 3 growth rings. Mean length of sailfish with 2 growth rings was marginally greater (66.3 in)

[2] Available online at Fluxus Engineering, www.fluxus-engineering.com

[Last accessed on 11/16/2006]

than mean length of sailfish with 3 growth rings (64.3 in.). Mean weight of sailfish with 2 growth rings (45.5 lbs) was less than mean weight of sailfish with 3 growth rings (49.3 lbs).

Blue marlin had 2-5 growth rings, 1 blue marlin had 2 growth rings, two fish had 3 growth rings, 3 fish had 4 growth rings and 2 fish had 5 growth rings. Mean length and weight of blue marlin with 3-5 growth rings increased as the number of growth rings increased. Blue marlin with 3 growth rings had mean length and weight of 101.6 in. and 354 lbs, fish with 4 growth rings had mean length and weight of 103.25 in. and 432.7 lbs and fish with 4 growth rings averaged 111.8 in. and 577 lbs. The blue marlin with 2 growth rings was 101.25 in. and 355 lbs. The single white marlin landed had 2 growth rings, was 70 inches and weighed 72 lbs.

Hydrated ova were identified in ovaries from 7 of 11 sailfish collected in 2005-06. Three of 6 sailfish collected in 2005 were hydrated, while 4 of 5 sailfish collected in 2006 were hydrated. Relative fecundity estimates for 6 of the 7 sailfish that had hydrated eggs ranged from 20 to 65 eggs/g gonad-free body weight, one fish had 7 eggs/g gonad-free body weight. Multiple oocyte stages were observed within an ovary from a single female, a characteristic of serial spawning fishes. The high percentage of hydrated females collected indicates sailfish may have a short spawning interval. Sailfish females appear to reach sexual maturity when they have 2-3 growth rings as 50 % of sailfish with 2 growth rings had hydrated ova, and 75 % of sailfish with 3 growth rings had hydrated ova.

No hydrated blue marlin were collected in either year. Ovaries of blue marlin collected in 2005 contained only primary growth oocytes, while fish collected in 2006 had ovaries with vitellogenic and late stage vitellogenic ova. Serafy et al. (2003) collected 2 to 17 day-old blue marlin larvae near the Bahama Islands in July 2000 indicating spawning occurred in at least late June through July. The presence of vitellogenic ova in some fish in late July and primary oocytes in others in late July to early August may indicate a transitional period from spawning to spent stage in late summer for blue marlin in the Gulf of Mexico.

Genetics results and discussion

The three species of billfish assayed had similar estimates of genetic diversity. In particular, haplotype diversity ranged between 0.71 (blue marlin) and 0.89 (sailfish and white marlin) (Table 3). These values are similar to those previously reported for blue marlin (Buonaccorsi et al., 2001) and overall (Graves and McDowell, 1995). A total of 25 unique billfish mtDNA haplotypes were identified among the three species, with sailfish having the highest number of haplotypes (10). Nucleotide diversity ranged from 0.0026 (white marlin) to 0.0074 (blue marlin).

Haplotype networks for each species were fully resolved, and contained no ambiguity or reticulation. For blue marlin, two divergent haplotype clades were identified (Fig. 1). The first contained five haplotypes from eight individuals; the second was nine mutations away from the first, and was less variable, with only three haplotypes found in 13 individuals. This two-clade structure is similar to that previously identified in blue marlin (Finnerty and Block, 1992; Buonaccorsi et al., 2001; Graves and McDowell, 2003), and typically represents an Atlantic-only clade, and a ubiquitous clade found both in the Atlantic and Pacific. Similarly, the sailfish haplotype network indicated a two-clade structure (Fig. 2). The first clade contained three

haplotypes found in five individuals. This clade was separated from the second clade by three mutations. The second clade consisted of seven haplotypes from 14 individuals, and also included two unsampled haplotypes, indicated as median-vectors on the haplotype network. Again, a two-clade structure has been previously reported in sailfish (Graves and McDowell, 2003), with the two clades representing Atlantic and Atlantic/Pacific distributions. Finally, the white marlin haplotype network indicated only a single lineage (Fig. 3), although a single highly divergent haplotype was identified that was four mutational steps away from the main body of haplotypes. Only 13 white marlin samples were sequenced, so it is unclear whether this divergent individual is a) a representative of a rarely sampled, divergent group of white marlin, or b) the result of four rare (and unsampled in this study) haplotypes between the divergent haplotype and the main body of haplotypes. Graves and McDowell (2003) indicated only a single mtDNA lineage of white marlin in the Atlantic, but also sampled a single individual which had a divergent haplotype.

Billfish are typically wide-spread in their distribution, and have the potential for extreme long-distance migration (Buonaccorsi et al., 1999). As a result, haplotypes for many species are shared among oceans (Finnerty and Block, 1992; Buonaccorsi et al., 2001; Graves and McDowell, 2003). Thus, one would predict that billfish have relatively high levels of genetic diversity. Indeed, all three species assayed here had haplotype diversity over 0.70, and estimates of diversity reported here are similar to those previously reported for blue marlin (Finnerty and Block, 1992; Graves and McDowell, 1995; Buonaccorsi et al., 2001), sailfish (Graves and McDowell, 1995), white marlin (Graves and McDowell, 1995, 2003) as well as those reported for striped marlin in the Pacific (Graves and McDowell, 1994; Graves and McDowell, 1995). In addition, haplotype networks for all three species are similar in general structure to those identified using control region sequences (Graves and McDowell, 2003). In the case of blue marlin, the two-clade structure recovered here has also been previously demonstrated in other mtDNA loci (Finnerty and Block, 1992; Buonaccorsi et al. 2001) as well as in nuclear microsatellites (Buonaccorsi et al. 2001). The concordance of this study with previous results examining global distributions is an indication that populations of billfish currently residing in the western Gulf of Mexico may be representative of Atlantic populations in general, and that blue marlin, and perhaps sailfish, residing in the Gulf are influenced by some measure of gene flow from Pacific populations. However, this point must be tempered with the understanding that the genetic clades recovered here were not directly compared to previous results; thus although the cladograms from this study closely resemble those of previous studies in general structure, we cannot explicitly say that both the “Atlantic” and “Ubiquitous” clades from earlier studies are represented in our results.

The sample sizes used in this study were large enough to roughly characterize the genetics of billfish in the western Gulf. However, these samples are not large enough to make a reasonable estimate of haplotype frequencies with any certainty, and have likely resulted in a number of rare haplotypes going unsampled in each species. An indication that this is the case is the two unsampled median haplotypes in the sailfish network, and the long branch separating a single haplotype in the white marlin network. Thus, the relative frequency of occurrence of two distinctive lineages in blue marlin and sailfish cannot be reasonably estimated, and measures of h and π for all three species would likely change at least slightly if sample sizes were enlarged.

This is beyond the scope of this study; however small sample sizes must be taken into account if these data are to be used in a management setting.

Conclusions

The project objectives were largely attained, as we were able to conduct age, reproductive and genetic analysis for fish sampled. Growth rings were identified in spines and should serve as an accurate representation of age based on previous reports (Chiang et al. 2004). The collection of sailfish with hydrated ova allowed us to make estimates of batch fecundity and age at maturity. Collection of blue marlin with vitellogenic ova provided estimates of age at maturity and possibly some insight to spawning season length. We were able to successfully conduct genetic analysis that indicates a resident population of billfish does not exist in the Gulf of Mexico. Small sample sizes of individual species did preclude the generation of meaningful growth curves.

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Table 1. Sample number, sample date, lower jaw fork length (LJFL), weight, type of capture and samples collected for three species of billfish collected off the Texas coast.

#	Species	Date	LJFL(in)	Wt. (lb)	status	tissue	spine	gonad	otolith
1	White Marlin	6/24/05	59	40	released	fin			
2	Blue Marlin	6/11/2005	74 - 78	150 - 165	released	fin			
3	Blue Marlin	7/3/2005	95	321	released	fin			
4	White Marlin	6/11/2005	70	72	landed	fin	yes	yes	yes
5	White Marlin	6/24/2005	nd	75	released	fin			
6	Blue Marlin	7/31/2004	100	nd	released	fin			
7	Sailfish	7/31/2004	68	nd	released	fin			
8	Sailfish	7/15/2005	69	58	landed	fin	yes	yes	yes
9	Sailfish	7/23/2005	59	30	released	fin			
10	Sailfish	7/8/2005	60'	60	released	fin			
11	Sailfish	7/30/2005	na	58	released	fin			
12	Sailfish	7/30/2005	na	54	released	fin			
13	Blue Marlin	7/22/2005	111.3	621	landed	fin	yes	yes	yes
14	Sailfish	7/23/2005	65.75	53.5	landed	fin	yes	yes	yes
15	Sailfish	7/23/2005	64	38.6	landed	fin	yes	yes	yes
16	Blue marlin	8/5/2005	72	75	released	fin			
17	White Marlin	8/6/2005	54	40	released	fin			
18	White Marlin	8/6/2005	58	45	released	fin			
19	White Marlin	8/5/2005	55	40	released	gill			
20	Sailfish	8/6/2005	58	40	released	fin			
21	Sailfish	8/20/2005	63	40	released	fin			
22	White Marlin	7/8/2005	48	40-45	released	fin			
23	Sailfish	9/10/2005	nd	40	released	fin			
24	Blue Marlin	8/5/2005	96	250	released	fin			
25	Sailfish	7/8/2005	58	40	released	fin			
26	Sailfish	7/28/2005	60	40	released	fin			
27	Blue Marlin	8/5/2005	108.25	463	landed	fin	yes	yes	yes
28	Sailfish	8/5/2005	62	nd	landed	fin	yes	yes	yes
29	Sailfish	8/5/2005	66	48.2	landed	fin	yes	yes	yes
30	Sailfish	8/5/2005	65.33	41.15	landed	fin	yes	yes	yes
31	Blue Marlin	8/6/2005	98.5	nd	released	fin			
32	Blue Marlin	8/6/2005	101.25	355	landed	fin	yes	yes	yes
33	White Marlin	8/20/2005	36	18	released	fin			
34	Blue Marlin	7/15/2006	99	375	released	fin			
35	Blue marlin	7/21/2006	112.25	533	landed	fin	yes	yes	yes
36	Blue Marlin	7/22/2006	101.75	368	landed	muscle	yes	yes	yes
#	Species	Date	LJFL(in)	Wt. (lb)	status	tissue	spine	gonad	otolith
37	Sailfish	7/2/2006	nd	30	released	fin			

38	Sailfish	8/25/2006	73.25	67	landed	fin	yes	yes	yes
39	Blue Marlin	7/14/2006	101.5	433	landed	fin	yes	yes	yes
40	Blue Marlin	7/14/2006	101.5	340	landed	fin	yes	yes	yes
41	Blue Marlin	7/14/2006	100	402	landed	fin	yes	yes	yes
42	Blue Marlin	7/15/2006	101	312	landed	fin	no	yes	yes
43	Blue Marlin	7/15/2006	111.5	583	landed	fin	no	yes	yes
44	omitted								
45	Sailfish	9/14/2006	83	nd	released	fin			
46	Blue Marlin	8/5/2006	60	85	released	fin			
47	Blue Marlin	8/4/2006	70	150	released	fin			
48	Blue Marlin	9/2/2006	95	250	released	fin			
49	Blue Marlin	9/3/2006	97	270	released	fin			
50	Blue Marlin	9/16/2006	98	325	released	fin			
51	White Marlin	8/5/2006	38	25	released	fin			
52	White Marlin	9/30/2006	66	45	released	fin			
53	White Marlin	9/30/2006	68	55	released	fin			
54	Sailfish	6/5/2006	65	45	released	fin			
55	Sailfish	7/29/2006	62.625	33	landed	fin	yes	no	yes
56	Sailfish	8/19/2006	64	40	released	fin			
57	Blue Marlin	10/29/2006	94	200	released	fin			
58	White Marlin	10/29/2006	75	85	released	fin			
59	Sailfish	8/4/2006	63.75	46.2	landed	fin	yes	yes	yes
60	Sailfish	8/5/2006	65.5	37	landed	fin	yes	yes	yes
61	Sailfish	8/5/2006	65	43.5	landed	fin	yes	yes	yes
62	Sailfish	8/4/2006	65	43	landed	fin	no	yes	yes

Table 2. Sample sizes (*n*), haplotype diversity (*h*) and nucleotide diversity (π) of blue marlin (*Makaira nigricans*), white marlin (*Tetrapturus albidus*) and sailfish (*Istiophorus platypterus*) in the western Gulf of Mexico.

	n *(individuals)*	n *(base pairs)*	*No. haplotypes*	h	π
Blue Marlin	21	716	8	0.71	0.0074
White Marlin	13	745	7	0.89	0.0026
Sailfish	19	755	10	0.89	0.0043

Table 3. Size, age and reproductive status data for billfish collected off the Texas coast (LJFL is lower jaw fork length, MI is spine marginal increment, GSI is gonadosomatic index. RF(a) and RF(p) are relative fecundity from the anterior and posterior gonad, respectively, * indicates a mean value).

Species	#	Date	Gonad Wt. (g)	LJLF (inches)	Weight (lbs)	rings	MI (mm)	ova stage	GSI	RF(p)	RF(a)	RF
White marlin	4	6/11/2005	184	70	72	2	0.196	1	0.57			
Sailfish	8	7/15/2005	1863	69	58	2	0.144	5	7.61			7.2
Blue marlin	13	7/22/2005	1523.5	111.3	621	5	0.204	1	0.54			
Sailfish	14	7/28/2005	1006.5	65.75	53.5	3	0.193	5	4.32			36.5
Sailfish	15	7/23/2005	95	64	38.6	2	0.105	1	0.55			
Blue marlin	27	8/5/2005	790	108.25	463	4	0.205	1	0.38			
Sailfish	28	8/5/2005	75.5	62	nd	3	0.189	1	nd			
Sailfish	29	8/5/2005	1507	66	48.2	3	0.238	5	7.40			43.0
Sailfish	30	8/5/2005	287	65.3	41.15	2	0.149	2	1.56			
Blue marlin	32	8/6/2005	1021.5	101.25	355	2	0.193	1	0.64			
Blue marlin	35	7/21/2006	1040	112.25	533	5	0.287	4	0.43			
Blue marlin	36	7/22/2006	860	101.75	368	3	0.198	4	0.52			
Sailfish	38	8/25/2006	2225	73.25	67	2	0.169	5	7.89	25.1	42.1	33.6
Blue marlin	39	7/14/2006	1420	101.5	433	4	0.202	3	0.73			
Blue marlin	40	7/14/2006	800	101.5	340	3	0.254	3	0.52			
Blue marlin	41	7/14/2006	810	100	402	4	0.131	3	0.45			
Blue marlin	42	7/15/2006	1165	101	312	nd		3	0.83			
Blue marlin	43	7/15/2006	1020	111.5	583	nd		3	0.39			
Sailfish	55	7/29/2006	nd	62.625	33	2	0.238	nd	nd			
Sailfish	59	8/4/2006	2345	63.75	46.2	3	0.158	5	12.59	25.6	20.5	23.0
Sailfish	60	8/5/2006	105	65.5	37	2	0.203	1	0.63			
Sailfish	61	8/5/2006	1600	65	43.5	2	0.153	5	8.82	52.3	46.9	49.6
Sailfish	62	8/4/2006	2200	65	43	nd		5	12.70	64.8	48.6	56.7

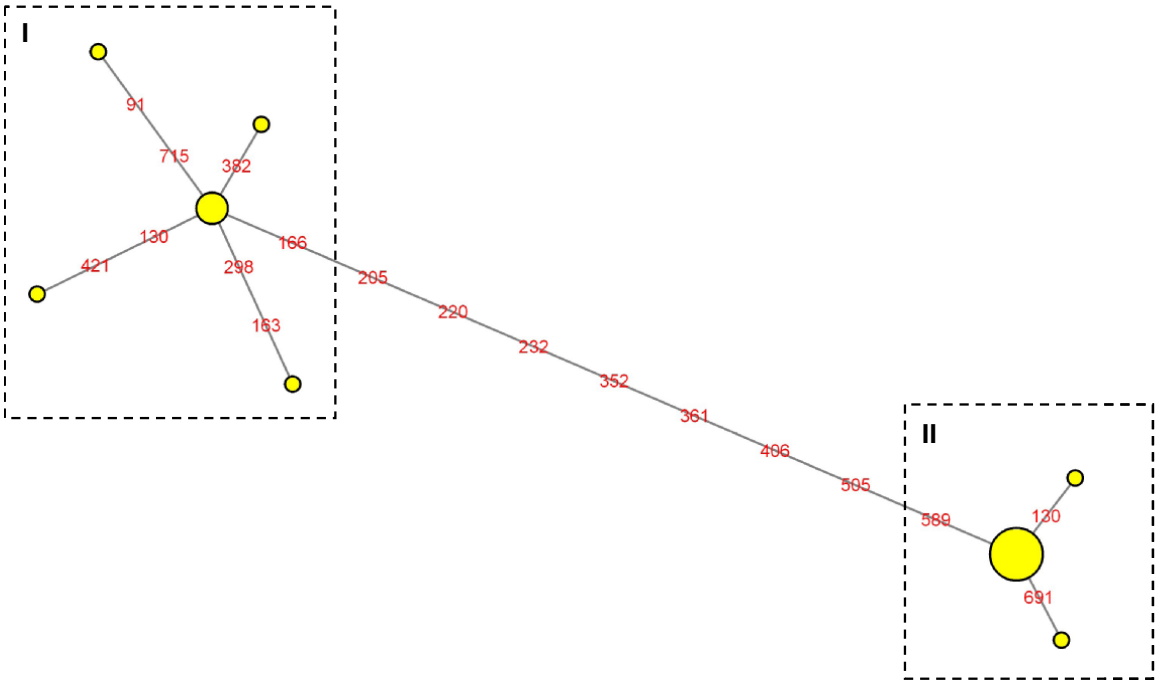

Figure 1. Network of haplotypes collected for blue marlin (*Makaira nigricans*) in the Gulf of Mexico. Mutations are indicated by numbers along network links (numbers correspond to mtDNA fragment position), and the frequency of a haplotype is reflected in the relative size of its circle (yellow circles). Roman numerals indicate clades I and II, respectively, in the order in which they are discussed in the text.

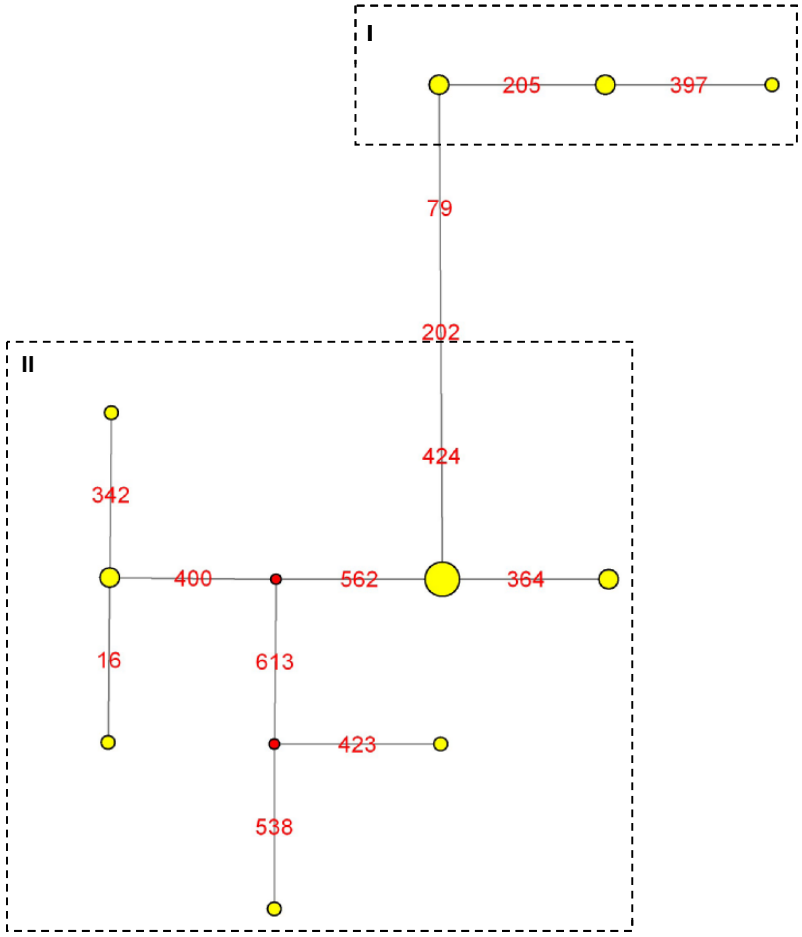

Figure 2. Network of haplotypes collected for sailfish (*Istiophorus platypterus*) in the Gulf of Mexico. Mutations are indicated by numbers along network links (numbers correspond to mtDNA fragment position), and the frequency of a haplotype is reflected in the relative size of its circle (yellow circles). Unsampled haplotypes are represented by small median vectors (red circles). Roman numerals indicate clades I and II, respectively, in the order in which they are discussed in the text.

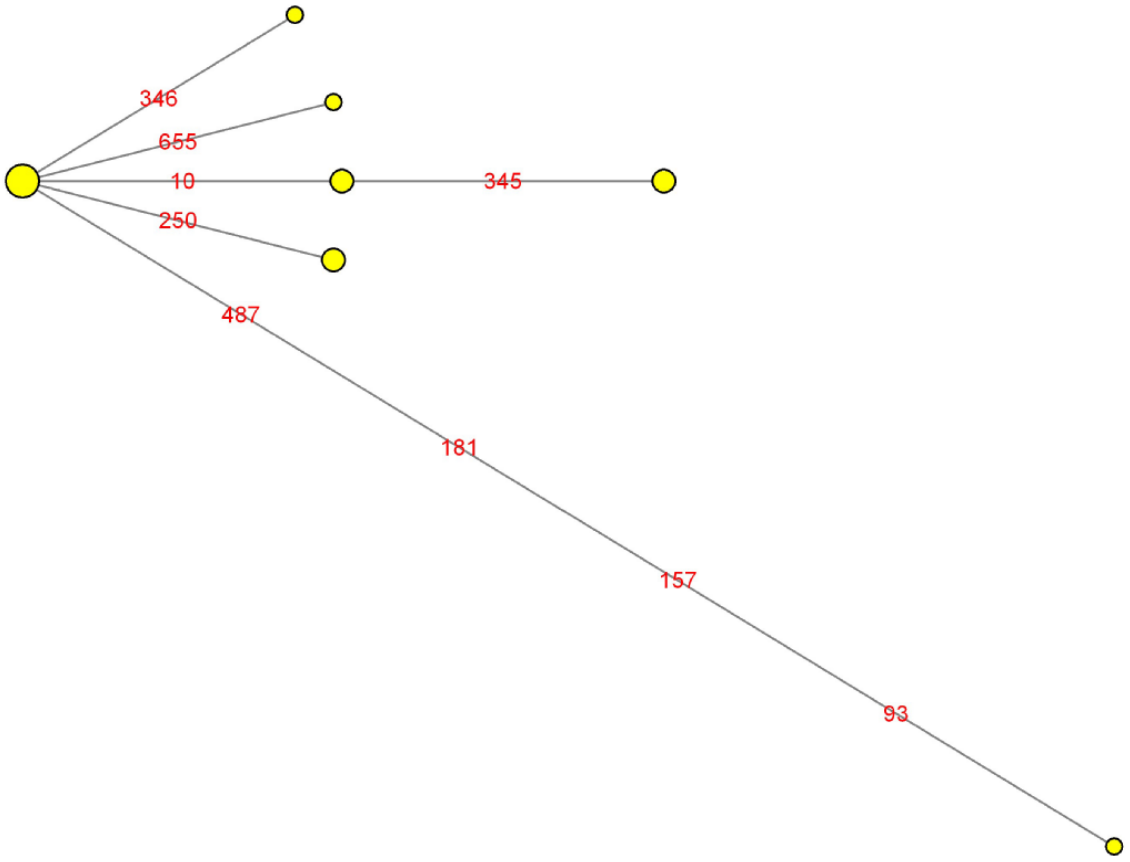

Figure 3. Network of haplotypes collected for white marlin (*Tetrapturus albidus*) in the Gulf of Mexico. Mutations are indicated by numbers along network links (numbers correspond to mtDNA fragment position), and the frequency of a haplotype is reflected in the relative size of its circle (yellow circles).

An Atlantic-wide study of age and growth of Atlantic marlins

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Abstract

Fin spines, fish size, and sex were collected from both blue and white marlin from several fisheries throughout the Atlantic. Cross-sections of the fin spines revealed alternating rings of opaque and translucent material. Relative marginal increment analysis of white marlin spine sections suggested that the translucent rings were formed once a year in the fall in this species but trends in marginal increment were not strong enough to consider frequency of ring formation is fully validated. The core of the spine in both species was composed of vascular tissue, which may have obscured the earliest rings in larger individuals. To correct for this phenomenon, k-means cluster analysis of the ring radii of white marlin was performed. The results indicated that rings had been lost in older fish, and that ring radii differed between males and females. The fitted growth model for white marlin also showed sexual dimorphism in growth parameters. Very few of the sampled fish were age one or two, suggesting that the majority of the fish available to the longline and artisanal fleets in our sample locations are older than two years of age.

Introduction

Atlantic White Marlin is endemic to the Atlantic where it constitutes a single stock. According to recent assessments (ICCAT 2003), the white marlin is the most depleted of all species of billfish. Atlantic blue marlin is also depleted below the level that produces MSY and is therefore overfished and suffering overfishing. The urgent need to recover these two stocks prompted the International Commission for the Conservation of Atlantic Tunas (ICCAT) to recommend large reductions in the harvest of these species by a sequence of management measures (ICCAT 2003). It will take years (ICCAT 2003), however, for data to be available to evaluate the success of the application of these measures, and for any reductions in harvest to translate into measurable increases in marlin abundance.

The lack of basic data on life history and catch of marlins has lead to stock assessments with a high degree of uncertainty. Both the level of overfishing and the responses of stocks to exploitation cannot be precisely explained. One of the major contributors for this uncertainty is the lack of information on growth rates and population age structure (ICCAT 2003). Knowledge of such parameters will open the way for age-structured population modeling, a technique that uses the available fishery data more effectively than the presently used aggregate biomass models. More precise and accurate assessments of the current status and productivity of the stock will help the design and implementation of management regulations aimed at reversing the depletion of marlin stocks. In addition, age and growth information can also enhance our understanding of natural survival, fishing mortality, spawning and migration. Knowledge of all these population parameters is directly relevant to the design of management measures such as minimum sizes and protected areas.

The wide geographical presence of adult marlin throughout the tropical and temperate Atlantic makes studies thatare able to give a stock level view of the life history of these fish more difficult. All previous studies have a limited spatial and/or time scale. This, combined with the lack of a population-level model for the migration of marlins hampers our ability to extend the results of local studies to the entire stock. Most of our knowledge of life history parameters for marlins therefore remains limited to certain areas and periods of time (ICCAT 2004).

There has been no comprehensive study of the age and growth of marlins, although a few studies have produced age estimates for Atlantic marlins in localized areas or more comprehensive studies for other related billfish species such as sailfish and swordfish. Wilson (1984) estimated growth curves for blue and white marlin from a small sample of otoliths obtained from recreational fisheries in the US. Age and growth models have been developed using hard parts for Atlantic swordfish (Ehrhardt 1992, Arocha, et al. 2002), and for several species of billfish, including the sailfish stock from the western Atlantic (Hedgepeth and Jolley 1983), western Pacific (Chiang, et al. 2004), the eastern pacific (Alvarado-Castillo and Felix-Uraga 1995, Vidaurri-Sotelo et al 2001), and also for the Pacific, for striped marlin (Melo-Barrera, et al. 2003) and black marlin (Speare 2003). Ehrhardt and Delevaux (2006) modeled the growth of Atlantic sailfish using tag-recapture data. This paper describes a comprehensive collection of fin spine samples of blue marlin and white marlin conducted throughout the Atlantic the ageing methodology and the results of the aging and growth estimation for white marlin.

Methods

Sample collection

Samples were collected from fishing operations (commercial, artisanal, and recreational) in Venezuela, Brazil, Martinique, the US, and the Gulf of Guinea. Observers, either at port or at sea, depending on the fishery, sampled white marlin and blue marlin that were caught in the course of normal fishery operations. For each fish, observers recorded the length (measured as lower-jaw fork length, in centimeters), the sex (determined from gonad morphology), and information on the time and point of capture (see Appendix 1 for the sampling protocol). Fins were frozen and taken to the appropriate lab for transport.

Sample processing and reading

Sample processing was done in Brazil and Venezuela for samples that originated in those countries, and in the US for samples from other countries. The second and third anal fin spines were removed, cleaned, and dried. Sections (~0.45 mm thick) were taken at a distance of one-half the width of the condyle base (Fig. 1) using a low-speed saw as described in Ehrhardt *et al.* (1992).

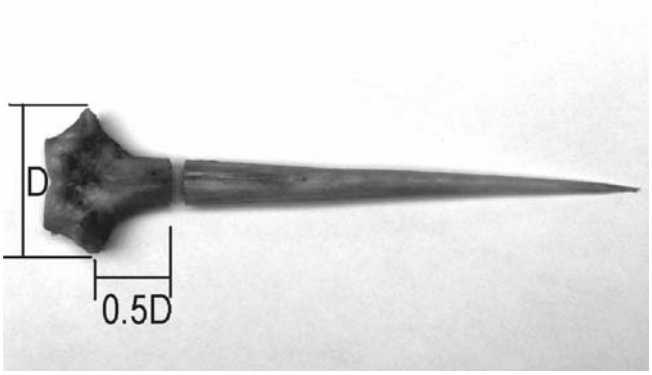


Figure 1: Second anal fin spine of a white marlin showing location of spine sections taken (Note: width of section not to scale)

Digital images of the spine sections (Fig. 2) were taken under magnification, and the Sigma Scan Pro software (Systat Software Inc, Richmond, CA, USA) was used to measure the cross-sectional area as well as the radius of the spine and of the vascularized tissue.

A standardized reading methodology was developed (Kopf *et al.* in press) with two other labs in the Pacific that are working on the age and growth of striped marlin (*Tetrapturus audax*), the sister species to the white marlin, with the intention of making cross-species comparisons of growth rates more meaningful.

We examined the precision of age determination of a subset of the white marlin samples. A total of 250 spines were chosen (approximately 25% of available samples), based in part on size: we included all of the samples from fish smaller than 140cm LJFL, all of the samples from fish larger than 180cm LJFL, and 25 males and 25 females chosen randomly from each of the 10cm length bins between 140cm and 180cm. Samples were distributed as evenly as possible over the size ranges to investigate whether precision changes as fish grow larger and the rings form closer together. In the end, 4 of the chosen samples were discarded because of problems with the images. This subset of images was aged independently by three to four trained readers, and the assigned ages were compared using the Average Percent Error (APE) and Coefficient of Variation (CV) statistics (Campana, *et al.* 2001).

In addition, all samples were reread by a single individual to ensure precision over time. When there was a disagreement in the assigned ages, the spine was read again and discarded if no consensus could be reached.

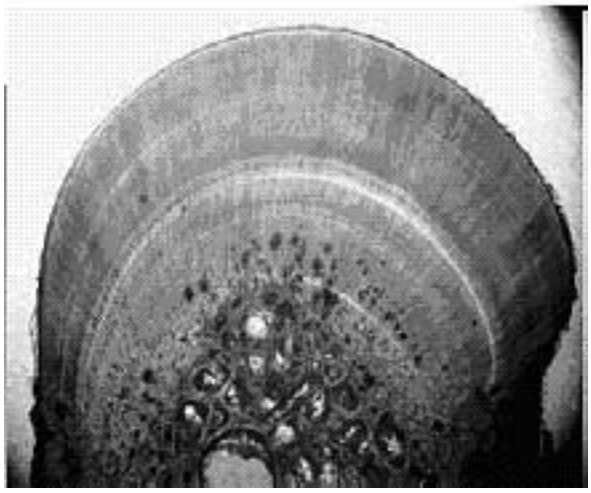


Figure 2: Digitized image of a fin spine section from a blue marlin showing the translucent bands considered as annual rings.

Correction for vascularization

Plotting the radius of the first visible ring against the radius of vascularization reveals an increasing trend; also, the radius of vascularization in larger spines is greater than the radius of the first visible ring in some of the smaller spines (Fig. 3). This suggests that rings are indeed being lost to vascularization over time. K-means clustering (MacQueen, 1967) was used to group the observed ring radii into clusters of similar values, which were interpreted as ages. This technique relies on the same assumption as mean ring radius method, namely that rings form at approximately the same distance in all spines. K-means clustering is an iterative, non-hierarchical clustering method that creates k clusters and assigns every observation into a cluster. The centers of the clusters and the observations in each cluster are recalculated at every step in order to minimize the within-cluster sum of squares, to result in k clusters whose members are more like each other than like the other observations in the data set.

Because this method is sensitive to the initial values of cluster centers, the clusters were seeded empirically, using the mean ring radius technique applied to a random subset of the data. Spines with a radius of vascularization less than the radius of the smallest observed ring were used to calculate the mean and standard deviation for the first three rings. Ring counts for spines whose radius of vascularization was less than the radius of the third ring were corrected for vascularization and used to estimate the mean and standard deviation for the next several rings. This process was repeated until all rings in the sub-sample had been assigned an age. The mean radius for each age was used to seed the cluster analysis.

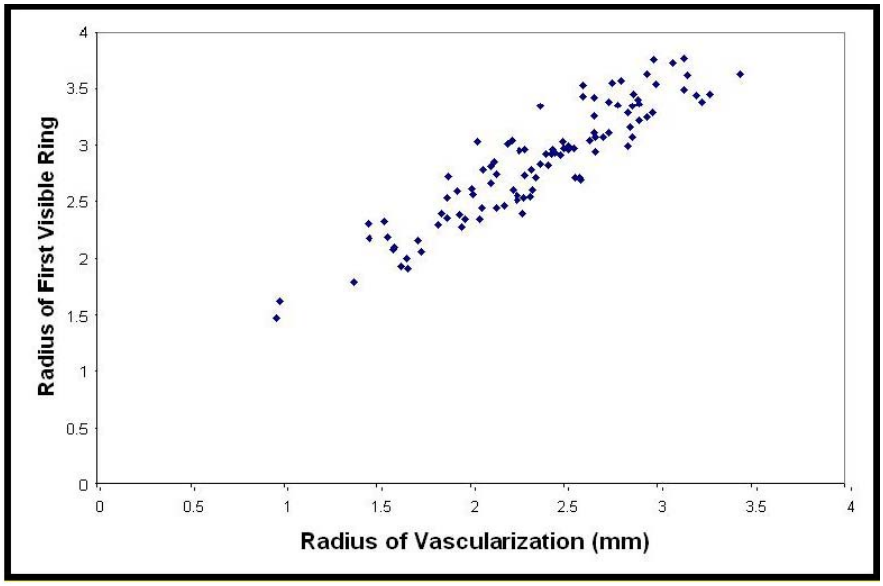


Figure 3: Radius of first visible ring vs. radius of vascularization

RMI analysis

To determine the periodicity of ring formation, relative marginal increment analysis is used (Campana, 2001). The marginal increment is calculated as the distance from the outer edge of the last ring formed to the edge of the spine section (Fig. 4). The relative marginal increment is calculated by dividing the marginal increment by the width of the last complete increment formed. This ratio is at a minimum just after a new ring has been formed and at a maximum just before a new ring is formed.

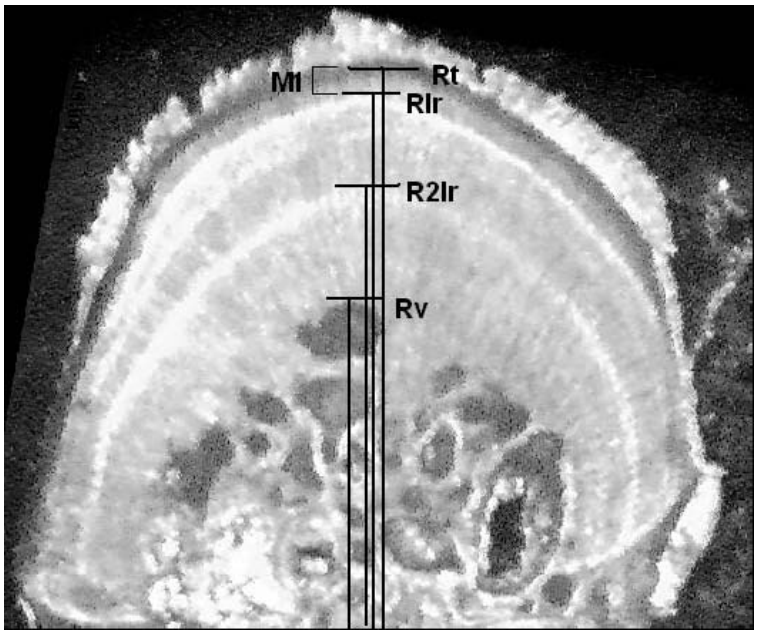


Figure 4: Spine measurements. Rt=Total radius of spine. R of last ring. R2lr=Radius to last ring

(in this case, since there are only two rings, it is also the radius of the first visible ring). Rv=Radius of vascularization. MI=Marginal increment (Rt-Rlr).

Fitting of growth curves and comparisons of growth curves by sex and location
Since the Brazilian white marlin samples had a significantly smaller lower jaw-fork length, than the Venezuelan samples, and since separate northern and southern stocks have been proposed for this species, we compared the Venezuelan samples to a subset of the Brazilian samples (males collected from latitudes south of 5^{o}S, the proposed stock boundary). For the Venezuelan samples, a total of 679 fish (390 females and 289) were used. A von Bertalanffy growth equation was fitted to the data set using proc nlin in SAS v.9.1, for each sex separately and for the pooled data. For the pooled data, ages were assigned to each fish in the sex-specific cluster analysis. The estimate parameters for each data set are shown in Table 2; separating by sex did reduce the sum of squares, suggesting there is sexual dimorphism in growth.

There were very few fish in the youngest age groups. To test the effects of these limited (and possibly biased) data points on the analysis, the growth curve was refitted, omitting age 1 fish, age 1 and 2 fish, and age 1, 2, and 3 fish. In all these cases, t_0 was constrained to zero; this is biologically reasonable, as size at hatching is very small and can be approximated as zero.

A total of 76 males were collected from south of 5^{o}S and included in this analysis. Ages were assigned using the k-means clustering technique. Three cases were examined for the Venezuelan and Brazilian male samples: (1) ages assigned and data fitted together in one model, (2) ages assigned for each location separately, but the model fitted together, and (3) age assignment and model fitting done separately for both sets of samples. Model fitting was done with proc nlin in SAS v.9.1.

Results
The distribution of marlins extends throughout the Atlantic Ocean but is concentrated in the tropical western Atlantic. Although we attempted to collect samples from the whole geographic range where these fish are found we were restricted by our ability to obtain samples from only some of the fleets that harvest this fish (Figure 5). This difficulty is due to a combination of factors including remoteness of fishing fleet operations, lack of contacts with certain fleets and the unusual occurrence of marlins in the catches of most fleets. The bulk of our samples were collected in three fisheries: the artisanal fishery occurring within Venezuelan Caribbean waters, and the pelagic longline fisheries of Venezuela and Brazil where marlins are a common bycatch. Additional samples were collected from the Martinique artisanal fleet, the US recreational fleet and the Spanish tropical purse seine fleet. The locations where marlins were collected for spine extraction reflect the activities of such fleets (Figure 5).

The second objective of the collection effort was to obtain enough spines of a wide range of fish sizes at different times of the year. Again the sizes collected were determined by the sizes available from the sampled fleets and reflect the availability of fish to those fleets (Figure 6). Length frequency distributions of both marlins were unimodal with most white marlins been sampled been between 140 cm and 180 cm long. Similarly most blue marlins sampled were between 175 cm and 245 cm. For both species, as expected, males sampled were on average smaller than females.

Collections of samples did take place throughout the year, however, three times as many samples of white marlin were obtained in the second part of the year than in the first half. Blue marlin samples were more evenly distributed, however twice as many blue marlins were sampled in the period between November and April than on the months of May to October (Figure 7).

We collected both the second and the third spine initially, but an analysis of 278 pairs of spines from white marlin indicates that the second spine is significantly more vascularized than the third spine (Student's t-test, $p < 0.001$, JMP IN software, SAS Institute). Thus, we used the third spine for aging.

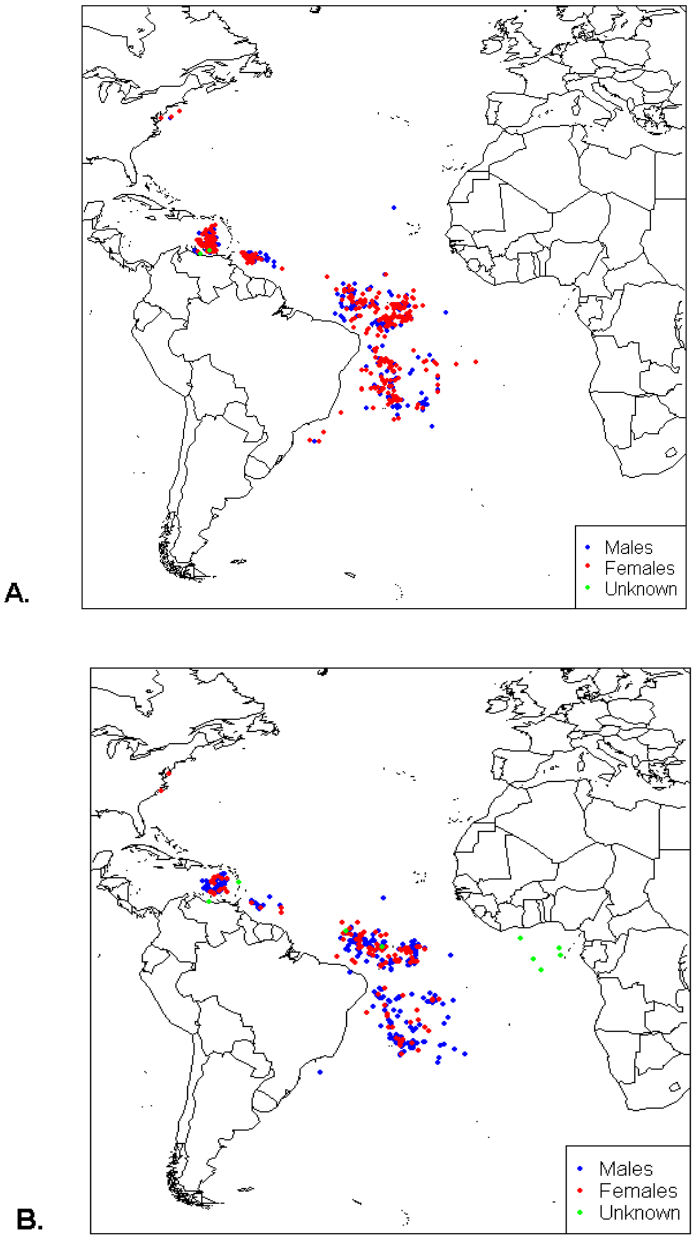
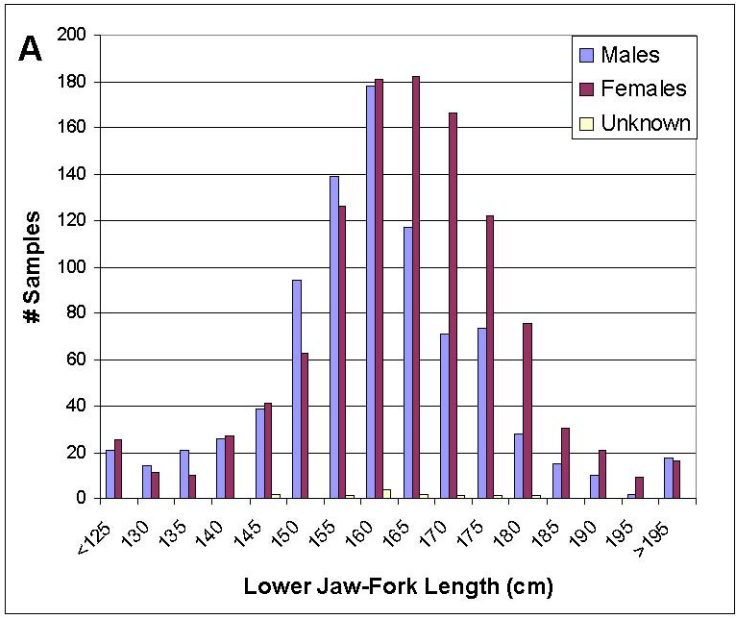


Figure 5: Location of samples collected for white marlin (A) and blue marlin (B)



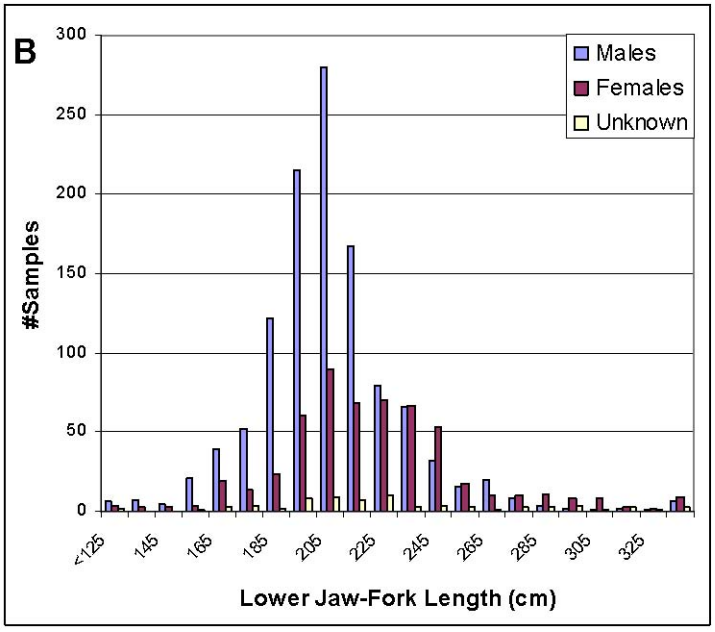


Figure 6: Total samples by sex and size for white marlin (A) and blue marlin (B)

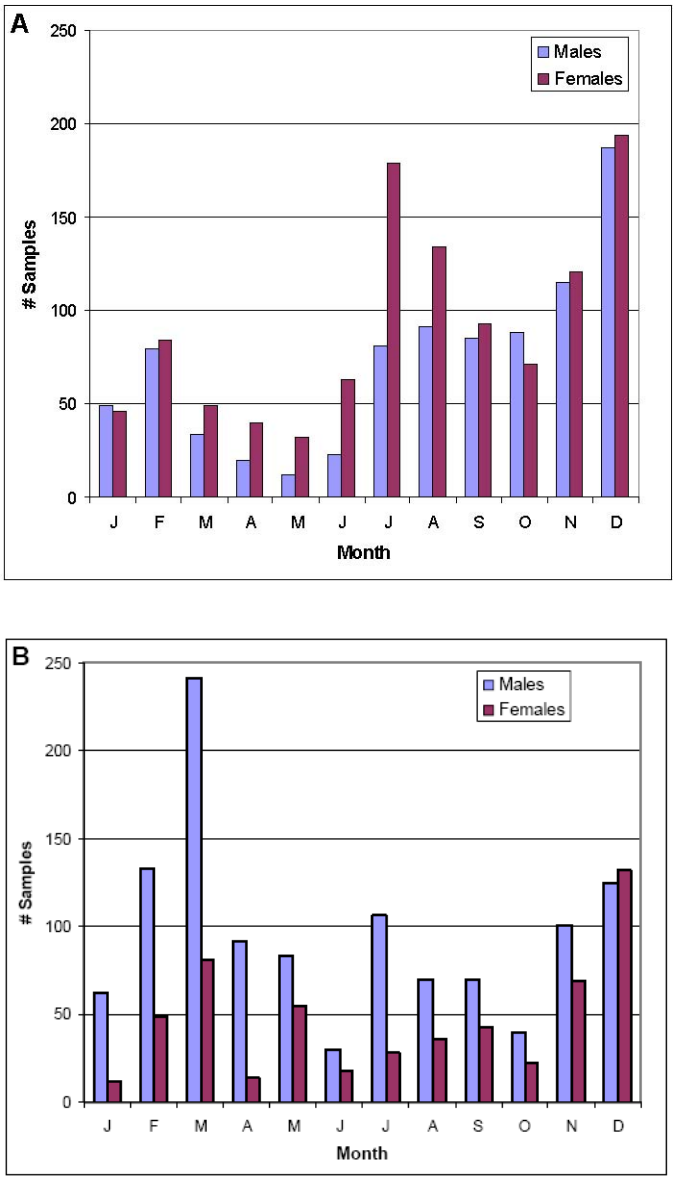


Figure 7: Total samples by month and sex for white marlins (A) and blue marlin (B)

Analysis of precision of age determination was completed for a subsample of white marlin. Overall, the average percent error (APE) was 20.8% and the coefficient of variation (CV) was 27.52%. Table 1 presents these indices of average precision broken down by sex and size. The smaller size ranges had the highest APE and CV values, indicating the lowest precision. These two indices are higher than reported in recent literature for billfish [e.g., Sun *et al.* (2002), Chiang *et al.* (2004)], although they do overlap with the range of values for a recent study of growth in yellowfin tuna (Leesa and Duarte-Neto, 2004). Our values are also comparable to the upper end of values reported in Compana's (2001) review of 117 published precision estimates.

Table 1: Summary of precision indices by size and sex

	APE		CV	
Size Range	**Males**	**Females**	**Males**	**Females**
<140 cm	26.26 (N=11)	39.82 (N=12)	34.431	50.84
140-149cm	23.17 (N=25)	27.80 (N=24)	30.39	37.17
150-159cm	15.23 (N=25)	17.44 (N=25)	20.76	22.54
160-169cm	22.58 (N=25)	14.27 (N=24)	30.34	18.71
170-179cm	28.57 (N=24)	13.73 (N=25)	38.14	18.15
≥180cm	25.00 (N=1)	13.08 (N=25)	28.87	17.66
All	22.74 (N=111)	19.21 (N=135)	30.27	25.26

N indicates sample size and is the same for both APE and CV.

Previously published age and growth studies in billfish have corrected for rings lost due to vascularization of fin spines by calculating the mean radius for the first several rings, using spines from smaller fish with minimal vascularization, and then estimating the number of rings lost from the radius of vascularized tissue. White marlin fin spines appear to be more extensively vascularized that previously studied billfish (Drew *et al.* 2006), and we have a low number of samples from younger animals, making this technique less reliable. Instead, we have used k-means clustering to assign rings to clusters representing ages.

This technique was applied to both the Venezuelan and Brazilian samples separately, and to the pooled sample. The separate samples had similar values for ring radius at age, particularly for the youngest ages, and separating the samples reduced the total sum of squares only slightly, from 4.75 to 4.63.

Most of the fish sampled are ages 3 to 8 but the youngest is one and the oldest thirteen. Assigned ages are consistent with the number of years at large for a subset of tagged fish that were recaptured and sampled.

For the Venezuelan white marlin samples, the relative marginal increment analysis was performed by season, rather than month, since the late spring months (April-June) were not well-sampled (due to the movement patterns of the fish). The analysis suggested a trend towards a minimum in the third quarter of the year, late summer to early fall for both sexes (Figure 8). Brazilian samples suggested a similar trend when analyzed separately, but like the Venezuelan samples had a high degree of variation (Figure 9).

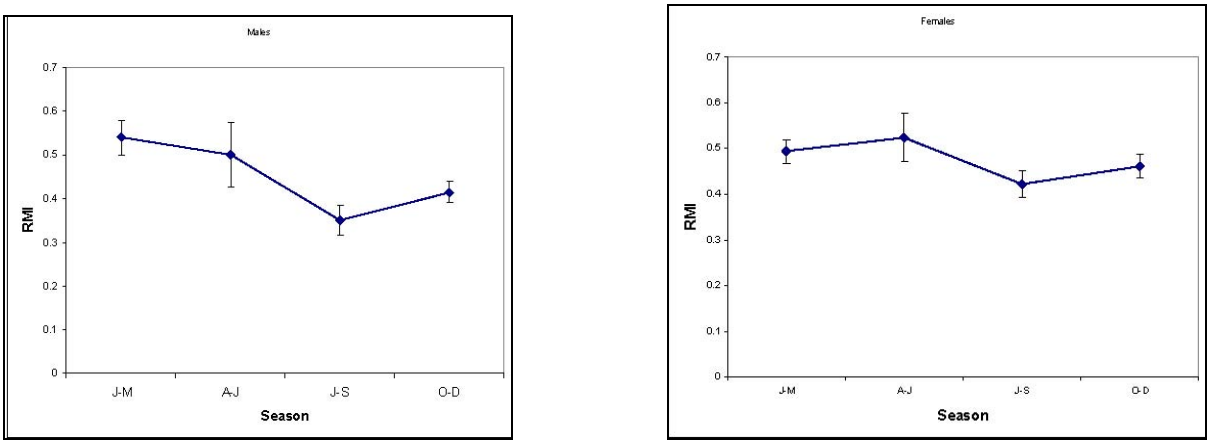


Figure 8: Relative marginal increments by season for male (left) and female (right) white marlin. Error bars indicate 95% confidence intervals.

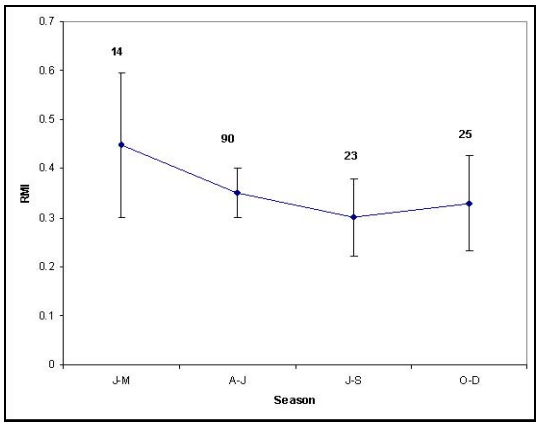


Figure 9: Relative marginal increment analysis for Brazilian samples (males and females combined due to low sample size). Error bars represent 95% confidence intervals and the numbers indicate sample size.

The results from these analyses suggest that RMI may not be a good tool for validation of ages for white marlin. This does not mean that the marks are not annual, but could be a reflection of a lack of synchrony in the formation of the mark. If the formation of the mark happens at different times of the year in individual marlin it could explain the patterns of seasonal RMI observed in this study. Although the ages estimated for the few individuals for which we have tagged information are consistent with the times at liberty, our estimated ages can not be considered to be fully validated.

We compared growth models fitted separately for each set of samples (separated by location and sex) to a growth model fitted for the combined samples (Table 2). As can be seen from Fig.10, there are few observations in the youngest age classes in the Venezuelan samples and a high degree of variance in the size-at-age data for the older age classes, which makes the model more difficult to fit. The predicted values for the youngest, underrepresented age classes are biologically unrealistic for the pooled data in particular. Omitting the youngest age groups did

not change the results for males, but did produce more biologically realistic parameters in females (Table 2, Fig. 10).

The similar means and high variance in the lengths of older age classes may be due to size-based selectivity of the sampling process (either due to the selectivity of the fishing gear, or more likely, the segment of the population available to the fishery), measurement error, or high natural variance in the population.

Three cases were examined for the Venezuelan and Brazilian male samples: (1) ages assigned and data fitted together in one model, (2) ages assigned for each location separately, but the model fitted together, and (3) age assignment and model fitting done separately for both sets of samples. Assigning ages to the Brazilian and Venezuelan samples separately reduced the residual sum of squares from 72,459.37 to 47,299.84 in the fitted growth curve for the pooled data. Fitting growth curves to the samples separately reduced the summed residual sum of squares to 45,535.43, but the parameters for all models were very similar suggesting no differences exist in the growth rates of white marlins sampled north or south of 5°S, supporting the notion that they are part of the same population (Figures 11 and 12).

Table 2: Estimates of growth parameters for white marlin by sex and as a function of which ages are included in the fit. L_∞ is reported in centimeters.

Data used	All ages	All ages (t_0=0)	Ages 2+	Ages 3+	Ages 4+
Males (VZ)	$L_\infty = 160.6$ $K = 0.76$ $t_0 = -0.45$	$L_\infty = 160.1$ $K = 0.90$ $t_0 = 0$	$L_\infty = 160.1$ $K = 0.90$ $t_0 = 0$	$L_\infty = 160.2$ $K = 0.88$ $t_0 = 0$	$L_\infty = 160.2$ $K = 0.88$ $t_0 = 0$
Females (VZ)	$L_\infty = 275.9$ $K = 0.031$ $t_0 = -22.15$	$L_\infty = 165.6$ $K = 0.78$ $t_0 = 0$	$L_\infty = 166.4$ $K = 0.72$ $t_0 = 0$	$L_\infty = 167.6$ $K = 0.64$ $t_0 = 0$	$L_\infty = 169.7$ $K = 0.55$ $t_0 = 0$
Both sexes (VZ)	$L_\infty = 178.2$ $K = 0.17$ $t_0 = -7.91$	$L_\infty = 163.2$ $K = 0.83$ $t_0 = 0$	$L_\infty = 163.6$ $K = 0.79$ $t_0 = 0$	$L_\infty = 164.4$ $K = 0.72$ $t_0 = 0$	$L_\infty = 165.5$ $K = 0.64$ $t_0 = 0$
Males (BR)	$L_\infty = 161.9$ $K = 0.35$ $t_0 = -2.62$				
Males (BR + VZ, ages assigned together)	$L_\infty = 160.9$ $K = 1.04$ $t_0 = -0.12$				
Males (BR + VZ ages assigned separately)	$L_\infty = 160.0$ $K = 0.58$ $t_0 = -1.13$				

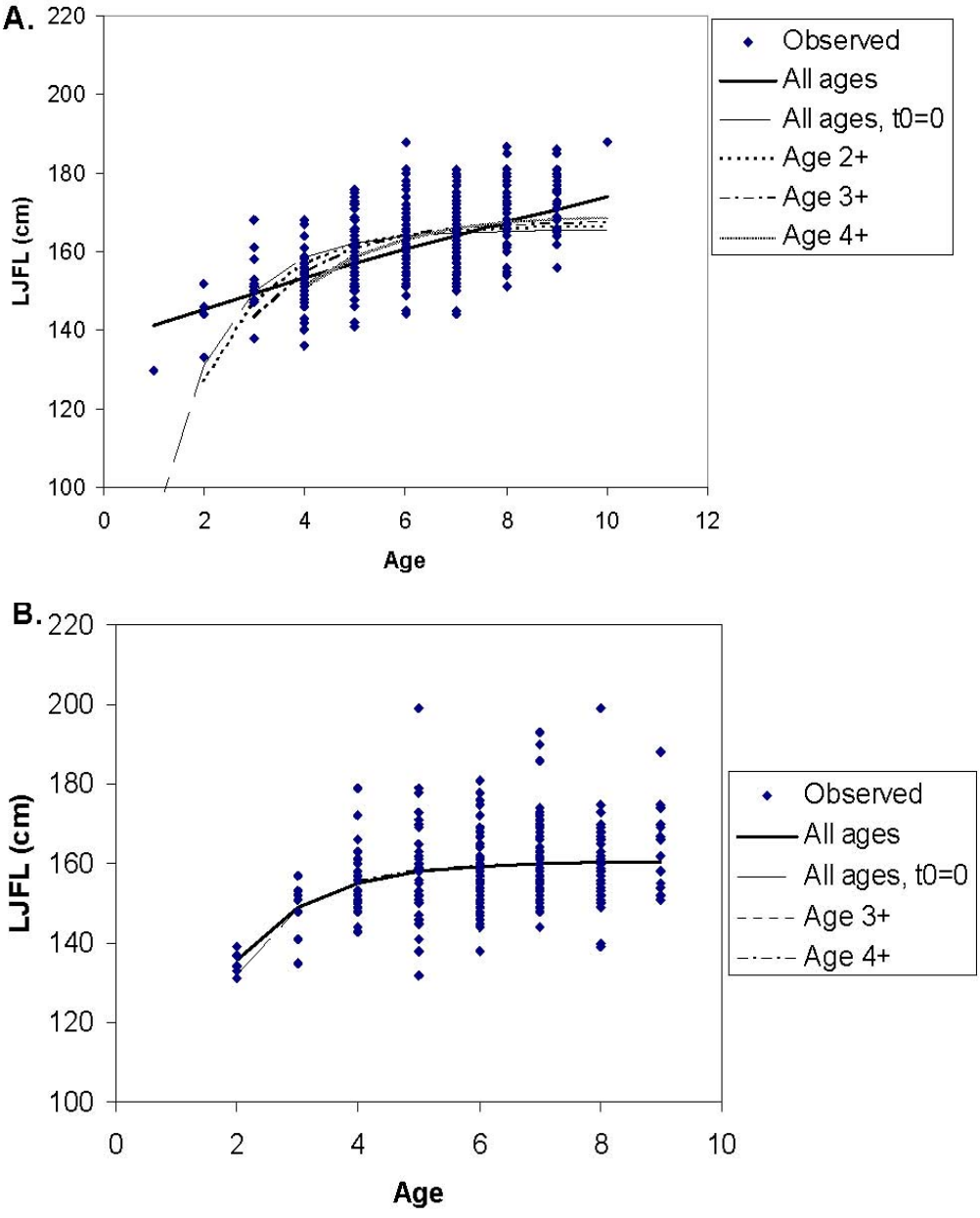


Figure 10: Observed and predicted values of length (LJFL) vs. age for white marlin females (A), males (B), and both sexes (C). Predicted values have been fitted by successively omitting the younger, less represented ages, as indicated in the legends.

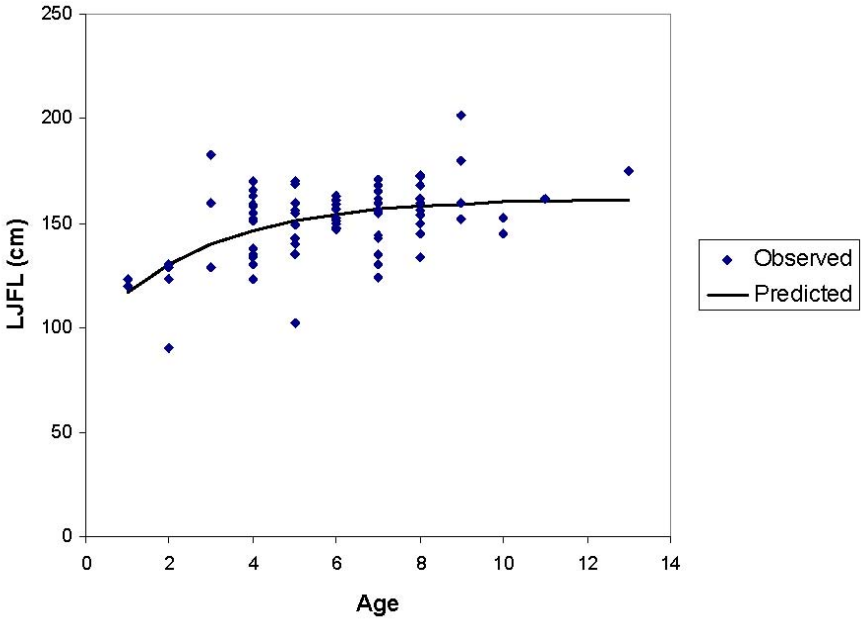


Figure 11: Observed and predicted values of lengths for the Brazilian white marlin samples (males caught below 5°S).

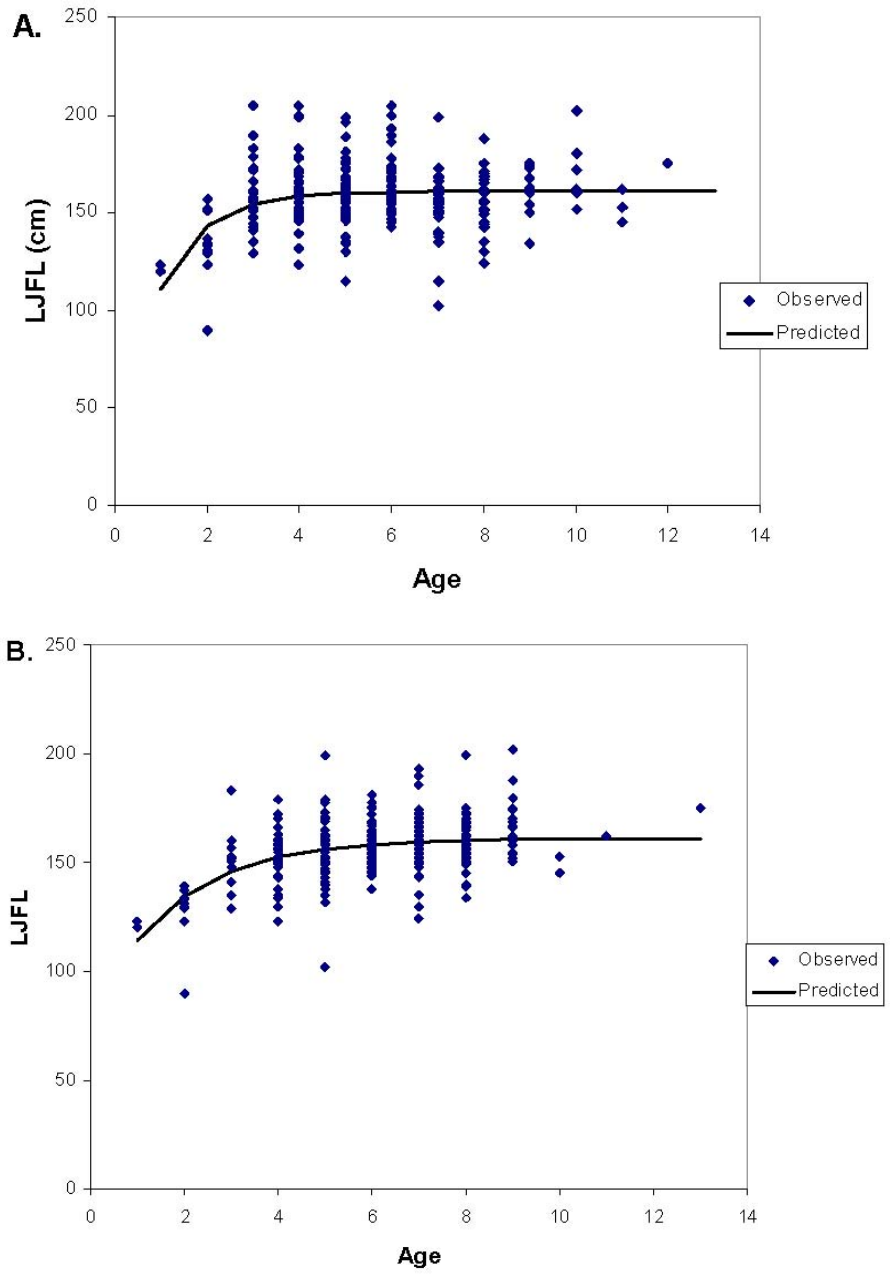


Figure 12: Observed and predicted lengths of male white marlin for Brazilian and Venezuelan samples together, with ages assigned to the pooled sample (A) and separately for each sample (B).

The samples analyzed here show a high degree of variability in length-at-age, the relationship between spine size and lower-law fork length, and seasonal RMIs. This variability in seasonal RMI results in only weak support for the use of RMI as a tool to validate the annual periodicity of ring formation in white marlin. The high degree of variability in length at age certainly will difficult the use of length as a predictor of age.

Part of this variation may be due to the geographical breath of distribution of marlins that migrate through the equator, and experiment seasonal cycles that are different to those from species that are confined to a single hemisphere. Recent work by Shivji *et al* (2006) suggests another possible source of variation. They identified a new species of spearfish in the same region we sampled. This species, while genetically distinct, is morphologically very similar to white marlin, and it is possible that our observers, who were not aware of the cryptic spearfish species during our sampling efforts, sampled both species as white marlin. The relative proportions of the two species in the catch are not well known. Beerkircher *et al.* (2008) found that roundscale spearfish made up the majority of the billfish catch in some areas, suggesting that their presence may not be trivial. Therefore, the extent to which possible misidentification of samples adds variability to the data is difficult to say. Genetically testing all of our samples would be unfeasible, but testing a sub-sample and then looking for patterns in spine morphology or morphometric relationships to distinguish the samples might be reasonable.

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USE OF POP-UP SATELLITE ARCHIVAL TAGS TO ESTIMATE POST-RELEASE SURVIVAL AND HABITAT PREFERENCES OF SAILFISH (*ISTIOPHORUS PLATYPTERUS*) FROM COMMERCIAL PELAGIC LONGLINE GEAR IN THE SOUTHERN GULF OF MEXICO

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Abstract
To estimate post-release survival of sailfish caught incidentally to regular commercial pelagic longline fishing operations in the southern Gulf of Mexico targeting mixed swordfish and tunas, 18 pop-up satellite archival tags were deployed on incidentally captured animals. A total of 17 tags transmitted following the full 10-day deployment period, transmitting 25-82% of the archived data. (Three tags were returned, allowing for 100% recovery of the archived data.) Repeated, short-duration movements to depth throughout the tag deployment duration and horizontal displacement data were consistent with survival for 15 of 17 animals (88.2%) for the ten-day deployment period. The short-duration behavior recorded by the tags also showed repeated movements to depths well below the measured effective fishing depths of the common types of pelagic longline fishing gear used in this geographic area. The results of this study clearly indicate that a large percentage of sailfish can survive the trauma resulting from interaction with pelagic longline gear, and that current domestic and international management measures promoting the release of sailfish from pelagic longline fisheries will reduce fishing mortality on the Atlantic sailfish stocks.

Purpose
Pelagic longline fishing gear is used throughout the world to commercially harvest broadbill swordfish *Xiphias gladius* and various tuna species. Incidental to these target species, pelagic longline gear also catches a variety of other animals, including istiophorid billfishes. Within the western Atlantic Ocean, it is estimated that the pelagic longline fishery is responsible for approximately one-third of the sailfish fishing mortality, with local gillnet, artisanal, and recreational fisheries comprising the other two thirds (ICCAT, 2001).

The current status of several highly migratory species stocks under the purview of the International Commission for the Conservation of Atlantic Tunas (ICCAT), is either fully exploited or over-exploited, including the western and eastern Atlantic stocks of sailfish. The present assessment methodology used for billfish species primarily utilizes long time-series of pelagic longline catch data combined with data from recreational and other commercial fisheries. The last formal assessment of the western Atlantic sailfish stock, which also included spearfish, occurred in 2001. The results of that assessment were considered unsatisfactory, however, but based on catch-per-unit-effort (CPUE) data from member states; the ICCAT Standing Committee for Research and Statistics (SCRS) recommended maintaining international catches

at the current level for the western stock. Based on other CPUE data in conjunction with local abundance indices, the SCRS also noted that the eastern Atlantic stock may be in decline.

Management measures requiring the release of live istiophorid billfishes have been suggested as one means to reduce fishing mortality on billfishes without sacrificing target catches (Kerstetter et al., 2002; Kerstetter and Graves, 2006). Jackson and Farber (1998) reported that about 32% of sailfish caught in the Venezuelan longline fishery were alive at the time of gear retrieval, and data from the U.S. observer program in the Gulf of Mexico report that 48% of sailfish were released alive from U.S. commercial pelagic longline gear in that area between 1985 and 1990 (Farber and Lee, 1990). In the United States, the National Marine Fisheries Service (NMFS) has required the release of live sailfish from the commercial longline fishery since 1988, although the fate of the released fish is unknown. The SCRS called for further work on the estimation of post-release survival for all billfishes at its 2005 meeting (ICCAT, 2005).

An increasing amount of information now exists about the post-release survival of billfish. Both blue marlin (Graves et al., 2002) and white marlin (Horodysky and Graves, 2005) caught in recreational fisheries have been shown to have high rates of post-release survival, especially when caught with circle hooks. Blue marlin (Kerstetter et al., 2003) and white marlin (Kerstetter and Graves, 2006) captured on pelagic longline gear also demonstrate relatively higher rates of post-release survival, albeit lower than recreational fisheries. Little is known, however, about post-release survival of sailfish. In general, recovery rates of sailfish tagged with conventional streamer tags by commercial and recreational fishermen have been quite low (<2% for all billfish species: Jones and Prince, 1998; Ortiz et al., 1998). While this observation is consistent with high post-release mortality, low recovery rates could also result from tag shedding and non-reporting of recovered tags (Bayley and Prince, 1994; Jones and Prince, 1998). Acoustic tracking of sailfish (Jolley and Irby, 1979) has shown post-release survival of recreationally-caught sailfish for periods ranging from approximately 3-28 hours, but these studies were limited by vessel time and environmental conditions, and may not have evidenced delayed mortality from capture-related injuries that can occur several days following release. Pop-up satellite archival tags (PSATs), which are programmed to record data for a set period of time, then independently release from the animal, float to the surface, and transmit the stored data through the Argos satellite system, provide a useful tool for the analysis of post-release survival.

Approach

Tag Model

The Microwave Telemetry, Inc. (Columbia, MD, USA) model PTT-100 HR satellite tag was used in this work (see Kerstetter and Graves, 2006 for the physical description of the tags and rigging details). The PTT-100 HR model tags sampled temperature, pressure (depth), and light level approximately every two minutes. All tags were pre-programmed to release from the fish after ten days, and the tags were activated prior to attachment to the animal by removing a small magnet from the side of the tag. This model also included emergency release software that automatically detached the tag if the pressure sensor indicated depths approaching the crush limit of the tag casing (ca. 2000 m). Due to delays in deployment opportunities, the batteries on several of the tags expired, requiring the rebuilding of the whole tags. This rebuilding provided an opportunity to update the on-board tag software, and the tags deployed in 2007 had an

additional program "fail-safe" that would trigger the release of the tag if it was at a constant depth (within 5 meters, suggesting a mortality resting on the ocean floor) for more than 24 hours.

Tagging Procedures

Tags were either activated prior to haulback or during haulback immediately following the tagging of a fish in preparation for another animal. The captain of the vessel identified incoming sailfish on the line during the morning haulback of the gear and fish were evaluated as live or dead based on movement (or lack thereof) alongside the vessel. All sailfish evaluated as alive were tagged, regardless of physical condition (i.e., no "high-grading" of animals, which would have biased the results by eliminating potential mortalities). The PSAT tagging procedures used were identical to the ones described in Kerstetter and Graves (2006), although with a shorter-length applicator tip to compensate for the much more laterally compressed sailfish body form. In addition to the PSAT, a conventional streamer tag was also attached to most sailfish in this study.

Sailfish were released as soon as possible after tagging by the standard commercial protocol of cutting the leader near the hook unless the hook was readily accessible for manual removal. No animals were resuscitated after tagging. Prior to release, hook type and hooking location (see Yamaguchi, 1989) were noted and fish lengths and weights were estimated. Each tagging procedure, from positive species identification to actual release from the gear, lasted less than ten minutes. All other pertinent individual fish data, including the time of day, vessel location, and sea surface water temperature were recorded immediately after tagging.

Data Analysis

Survival of tagged animals was inferred from three types of environmental data provided by the tag: water temperature changes, depth changes, and ambient light intensity. Frequent short-scale (< 15 minutes) variations in both depth and temperature throughout the ten-day tag deployment period were used as indicators of a live sailfish. The survival of individual animals was also supported by the net horizontal displacement, calculated as the distance from the location of the vessel at the time the sailfish was released to the location of the first good transmission from the free-floating PSAT to the ARGOS satellite system (see Kerstetter et al., 2003). All distances were calculated with PROGRAM INVERSE (NGS, 1975; modified by M. Ortiz, NMFS SEFSC, Miami, FL).

The 95% confidence intervals associated with these estimates were calculated using the RELEASE MORTALITY version 1.1.0 software developed by Goodyear (2002). These confidence intervals were based on 10,000 simulations with assumed underlying post-release mortality rates derived from the transmitted data with no error sources (e.g., no premature releases or tag-induced mortality). For the purpose of these simulations, natural mortality was also assumed to be zero because of the relatively short duration of the tagging deployment period. Unless otherwise noted, all statistical analyses for this study were conducted using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

All but one of the tagging deployments in this study occurred opportunistically aboard the commercial pelagic longline fishing vessel F/V *Kristin Lee* (16.5 m length) during trips in the

southern Gulf of Mexico during November 2005 (n = 1 PSAT), May 2006 (n = 9 PSATs), and June 2007 (n = 7 PSATs). The only tagging not conducted on the F/V *Kristin Lee* was done on the F/V *Alfa* off the coast of northeast Brazil in March 2006 (n = 1 PSAT). Despite possessing on-board emergency release software, this tag did not report. Several possibilities exist for non-reporting tags (see Kerstetter et al., 2004), but because a definitive causative agent for the lack of transmissions cannot be identified, this tag is not discussed further. Other trips were taken to deploy tags, but, in 2005 and 2006 off the Florida East Coast and offshore of South Carolina in the area of the Charleston Bump. These additional trips were ultimately unsuccessful for tagging purposes, however, as no live sailfish were encountered during those sets. All of the sailfish tag data presented in this study was obtained from fish tagged in the southern Gulf of Mexico (GOM), approximately 95 km south-southwest of Key West, Florida, an area traditionally fished by the U.S. coastal pelagic longline fleet. The F/V *Kristin Lee* used 10 fathom (fa; approximately 60 feet) buoy line lengths and both size 16/0 non-offset and size 18/0 10°-offset circle hooks on all of its trips. Bait was usually frozen squid (*Illex* sp.), but occasionally included small amounts of frozen Atlantic mackerel (*Scomber scombrus*).

Findings

Post-Release Survival

The first 18 sailfish deemed to be alive at haulback were tagged, regardless of physical condition. During the U.S. fishing trips used in this study, 20 of 29 sailfish (68.9%) were alive at the time of haulback (this total includes three fish that broke off the leader prior to tagging). Estimated weights of fish released alive ranged from 25-65 pounds. Four animals were foul-hooked, and another was entangled in the leader itself. Only the first sailfish tagged had swallowed the hook such that it was not visible upon examination alongside the vessel. Excluding the fish tagged in Brazil, the second nine fish were tagged within a five-day period and the third group of seven fish within a six-day period. In the end, all 17 of the reporting tags were from fish tagged within the same general geographic area, and additional information on each animal is found in Table 2.

Of the 17 tags that reported, it was inferred that 15 of them survived for the full duration of the 10-day deployment period. The first sailfish tagged in this work (gut-hooked) showed some vertical movements during the first 12 hours, and then rested along the seafloor for approximately two days before undergoing vertical movements while showing no light level, even during times of daylight. Based on previous research (Kerstetter et al., 2004), we concluded that this tag (and part of the sailfish) was probably eaten by a scavenging animal such as a shark. One of the last fish to be tagged in 2007 also apparently died soon following release from the fishing gear. The other fifteen fish all showed similar, short-scale (< 15 minutes) vertical movements with underlying diurnal patterns. Although the majority of time was spent within 50 m of the surface, several animals demonstrated frequent movements to depths in excess of 100 m. Additionally, all of the reporting tags showed movements to depth that were remarkably similar, if somewhat shallower, than the movement types described in Horodysky et al. (2007). Figure 1 shows a short track with the identified movement types.

Minimum straight-line distances over the ten-day deployment period varied widely between individuals, ranging from 36.4 to 387.3 nautical miles. Most of the tagged animals remained within the U.S. Exclusive Economic Zone (EEZ), with many in the 2007 tagging season moving

east-northeast with the Gulf Stream along the Florida East Coast shelf towards West Palm Beach and northward. Only one animal in the study went southward, crossing the Gulf Stream and ending up in the shelf waters of the north Cuban coast just west of the Bahia de Cabañas.

These data result in an overall post-release survival rate of 88.2%. Assuming an underlying true post-release mortality rate of 11.8% and a study of 17 total satellite tags, the results of the Goodyear (2002) simulations indicate a 95% C.I. range of mortality of 0-23.5% for released animals after ten days. To date, none of the conventional ("spaghetti") tags applied to these PSAT-tagged animals have been reported or returned to the NMFS Cooperative Tagging Center.

Interactions with Pelagic Longline Fishing Gear

During the course of the research, small temperature-depth recorders (TDRs; Lotek Wireless, Inc. model LTD_1100) were deployed opportunistically along with the pelagic longline gear to determine the effective fishing depths. Figure 2 shows the potential interactions between pelagic longline gear and the vertical movements to depth by sailfish in the southern Gulf of Mexico.

The most significant problem encountered in this study was deploying tags on live animals. A large component of this problem was the difficulty in obtaining vessel time for the project. Many of the vessels that fish this southern Gulf of Mexico area are small boats with limited space for an additional crewmember, much less a dedicated scientist. One of the PIs (DWK) occasionally circumvented this problem by agreeing to work as a full crewmember in exchange for the opportunity to tag a sailfish, a very small percentage of the total time aboard. However, the project was also partly a victim of unfortunate timing. In spring 2005, most of the pelagic longline vessels that have cooperated in similar research in the past became involved with a cooperative research program with the National Marine Fisheries Service that included experimental fishing within politically sensitive areas. A compromise was reached that allowed the experimental fishing to occur within those areas, but only with federal Pelagic Observer Program fisheries observers aboard, rather than one of the principal investigators, despite their being part of the research design team. Therefore, most of the vessels with which the Principal investigators had participated in past research, and which had agreed at the time of the original proposal to participate in this tagging work, were otherwise unavailable during most of 2005. One trip was taken in July 2005 off South Carolina, although no live sailfish were encountered during that week of fishing effort. Another trip was taken in November 2005 during which one sailfish was tagged, although it died shortly after release.

A potential compromise presented itself when other cooperative work with the Brazilian pelagic longline fleet commenced during the spring of 2006. During the first trip from Natal on the northeast Brazilian coast, the vessel encountered mechanical problems and the fishing ended prematurely. One sailfish was tagged on a subsequent trip, although that tag never reported. The low encounter rates of sailfish and declining battery strength of the remaining tags led to the abandonment of this avenue, and a trip was taken into the southern Gulf of Mexico in May 2006. During that trip, nine sailfish were tagged (all nine tags reported, indicating that all of the fish had survived for the 10-day deployment duration). The efforts of the fleet for the remainder of the summer and fall were directed elsewhere, precluding any additional efforts in the southern Gulf of Mexico. Another two trips were taken in summer 2006 off the Florida East Coast, but no live sailfish were encountered. Following this time, the remaining tags were returned to the

manufacturer for rebuilding with new batteries. A final trip was arranged for the southern Gulf of Mexico in June 2007, during which seven sailfish were tagged (all of the tags reported, although only six of the seven fish survived). As with the 2006 season, the efforts of the fleet for the remainder of the 2007 summer and fall seasons were planned to occur elsewhere, whether off northeastern Florida, South Carolina, or even further north into the Mid-Atlantic Bight.

In conclusion, changes in seasonal effort and the unexpected requirements for mandatory federal observer program coverage within the fleet during the research period of 2005 created problems with opportunities for tag deployments, even though several vessels at the time of the proposal submission had agreed to participate with the research program. Nonetheless, 18 of the 20 tags were eventually deployed.

Conclusions

The goal of this research was to obtain an estimate of post-release survival of sailfish released from pelagic longline gear in the southern Gulf of Mexico, a goal that was clearly achieved. Although the number of PSATs deployed was limited by costs, the objective of this work was as much political as scientific in demonstrating a relatively high survival rate for sailfish released from pelagic longline gear in the warm waters of the southern Gulf of Mexico/Florida Straits. Calls for mandatory release of sailfish by pelagic longline fisheries has been opposed in international fisheries management forums, with arguments against such release based on anecdotal evidence of high mortality rates for released sailfish from this gear type. This research supports on-going efforts to promote the release of sailfish, which are currently retained by many pelagic longline fisheries of Caribbean and other tropical nations, and which would have positive benefits to the Atlantic Ocean stocks. In addition, the results of this study may provide a missing element to international stock assessments for sailfish: an experimentally-generated estimate of post-release mortality for the pelagic longline fishery.

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Table 1. Summary of tagging efforts for sailfish during project period. Rows of mortalities have been shaded. "GOM" is the Gulf of Mexico. Distance traveled is calculated as the "Minimum Straight-Line Distance" (MSLD) in nautical miles between the location that the fish was tagged and the location at which the tag first transmitted to the ARGOS satellite system.

Sailfish Number	Month Deployed	Tagging Location	Reported?	Reporting Percentage	Distance Traveled	Survived?
SAIL-05-01	11/05	GOM	Yes	25%	28.3	No
[none]	3/06	Brazil	No	n/a	n/a	n/a
SAIL-06-01	5/06	GOM	Yes	59%	241.9	Yes
SAIL-06-02	5/06	GOM	Yes	82%	202.7	Yes
SAIL-06-03	5/06	GOM	Yes	63%	81.0	Yes
SAIL-06-04	5/06	GOM	Yes	55%	101.8	Yes
SAIL-06-05	5/06	GOM	Yes	68%	179.5	Yes
SAIL-06-06	5/06	GOM	Yes	75%	299.6	Yes
SAIL-06-07	5/06	GOM	Yes	65%*	52.5	Yes
SAIL-06-08	5/06	GOM	Yes	40%	104.5	Yes
SAIL-06-09	5/06	GOM	Yes	68%	241.3	Yes
SAIL-07-01	6/07	GOM	Yes	75%	282.4	Yes
SAIL-07-02	6/07	GOM	Yes	88%	63.2	No
SAIL-07-03	6/07	GOM	Yes	87%	67.5	Yes
SAIL-07-04	6/07	GOM	Yes	74%*	219.3	Yes
SAIL-07-05	6/07	GOM	Yes	86%	304.6	Yes
SAIL-07-06	6/07	GOM	Yes	88%	387.3	Yes
SAIL-07-07	6/07	GOM	Yes	88%*	36.4	Yes

* Original reporting percentage – tags were later returned, allowing a 100% data recovery rate

Table 2. Summary of conditions for sailfish tagged during project period. Rows of mortalities have been shaded. "ACESS Score" refers to a physical condition index based on a ten-point scale ("10" being the best possible score, indicating a very healthy animal; see Kerstetter et al., 2002 for further details on the scale). Estimated lengths are "lower jaw-fork lengths" (LJFL), the standard metric for length in billfishes. For clarity, the non-reporting tag from Brazil has been omitted from the table.

Sailfish Number	Hooking Location	Hook Size and Type	Hook Removed?	ACESS Score	Estimated Length (LJFL)	Survived?
SAIL-05-01	(not visible)	18/0 Circle	N	9	60"	No
SAIL-06-01	Corner	16/0 Circle	Y	9	54"	Yes
SAIL-06-02	Lower Jaw	18/0 Circle	Y	9	72"	Yes
SAIL-06-03	Fouled	16/0 Circle	N	8	66"	Yes
SAIL-06-04	Isthmus	18/0 Circle	Y	10	72"	Yes
SAIL-06-05	Corner	16/0 Circle	N	10	66"	Yes
SAIL-06-06	Eye Socket	16/0 Circle	N	9	60"	Yes
SAIL-06-07	Fouled	16/0 Circle	n/a	8	60"	Yes
SAIL-06-08	Lower Jaw	18/0 Circle	Y	8	66"	Yes
SAIL-06-09	Corner	18/0 Circle	Y	8	60"	Yes
SAIL-07-01	Corner	16/0 Circle	N	9	48"	Yes
SAIL-07-02	Corner	16/0 Circle	N	8	48"	No
SAIL-07-03	Corner	16/0 Circle	N	6	48"	Yes
SAIL-07-04	Corner	16/0 Circle	N	10	48"	Yes
SAIL-07-05	Corner	16/0 Circle	N	10	54"	Yes
SAIL-07-06	Corner	16/0 Circle	N	5	42"	Yes
SAIL-07-07	Corner	16/0 Circle	N	6	48"	Yes

Figure 1. 12-hour period of activity for a sailfish tagged with a PSAT in the southern Gulf of Mexico, May 2006. Bracketed regions show segments of the recorded track of the animal that are identical to segments of recorded tracks from white marlin (see Horodysky et al., 2007).

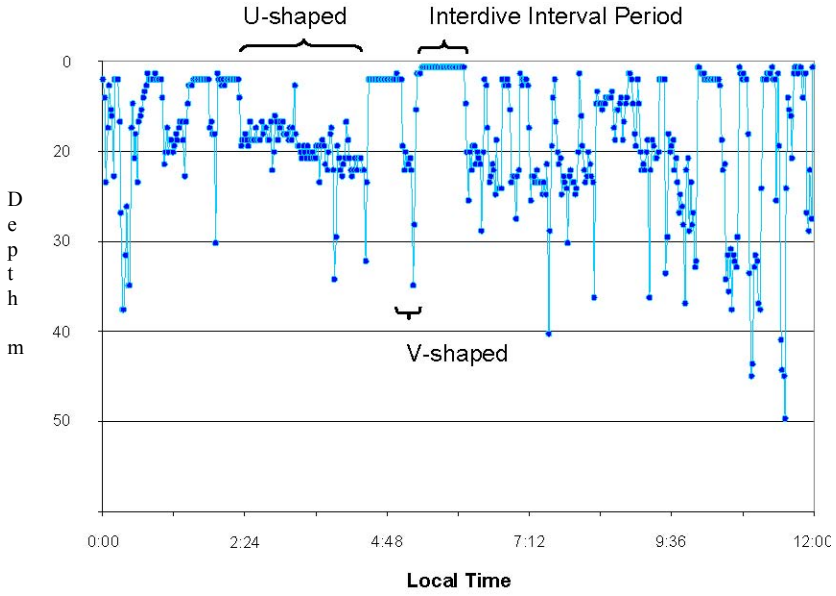
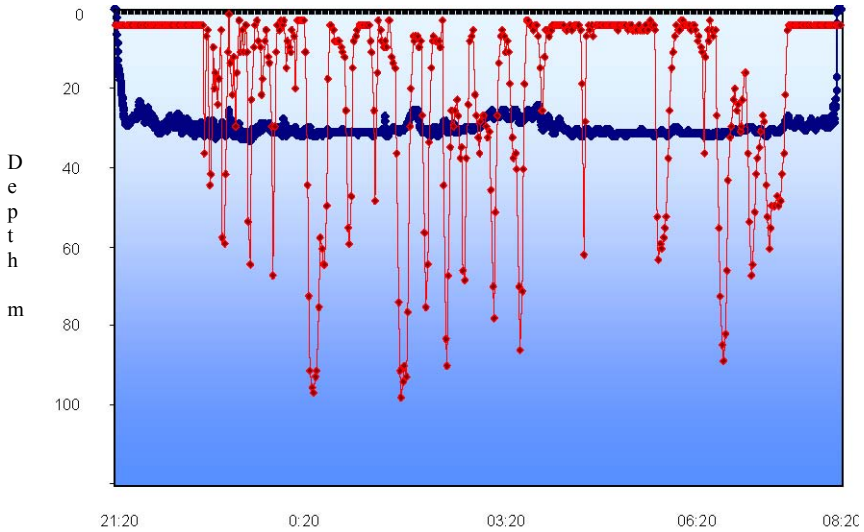


Figure 2. Short-duration behavior and pelagic longline fishing gear interaction potential of a sailfish tagged off the Florida Keys, May 2006. Red line is the track at 90-second intervals of a sailfish tagged with a pop-up satellite archival tag, while the blue line indicates the monitored fishing depth at 14-seond intervals of commercial coastal pelagic longline fishing gear deployed concurrently with the tag deployment duration. Repeated, short-duration movements through the effective fishing depth range of the longline gear suggests a high interaction potential for this individual.



REPRODUCTIVE BIOLOGY, POTENTIAL SPAWNING AND NURSERY AREAS AND LARVAL IDENTIFICATION OF BLUE MARLIN, *MAKAIRA NIGRICANS*, IN THE NORTHCENTRAL GULF OF MEXICO

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Abstract

Blue marlin (*Makaira nigricans*) captured in fishing tournaments throughout the northcentral Gulf of Mexico (ncGOM, Panama City, FL through Venice, LA) from 2000 – 2007 were sampled to determine their reproductive status. Only females were captured, and a total of 78 ovarian samples taken from May through September revealed no blue marlin captured in the ncGOM were spawning capable. Gonadosomatic Index (GSI) values were < 1 for all fish. Histological examination showed that the ovaries of many females were in the early developing and developing reproductive classes from May through July but none contained large vitellogenic oocytes or postovulatory follicles. One female captured in September had atretic, hydrated oocytes in the ovary, suggesting a possible spawning event earlier in the year. Histological evidence strongly suggests blue marlin do not spawn in the ncGOM. Two research cruises (2005 and 2006) were undertaken to capture and identify istiophorid larvae in the ncGOM. Additionally, istiophorid larvae captured during previous research cruises (2000 – 2005) targeting *Sargassum* habitat in the ncGOM were analyzed. Most istiophorid larvae were captured at the Loop Current in August 2005; only 4 larvae were captured in *Sargassum* habitat. Genetic analysis of total DNA extracted from istiophorid larvae confirmed morphological identifications and also allowed species identification of larvae that had intermediate morphological characteristics. A total of 43 larval blue marlin were identified, and all but one of these larvae were captured in association with the Loop Current. Larvae ranged in size from 2.5 - 5.2 mm body length. Daily growth increments from the saggital otoliths were used to estimate ages of the larvae which ranged from 4 – 12 days. Growth curves were used to calculated a mean growth rate of 0.25 mm/day. To estimate spawning locations of the larvae collected during this study, larval transport from capture locations to potential spawning sites was computed using archived data from the Inter-American Seas Nowcast/Forecast System (IASNFS). Results of the model predicted that all but one of the larvae were spawned along the shelf/slope region of the Yucatan Peninsula off Mexico along the edge of the Loop Current. These predictions were unexpected, and suggest a previously unknown area of blue marlin spawning.

Purpose

The objectives of this project were utilize GSI and histology to determine gonadal condition and spawning seasonality of female blue marlin in the northcentral Gulf of Mexico (ncGOM); collect larval blue marlin and other istiophorids from the ncGOM; identify larval blue marlin and other

istiophorids collected from the ncGOM using morphometric and molecular techniques; estimate age and growth rates of larval blue marlin in the ncGOM; and identify possible blue marlin spawning and nursery areas in the ncGOM through the combination of spawning seasonality, ichthyoplankton collections, larval ageing and larval transport models.

Some information on the reproductive biology of blue marlin, *Makaira nigricans*, in the Atlantic Ocean and Caribbean Sea is available (Luckhurst et al. 2006; Prince et al. 2005; deSylva and Breder 1997; Cyr 1987; Yeo 1978; Erdman 1968). The spawning season from South Carolina through Puerto Rico appears to occur from May through early October based on gonadal and some histological observations, although these estimates are based on limited data sets. Blue marlin spawn off Bermuda, based on histological observations (Luckhurst et al. 2006). Erdman (1968) considered the peak spawning season for blue marlin off Puerto Rico to be July and August. Collections of larval blue marlin from Exuma Sound, Bahamas in July also suggest a summer spawning season for the species (Serafy et al. 2003). However, blue marlin larvae were collected in April and May off the Dominican Republic (Prince et al. 2005). Blue marlin have been reported to be capable of spawning up to 4 times a year (deSylva and Breder 1997), although there are no estimates of batch fecundity available and accurate spawning frequency estimates are lacking. Male Atlantic blue marlin reach sexual maturity at a smaller size (174 cm FL) than females (193 cm FL, 120 kg), and both males and females are considered to reach sexual maturity between 2-4 years of age (NMFS 1999; ICCAT 2000; deSylva and Breder 1997).

The early life history and environmental requirements of blue marlin and other istiophorids are not well known because of the relative lack of collections and problems of larval identification (de Sylva et al. 2000). Identification of billfish larvae using morphological characteristics continues to be a serious challenge, however, recent use of molecular techniques has facilitated identification of early life history stages of billfish. McDowell and Graves (2002) developed molecular markers based on mitochondrial and nuclear DNA to identify istiophorids. Luthy et al. (2005) used these molecular techniques to identify larval white marlin, blue marlin and sailfish. Morphometric measurements and pigment patterns were then described for a subset of these identified larvae (Luthy et al. 2005). Lower jaw pigments alone could be used to identify 40% of the pre-flexion blue marlin and 60% of the sailfish larvae. Based on an identification key that included pigment patterns, month of capture, and relationships between fish length (SL) and the snout length to eye-orbit diameter ratio, Luthy et al. (2005) was able to identify 70% (n=304) of larvae examined. Only one specimen was incorrectly identified. Of the 93 larvae that could not be identified, 71 could be identified using a Canonical Variates Analysis that combined morphometric, pigment pattern and month of capture information.

As with most pelagic fishes, billfish are difficult to age, and there has been no successful validation of daily marks on larval billfish otoliths to date. However, Prince et al. (1991) used microstructural features of larval blue marlin sagittal otoliths to estimate age and growth rate and found strong indications for daily increment formation. The deposition of daily increments has not been directly validated, but a deposition rate of one increment per day was the only periodicity that resulted in back-calculated spawning times occurring in the summer and fall, known spawning periods of Atlantic blue marlin (Mahon and Mahon 1986). In addition, otolith microstructure was very similar to other species for which daily periodicity of increment

formation has been validated, i.e. Atlantic bumper (Comyns et al. 2003; Leffler and Shaw 1992) and red snapper (Szedlmayer 1998). Sponaugle et al. (2005) used these techniques to document differences in growth rates of larval blue marlin collected from Exuma Sound and the Straits of Florida.

There is virtually no published information on blue marlin biology and life history from the GOM (deSylva et al. 2000). The northcentral Gulf of Mexico (ncGOM) supports an active sport fishery for the species from May through September (Brown-Peterson et al. 2004); from 1996 – 2000, a total of 1,297 blue marlin were caught during GOM fishing tournaments, although the vast majority of these fish were tagged and released (Avrigian and Venizelos 2003). The ncGOM produced most of these fish, primarily in the region between the Mississippi River and the Desoto Canyon. Preliminary research on the reproduction of blue marlin in the ncGOM was based on 5 females captured during June, of which 60% were in the early developing reproductive phase (Brown-Peterson et al. 2004), suggesting spawning probably begins in the northern GOM in July. Additionally, unidentified istiophorid larvae collected in the ncGOM in July and August (Hoffmayer et al. 2005; Franks et al. 2004; Comyns et al. 2002, Franks et al. 2002) indicate the importance of the ncGOM as a billfish spawning area. Species-specific knowledge of the distribution and abundance of billfish larvae is important because: 1) the presence of very young larvae is the only conclusive evidence that successful adult spawning activity has occurred at, or near, the waters of their collection; and 2) larval abundance may hold promise as an indicator of the number of spawning adults that produced them.

The identification of potential billfish spawning and nursery areas in the GOM is critical for the protection and conservation of the species. Recent work in the Atlantic suggests semi-enclosed pelagic waters function as spawning and nursery areas for blue marlin (Serafy et al. 2003). However, the pelagic waters of the northern GOM are an open ocean ecosystem with no semi-enclosed areas. Dynamics of the central GOM are dominated by the Loop Current (LC) and its associated eddies. The LC is part of the strong western boundary current that intrudes into the GOM through the Yucatan Channel, forms a loop and exits through the Florida Straits (Johnson et al. 1992). The LC frequently extends northward of latitude 28°N into the north-central GOM (Figure 1). It is baroclinically unstable, and at intervals of characteristically 9-13 months, the loop intrudes far to the north, folds back on itself and breaks off as a separate eddy of several hundred kilometer diameter. When a spin-off eddy occurs, flow passing through the Yucatan Channel reconnects with the Florida Straits and a new loop is formed. The LC and its spinoff eddies contain currents characteristically 200 cm/s with

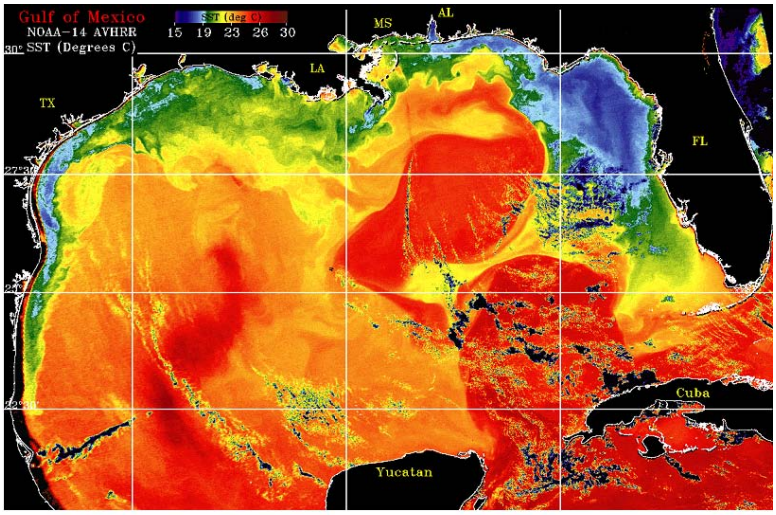

Fig. 1. Loop Current and associated eddy intrusion into the north-central Gulf of Mexico.

strong shears and convergences along the outer edges (boundary). It is in these outer edges that oligotrophic waters of the Caribbean meet more nutrient rich waters (entrained from the Campeche Bank or upwelled from depths) where planktonic organisms, including fish eggs and larvae, can concentrate (NMFS 1999). The role of the LC boundary as a potential spawning and nursery area for billfishes has not been investigated, although fish larvae are known to be associated with the hydrographic features of the LC (Richards et al. 1989). Billfish show affinities for such physiographic features in the water column (Seki et al. 2002; NMFS 1999), although information on how such features might influence blue marlin spawning is lacking for the northern GOM region.

Pelagic *Sargassum*, a brown macroalga (Phaeophyceae) comprised of two species in the GOM (*S. natans* and *S. fluitans*), represents a ubiquitous surface feature in the ncGOM, and recent work has demonstrated that pelagic *Sargassum* in the GOM represents important habitat, and possibly Essential Fish habitat (EFH) for several members of the pelagic fish community including young billfishes (Hoffmayer et al. 2005; Rooker et al. 2004; Franks et al. 2004; Wells and Rooker 2003; Franks et al. 2002; Comyns et al. 2002). However, the importance of *Sargassum* habitat as a nursery area for young billfishes has not been the subject of direct investigation.

The 2000 stock assessment for Atlantic highly migratory species identified several research priorities, including determining spawning site fidelity and the age at first reproduction for pelagic species (NMFS 2000). Additionally, the Atlantic billfish management plan states data are needed to describe and identify EFH (NMFS 1999). Given the paucity of information on reproduction, spawning, and larval distribution of blue marlin, particularly in the GOM, the NMFS priorities need to be addressed in the framework of increasing the knowledge of all aspects of blue marlin reproduction in the GOM. Since GOM blue marlin are not specifically addressed in the billfish FMP, data are needed to provide a baseline for assessments of the GOM blue marlin population for inclusion in future updates of the billfish FMP.

Approach

Gonadal collections and reproductive biology analysis of blue marlin from the north-central GOM

Archived ovarian samples and associated catch data of GOM blue marlin collected by the National Marine Fisheries Service (NMFS), Southeast Fisheries Science Center, Migratory Fisheries Biology Branch from 2002-2004 were used for this study. Additionally, archived ovarian tissues and associated catch data of GOM blue marlin collected by personnel with The University of Southern Mississippi's Gulf Coast Research Laboratory (GCRL) from 2000-2003 were also used. These archived samples represented data from 34 fish. Most of the NMFS archived samples were frozen, while those from GCRL were stored in 10% neutral buffered formalin (NBF). The frozen samples were placed in 10% NBF and allowed to slowly thaw in the refrigerator, in an attempt to minimize cellular damage due to freezing.

Fresh ovarian samples were obtained from 2005 – 2007 from fish landed at billfish tournaments throughout the ncGOM through cooperation with NMFS Billfish Recreational Survey port samplers at these tournaments. All these billfish were captured with hook and line (Figure 2).

Figure 2. Blue marlin captured and released in August, 2005 in the north-central Gulf of Mexico during the 2005 billfish research cruise.

A small cross sectional slice of the middle section of one ovary was preserved in 10% NBF within 24 h of capture. A total of 46 ovarian samples were obtained during these collections; Table 1 lists the dates, locations, and number of fish from each collection.

Table 1. Collections of gonadal samples from blue marlin captured in fishing tournaments in the north-central Gulf of Mexico.

Date	**Tournament, Location**	**Number fish collected**
29 May 2005	Mobile BGFC Tournament, Orange Beach AL	2
9 June 2005	MS Gulf Coast Billfish Classic, Biloxi MS	1
25 June 2005	Emerald Coast Blue Marlin Classic, Destin FL	2
1-2 July 2005	Pensacola International Billfish Tournament, FL	4
22-23 July 2005	Bay Point Invitational Billfish Tournament, Panama City, FL	3
19 August 2005	Outcast Blue Marlin Classic, Pensacola FL	1
26 May 2006	Mobile BGFC Tournament, Orange Beach AL	3
9-10 June 2006	MS Gulf Coast Billfish Classic, Biloxi MS	6
23-24 June 2006	Emerald Coast Blue Marlin Classic, Destin FL	2
30 June – 1 July 2006	Pensacola International Billfish Tournament, FL	6

11 July 2006	Bay Point Invitational Billfish Tournament, Panama City, FL	3
3 Sept 2006	Mobile BGFC Tournament, Orange Beach AL	1
1 June 2007	Cajun Canyons Billfish Classic, Venice LA	1
8 – 9 June 2007	MS Gulf Coast Billfish Classic, Biloxi MS	3
22 June 2007	Emerald Coast Blue Marlin Classic, Destin FL	4
29 June 2007	Pensacola International Billfish Tournament, FL	1
1-2 Sept 2007	Mobile BGFC Tournament, Orange Beach AL	3

Data collected from all fish included lower jaw fork length (LJFL, 0.1 cm), total weight (W, 0.1 lb) and gonad weight (GW, 0.02 lb). The Gonadosomatic Index (GSI) was calculated for each fish (GSI = [GW/(W-GW)] x 100) and monthly means were plotted as an indicator of spawning seasonality and reproductive readiness.

A small (1 cm^3) section of the central portion of each ovary was removed and placed in an individually labeled cassette for histological processing. Tissues were rinsed in running water overnight, dehydrated in a series of ethanols and placed in a tissue processor for further dehydration, clearing and infiltration with paraffin following standard histological techniques. Tissues were then embedded in paraffin, sectioned at 4 μm on a micrometer, mounted on slides and stained with hematoxylin and eosin. Histological slides were inspected microscopically for oocyte development and classified into the following reproductive phases following Brown-Peterson et al. (2007a): early developing, developing, spawning capable, actively spawning, regressing, and regenerating. Additionally, any abnormalities in the ovarian tissue were noted during microscopic histopathological inspections.

Collection of larval blue marlin and other istiophorids from the north central GOM

Research cruise activities were conducted at *Sargassum* habitat (2000 – 2005), the Loop Current (2003 – 2005) and the DeSoto Canyon area (2006) within a broad study area defined as north of 24.7^0 N Latitude and between 86^0 W and 89.5^0 W Longitude**.** Research cruises conducted during 2005 and 2006 were specifically associated with the Billfish Project. Research was conducted aboard the Gulf Coast Research Laboratory's 30.5m research vessel *R/V Tommy Munro* during multi-day research cruises, the majority of which occurred during spring and summer months. For all ichthyopolankton cruises, fishes collected in net tows were washed from the nets and placed in labeled containers with 95% ethanol. The samples were then sorted in the laboratory, where istiophorid larvae were removed for identification.

2000 – 2005 collections in *Sargassum*

Sargassum sampling stations occupied during 2000 – 2005 were geographically located in the ncGOM and at the Loop Current (LC) within the broad areas shown in Figure 3. Sampling locations were selected based upon: 1) satellite-derived remote sensing of sea surface temperature (SST), 2) aerial surveys of the study area prior to and during research cruises, 3) reported sightings of *Sargassum* (weedlines or mats) and oceanic fronts by local anglers, charter boat captains, and offshore vessel operators, and 4) opportunistic encounters with *Sargassum* during our research cruises. Satellite sea surface temperature images and data were made

available to the project by Ocean Technologies, Bay St. Louis, MS at no cost to the project. *Sargassum* habitat types sampled for the presence of larval and juvenile fishes were mats and windrows.

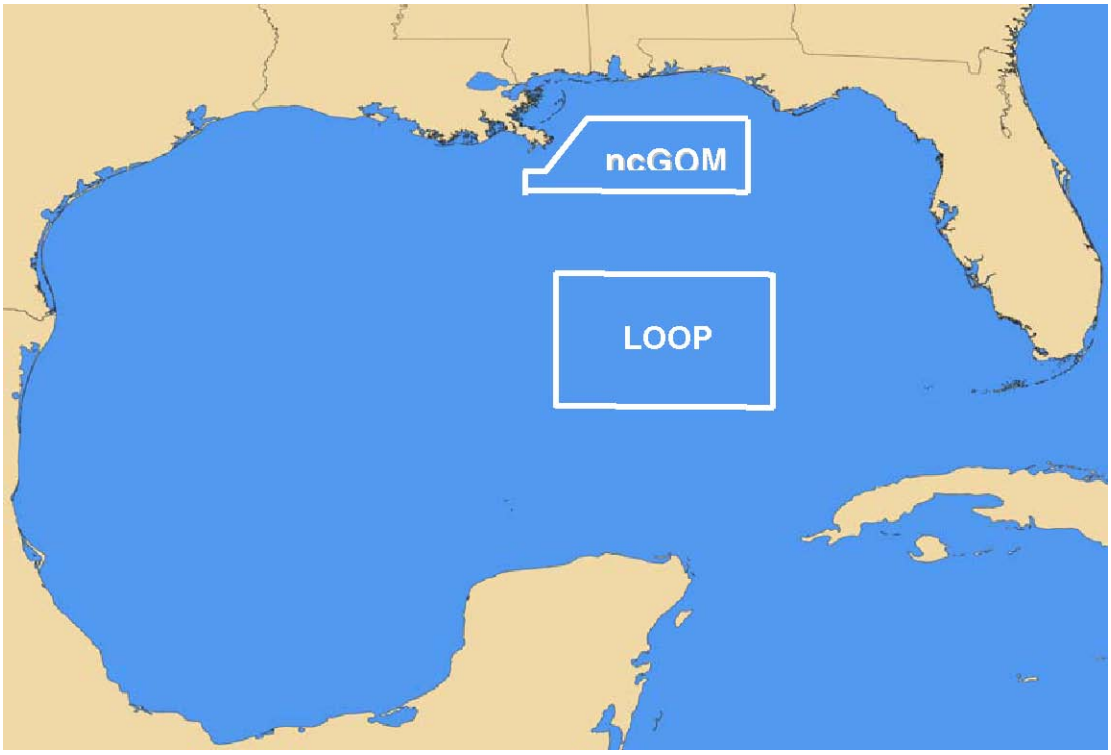

Figure 3. General location of stations sampling *Sargassum* habitat in the northcentral GOM.

In summer of 2003 and 2004 the LC protruded northward into the Gulf which provided a unique opportunity to sample the Loop's western and northern boundaries. The general location of the LC western and northern boundaries was determined using sea surface temperature (SST) data acquired via satellite imagery (AVHRR) provided by Ocean Technologies of Bay St. Louis, MS. Additional satellite images were provided by Roff's Ocean Fishing Forecasting Service, Miami, Florida. To more accurately determine the location of the LC boundary, CTD and XBT water column profiles were taken to locate the 20^{o}C isotherm at 100m, reported by Richards et al. (1989) to be a good representation of the LC boundary.

The biological sampling gear and sampling procedures varied slightly based on the geographical area of collection. Neuston nets (60 collections) and Bongo nets (46 colections were used in the ncGOM. Neuston nets (0.505 mm mesh net and a 0.333 mesh net) were used to sample the surface interface (upper 1 m of the water column) (Figure 4). These nets were 4 m in length and were fitted to a 1 m X 2 m frame. The nets were towed (not simultaneously) for a period of 10 min at an approximate speed of 2 knots around *Sargassum* mats and along oceanic fronts associated with *Sargassum.* The 0.505 mm net was the primary gear used for 52 of the collections. Bongo nets (0.333 mm mesh netting) sampled the surface interface and were towed for a period of ~10 min. at an approximate speed of 2 knots at *Sargassum* mats and windrows

and along each boundary of oceanic fronts associated with *Sargassum.* The bongo nets were fitted with mechanical flow meters to record the volume of water filtered.

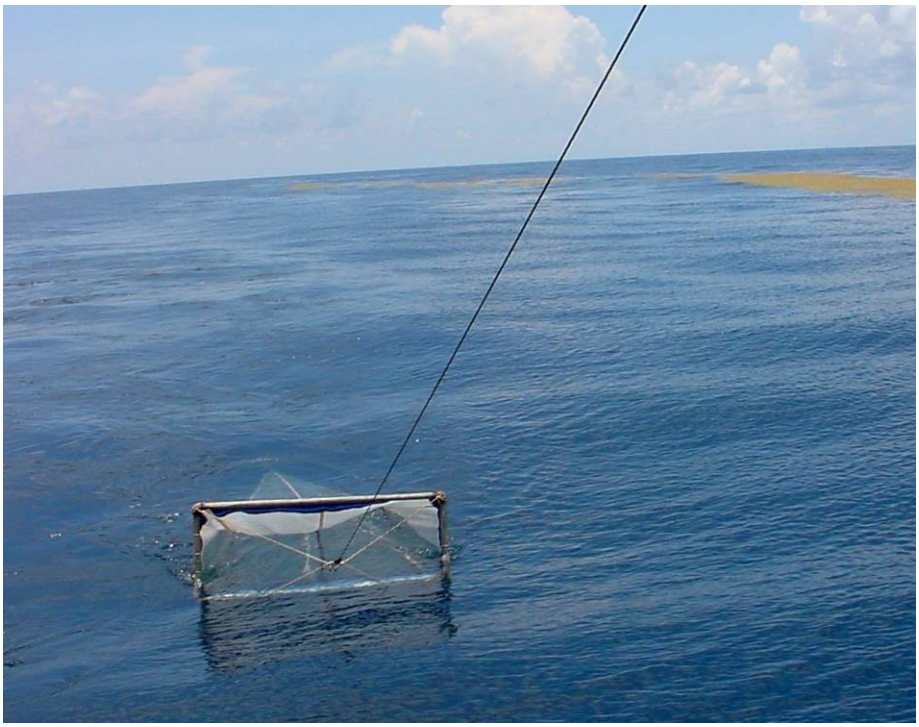

Figure 4. Neuston net used to sample the top 1 m of water for larval fishes.

In Loop Current areas, a Tucker trawl was used for 90 collections (Figure 5). A frame rigged with 3 Tucker trawls (0.333 mm mesh netting) was towed at three discrete depths (1 m, 10 m, and 20 m) at designated stations located along LC transects (~25 km in length) in 2003 (n = 2) and 2004 (n = 1). A total of 57 collections were taken by this gear. Furthermore, a single Tucker trawl was towed obliquely to a depth of 30 m at LC transect stations in 2004 (33 collections). Tucker trawls were fitted with a mechanical flow meter to record the volume of water filtered.

Hydrographic and environmental data were taken at each collection site. The location of sampling sites was recorded using GPS coordinates. Sea surface temperature, salinity, and dissolved oxygen were recorded at each station using a YSI meter (Model 85). XBTs were deployed to record water column temperature profiles. Other field data included air temperature, sea state, wind speed and direction, cloud cover, time of day/night, water clarity (via Secchi disc), general physical characteristics of any frontal zone features (including the estimated size, i.e., length, width, and depth) and *Sargassum* weedlines/mats sampled, and the general direction of frontal zone movements.

Figure 5. Tucker trawl used to collect larval fish samples at the Loop Current.

2005 collections in Loop Current

Sampling was conducted at the LC during a 7-day cruise in August 2005, during the first year of the Billfish Project. Collections were taken at stations positioned along 7 transects (T) which intersected the western (T2 – T5) and eastern (T6 – T8) boundary of the LC (Figure 4). Additional samples were taken northward of the LC along a transect (T1) positioned at the northern boundary of a LC spin-off eddy (Figure 6)**.** Six collection stations were occupied on transects 1 – 7 and 8 stations were occupied on transect 8. Transect stations were located outside, on and inside the boundary area.

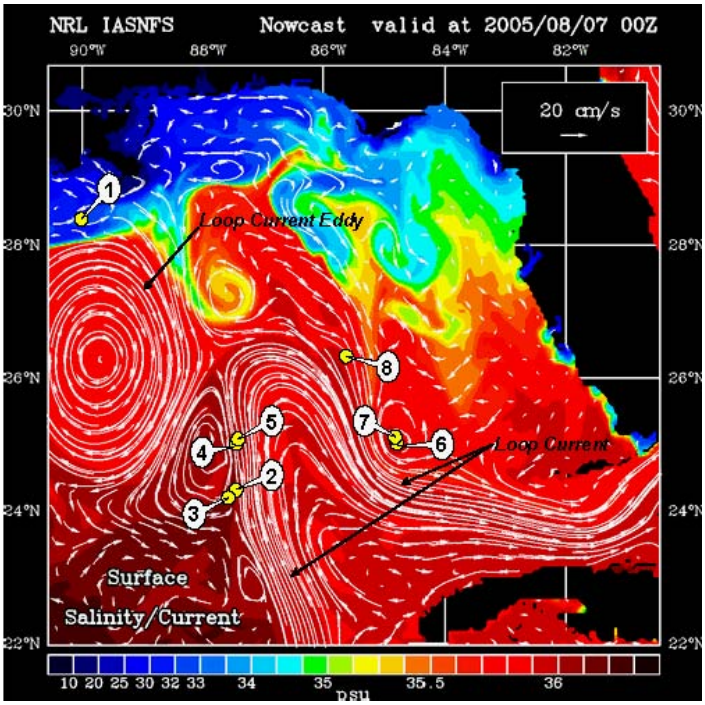

Figure 6. Location of collection transects during the 2005 billfish cruise as related to a salinity and temperature graphic of the Loop Current.

Sampling locations were selected using web-based displays of forecast current in the GOM from the Naval Research Laboratory-Stennis Space Center (NRL-SSC) model (http://www7320.nrlssc.navy.mil/IASNFS_WWW/), as well as Chlorophyll-a and AVHRR images from NRL-SSC web sites (http://web7240.nrlssc.navy.mil/), AVHRR images and analysis from Ocean Technologies, Bay St. Louis, MS, and sea surface height (SSH) analysis for the GOM using satellite altimetry via http://www.ccar.colorado.edu/ ~altimetry/.

A 0.333 mm mesh neuston net (1 m X 2 m frame; 4 m net length) was used to sample the surface interface (upper 1 m of the water column) at each station. The net was towed for a period of 10 min. at an approximate speed of 2 knots. A total of 48 collections were taken during this cruise.
The location of sampling sites was recorded using GPS coordinates. Sea surface temperature, salinity, and dissolved oxygen was recorded at each sample location using a YSI meter (Model 85). In 2003 a CTD (Hydrolab DataSonde-4) was deployed to obtain water column data on temperature, salinity, and dissolved oxygen and to more accurately determine the location of the LC boundary, typically represented by the 20 ^{o}C isotherm at 100 m. XBTs were deployed to record water column temperature profiles. Chlorophyll fluorescence was determined at sampling stations, and a GPS-logging surface drifter was deployed at each station to measure current flow. Other field data included air temperature, sea state, wind speed and direction, cloud cover, time of day/night, and water clarity (via Secchi disc).

2006 collections in DeSoto Canyon

Sampling was conducted in an area of the ncGOM located between the Mississippi River delta and the north-eastern boundary of the DeSoto Canyon (Fig. 7), defined as between Longitude

86.5°W and 89°W and Latitude 28.5°N and 30.0N. Sampling was conducted at *Sargassum* features, including mats, windrows and frontal zones, during a 7-day cruise in August 2006. Half of the samples were taken in areas associated with *Sargassum* and half were taken in areas associated with oceanic fronts but with no *Sargassum* present.

Figure 7. Area of collection of larval fish in the DeSoto Canyon area of the northern Gulf of Mexico during August 2006.

Web-based displays of forecast current in the DeSoto Canyon region and satellite imagery were used to assist in locating collection sites. Additionally, AVHRR images and analysis from Roff's Ocean Fishing Forecasting Service, Miami, Florida were used to more accurately determine prospective sampling locations, and aerial surveys were conducted prior to and during the research cruise to visually pinpoint optimal locations. The location (GPS) of *Sargassum*-frontal zone features was radioed directly from the aircraft to the research vessel.

A 0.333 mm mesh neuston net (1 m X 2 m frame; 4 m net length) was used to sample the surface interface (upper 1 m of the water column) at each station. The net was towed for a period of 10 min. at an approximate speed of 2 knots for a total of 48 collections.

Bongo nets (0.333 mm mesh netting) sampled the surface interface at only two stations and were towed for a period of ~10 min. at an approximate speed of 2 knots. One station was located along the perimeter of a large patch of *Sargassum* (Figure 8). The other station was designated a 'control' for the *Sargassum* station sample. The bongo nets were fitted with mechanical flow meters to record the volume of water filtered.

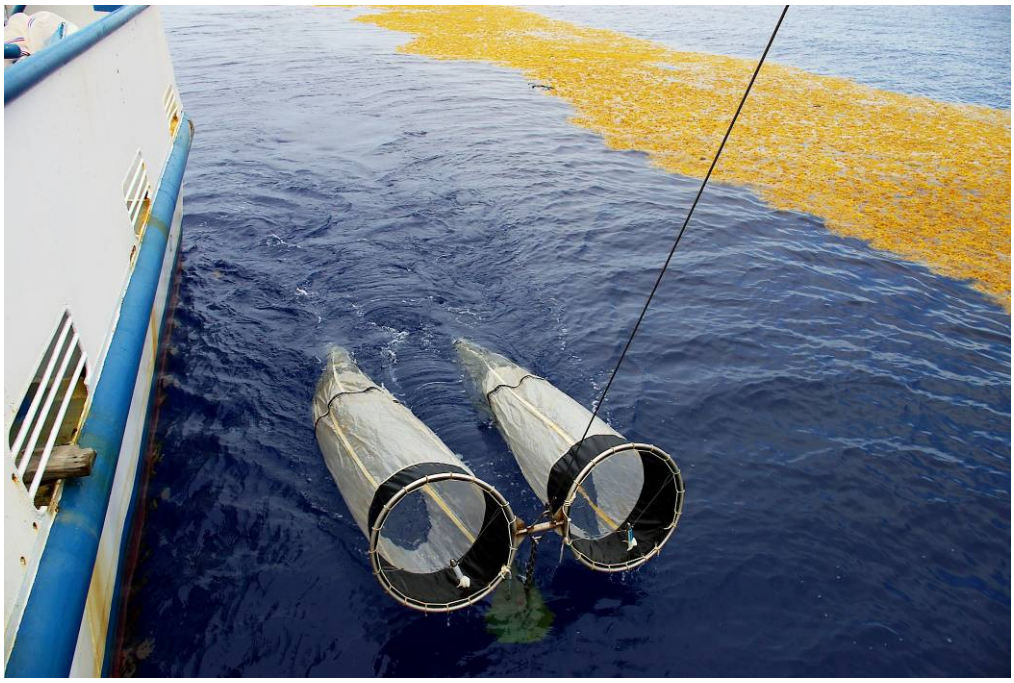

Figure 8. Bongo nets used to collect larval fishes.

The location of sampling sites was recorded using GPS coordinates. Sea surface temperature, salinity, and dissolved oxygen were recorded at each sample location using a YSI meter (Model 85). Other field data included air temperature, sea state, wind speed and direction, cloud cover, time of day/night, water clarity (with a Secchi disc), general physical characteristics of any frontal zone features (including the estimated size, i.e., length, width, and depth) and *Sargassum* weedlines/mats sampled, and the general direction of frontal zone movements.

Identification of larval blue marlin and other istiophorids using morphometric and molecular techniques

Morphometric identification

Four istiophorids occur in the Gulf of Mexico: blue marlin (*Makaira nigricans*), white marlin (*Tetrapturus albidus*), sailfish (*Istiophorus platypterus*) and longbill spearfish (*Tetrapturus pfluegeri*). The longbill spearfish was not of concern for this project, because this species is a winter spawner (Robins 1975; de Sylva and Breder 1997), and our collections were taken during summer months. In addition, larval longbill spearfish are the only larvae that possess pigment on the branchiostegal membranes, and none of our larvae displayed this pigment. We were able to identify some blue marlin and sailfish using morphological characteristics. Approximately 40% of pre-flexion blue marlin larvae can be identified by having only one pigment spot on each side of the tip of the lower jaw as shown in Figure 9 (Luthy et al. 2005), and ~60% of pre-flexion sailfish can be identified by having more than three pigment spots on the posterior 2/3 of the

lower jaw (Richards and Luthy 2006). Larvae that had these specific pigment markings were identified as either blue marlin or sailfish; voucher specimens were also sent for molecular identification to confirm the morphological identification. Pre-flexion larvae that showed intermediate lower jaw pigment and thus could not be identified definitively using morphological methods (a group which includes white marlin) were identified using genetic analyses. Since many larvae were small (3-4 mm), both eyeballs and body tissue below the head were removed for DNA analysis. Heads of those larvae with intermediate lower jaw pigment were retained for future examination after their identity was genetically determined. For larvae >10 mm, species can be identified by snout length / orbit diameter. Because we had very few larvae of this size, we verified the identity of these larger specimens using genetic analyses.

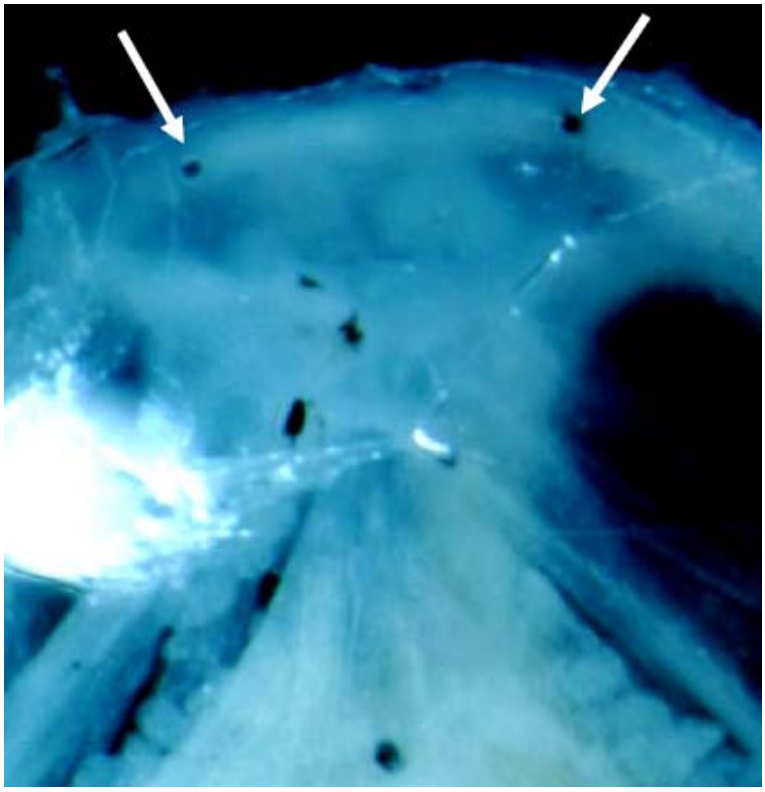

Figure 9. Distinctive lower jaw pigmentation (arrows) used to identify pre-flexion blue marlin larvae.

Molecular identification

Total genomic DNA was extracted from 115 preserved larval billfish eyes using a Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's directions. After isolation, larvae were identified following one of several methods depending on the quality of the DNA extraction. The first identification method followed McDowell and Graves (2002). Briefly, a 1200 bp band was amplified using primers for the nuclear locus MN32-2 (Buonaccorsi et al., 1999). The resulting band was digested with the restriction endonucleases *Dra* I and *Dde* I, (Invitrogen Corporation, Carlsbad, CA) and larvae were identified based on published

restriction profiles of adult billfishes (McDowell and Graves 2002). Digested DNA from adult sailfish, blue marlin, and white marlin were run on each gel as controls.

In a few cases, amplification of the 1200 bp band was not possible due to sample degradation. In these cases, an alternate protocol using species-specific multiplex primers developed by J. Magnussen and M. Shivji (pers. comm..) were used to identify samples. As above, controls consisted of DNA from adults of known identity. In cases where both of these simpler methods failed to result in unambiguous identification, larval DNA was amplified using the primers AllBill dloop F: 5' TTC CTG GCA TTT GGY TCC 3' and AllBill dloop R: 5' TAA GCT GTT AGA GGT TTT CC 3' designed for this study to amplify a short section of the mitochondrial control region.

Column cleaned PCR products were prepared for sequencing using the ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) at a 1/8 dilution according to the manufacturer's protocol. Amplfication products were subsequently sequenced on an ABI 313031300x*I* genetic analyzer (Applied Biosystems, Foster City, CA) using standard conditions. Standard chromatographic format (SCF) curves of each forward and reverse sequence were created using Sequencing Analysis v. 5.1.1 software (Applied Biosystems, Warrington, UK). The SCF curves of forward and reverse reactions were used to form a consensus sequence for each individual and bases were edited using Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, MI). Consensus sequences were aligned with control region sequences from unambiguously identified adult billfishes using the CLUSTALW algorithm (Thompson *et al.* 1994) in MacVector 7.2 (Oxford Molecular Ltd, Madison, WI). Sequences of adults included all Atlantic billfish species; blue marlin, sailfish, white marlin, longbill spearfish, and roundscale spearfish. Identification of larval billfishes was based on sequence similarity, visualized using a neighbor-joining algorithm (Saitou and Nei, 1987).

Estimate age and growth rates of larval blue marlin

Blue marlin larvae were measured to the nearest 0.1 mm body length (BL) using a dissecting microscope. Ethanol-preserved blue marlin larvae were soaked in medium-weight immersion oil for several days to prepare otoliths for ageing. Larvae were then transferred individually to a drop of oil on a microscope slide and examined under a dissecting microscope using polarized light. The polarized light enhanced the appearance of the otoliths. Sagittae were removed from larvae using very fine dissecting needles, and placed within a small circle that had been marked on the underside of the microscope slide. The circle was needed as a reference point so that the location on the slide of tiny otoliths would be known. The otoliths were then covered with another drop of oil and soaked for several more days following Sponaugle et al. (2005). Sagittae were used instead of lapilli because they provided overall higher clarity (Sponaugle et al. 2005). Daily otolith growth increments were observed by "optically sectioning" otoliths in the sagittal plane under a compound light microscope at 1,250x magnification using oil-immersion. Growth increments were most distinct when observed in a plane that passed though the otolith primordium.

Sponaugle et al. (2005) aged blue marlin larvae collected from Exuma Sound, Bahamas and the Straits of Florida. They presumed otolith growth increments were formed daily because back-calculated spawning dates of blue marlin larvae were in general agreement with adult spawning

patterns (Prince et al. 1991), and daily periodicity of otolith growth increments has been validated for many other pelagic fish (Tanabe et al. 2003; Jones 2002; Jenkins and Davis 1990). We subsequently assumed a diel periodicity of blue marlin otolith growth increments. However, it is important to note that subdaily rings are sometimes observed.

Because otolith growth increments were frequently not distinct we tried an alternate method to enhance detection of the growth increments. Otoliths were prepared to provide a cross-section through the otolith primordium by infiltrating larvae with epoxy resin, and then embedding them in small resin blocks following methodology outlined in Comyns et al. (1989) (Figure 10). Each resin block was cut with a Buehler isomet saw to obtain a small section containing the larval head and enclosed otoliths. The section was coarse sanded until the sagittae were near the surface of the sanded side, and then mounted sanded side down on a glass microscope slide with Crystal-Bond adhesive. The inverted block was carefully sanded with fine steel wool and polished until a cross section through the otolith primordium (center) was obtained. Otolith cross sections were viewed with a compound light microscope at 1,250x magnification under oil immersion and growth increments were counted. However, this methodology did not enhance the growth increments and was very time consuming, so ages were determined from whole sagittae that had been soaked in oil.

Daily otolith growth increments were often not well defined and the innermost couple of increments were frequently not visible. Growth increments were read along the longest otolith axis, and measurements (ocular units) from the primordium to each ring were recorded. Measurements taken for otoliths that had distinct innermost increments made it possible to account for these increments in otoliths for which they were not visible.

The growth of larval blue marlin was estimated only for larvae collected in the Loop Current during August 2005 because the majority of larvae were collected during this cruise. Larval length was regressed on age to estimate an exponential curve of blue marlin growth following the formula $L_t = L_0 * e^{Gt}$, where L_t = length at time t, L_0 = length at hatch and G = the instantaneous growth rate.

Figure 10. A 3.1 mm BL blue marlin larva embedded in a resin block for cross sectioning of the otolith. Otolith location is indicated by the arrow.

Identify potential blue marlin spawning and nursery areas

Larval transport from capture locations to potential spawning sites was computed using archived data from the Inter-American Seas Nowcast/Forecast System (IASNFS) developed and run by Dr. Dong-Shan Ko of the Naval Research Laboratory – Stennis Space Center. This model has been run and the currents archived at 6 hourly intervals from 2001 to the present. Larval capture dates within this period were taken as the starting times, and capture locations were taken as the starting locations for larval transport calculations. At each capture location and date, propagules were launched and traced backward for the number of days determined by ootilith aging as the larval age. Twelve (12) backward launches were made for each larva with sub-grid scale turbulence added to the archived field of currents. This turbulence was calculated as 20% of the current vector times a probability density function (random number) with variance of one.

IASNFS consists of an 1/24 degree (~4-6 km), 41-level sigma-z data-assimilating ocean model based on the Navy's Coastal Ocean Model (NCOM). Model topography is from the Naval Research Laboratory's high resolution DBDB2. The model continuously assimilates synthetic temperature/salinity profiles to produce a nowcast. In order to lock the model into real events, real-time data are assimilated from satellite altimeter (GFO, Jason-1, ERS-2) sea surface height (global) anomaly and AVHRR sea surface temperature (global). Three hourly surface heat fluxes, including solar radiation, wind forcing. To capture non-local events which propagate into the regime, the model is nested within a lower resolution global ocean model.

Findings

Gonadal collections and reproductive biology analysis of blue marlin from the northcentral GOM

Reproductive Seasonality

Blue marlin were captured in the ncGOM at tournaments from mid May through early September, and ranged in size from 253.4 – 351.3 cm LJFL and in weight from 140.4 – 478.8 kg. Only female fish were captured. Ovarian tissue for histological analysis was collected from 78 of the 80 fish captured during 2000 – 2007 (Figure 11); ovarian weights were obtained for 56 specimens. The mean GSI was very low during all months and showed a slight decrease from May to September (Figure 12). All fish had GSI values $\leq$ 1.0.

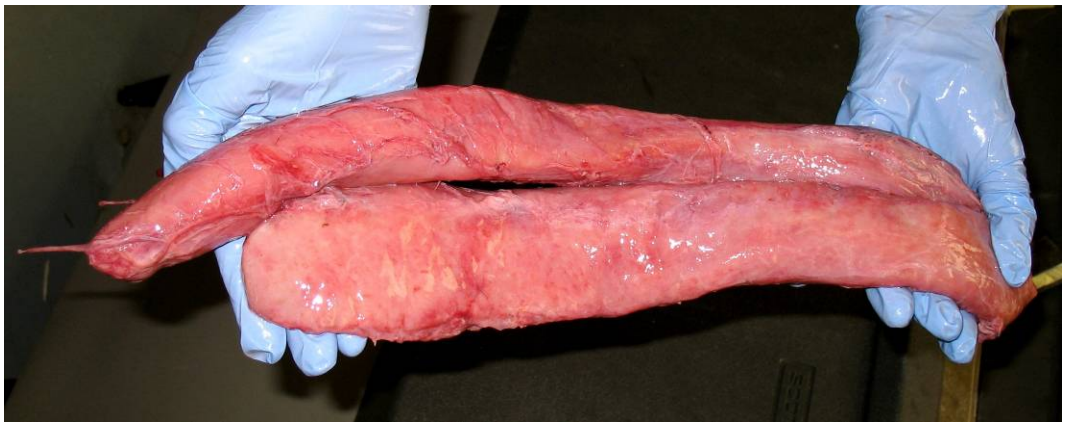

Figure 11. Ovaries from a 262.1 kg blue marlin.

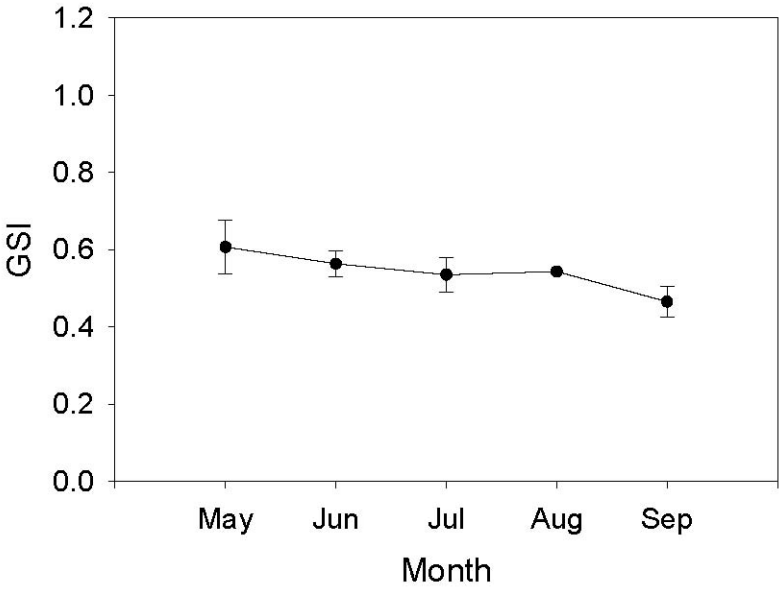

Figure 12. Mean (± S.E.) monthly Gonadosomatic index (GSI) values of female blue marlin from the northcentral Gulf of Mexico. Data represent fish collected from 2000 – 2007.

Blue marlin are relatively abundant in the northern GOM during the summer, and this study was fortunate to be able to obtain ovarian samples from 78 individuals during the May through September resident period. Furthermore, all blue marlin examined were sexually mature; weight

at sexual maturity for female blue marlin has been reported to range from 61.3 to 120 kg (Yeo 1978; Hopper 1990; deSylva and Breder 1997), well below the size of the smallest female captured in this study. Thus, all fish captured in this study were certainly capable of spawning. Previous reports of blue marlin reproduction have indicated that females with GSI values > 3 are reproductively active (Hopper 1990; Luckhurst et al. 2006). However, all of the females sampled from the northeastern Gulf of Mexico had GSI values $\leq$ 1, suggesting no spawning activity occurred in the area. Similarly low GSI values have been found in tournament-captured blue marlin from South Carolina (Cyr 1987) and Cabo San Lucas, Mexico (Ortega-García et al. 2006); fish from these areas were also considered reproductively inactive based on GSI values.

Gonadal Histology

All females captured were sexually mature. Histological inspection of ovarian tissue showed that none of the females captured from the ncGOM were spawning capable (Table 2). Furthermore, there were some females in the Regenerating phase, not undergoing ovarian recrudescence, in May, June, July and August. This suggests some female blue marlin may not spawn every year. The most commonly observed reproductive phase from May through July was the Early Developing subphase (Figure 13A), characterized by cortical alveolar oocytes and no vitellogenesis. The majority of fish in May and June were in this subphase (Table 2). The most advanced ovarian development seen was the Mid Developing subphase, with oocytes just beginning to sequester vitellogenin (Figure 13B); the highest percentage of fish in this subphase was seen in June (Table 2). Fully grown vitellogenic oocytes not undergoing atresia were not observed in any blue marlin captured from the ncGOM. However, evidence that blue marlin captured in the nGOM are capable of fully maturing oocytes comes from a fish in the Regressing phase, captured in early September, 2007. This female had masses of hydrated, but atretic, oocytes, remnants of an incomplete or unsuccessful spawning event earlier in the year (Figure 13C). A fish captured in early June, 2007 in the Early Developing subphase also had a mass of hardened, atretic hydrated oocytes, no doubt left over from a spawning event that occurred during summer 2006. However, it is unclear where these fish may have spawned and if they successfully released hydrated oocytes.

Table 2. Ovarian histological maturity phases of blue marlin collected in the northern Gulf of Mexico, 2000-2007. Data represents monthly percentages of females in each phase.

Month	N	Developing—Early*	Developing—Mid*	Spawning Capable	Regressing	Regenerating
May	8	75	12	0	0	13
June	41	60	18	0	4	18
July	23	35	13	0	17	35
August	3	33	0	0	33	34
September	3	0	0	0	100	0

*Ovaries in the early subphase are characterized by cortical alveolar oocytes and no vitellogenesis; ovaries in the mid subphase have oocytes beginning to sequester vitellogenin.

By July, 17% of the females were in the Regressing phase, with histological evidence of some atretic vitellogenic oocytes in the ovary (Figure 13D); this percentage increased in August and

early September. The presence of females in the Regressing phase suggests spawning had occurred several weeks prior to capture based on lack of post-ovulatory follicles (POF) and the advanced atretic stages of the oocytes in all fish in this phase (Figures 13C, 13D). The greatest percentage of fish in July and August were in the Regenerating phase, indicating cessation of spawning for the season. Thus, histological evidence suggests blue marlin in the ncGOM have a relatively short spawning season from late June through August, based on histological assessment of ovarian development. This conclusion is supported by a single report of a female blue marlin in the Actively Spawning phase, with hydrated oocytes, captured west of the Mississippi River in Louisiana in July, 2005 (J. Yurt, Louisiana Department of Fish and Wildlife, pers. comm.). Unfortunately, the ovarian sample collected from this fish was lost during Hurricane Katrina before it could be processed and examined. Interestingly, no fish captured east of the Mississippi River showed any indications of active spawning.

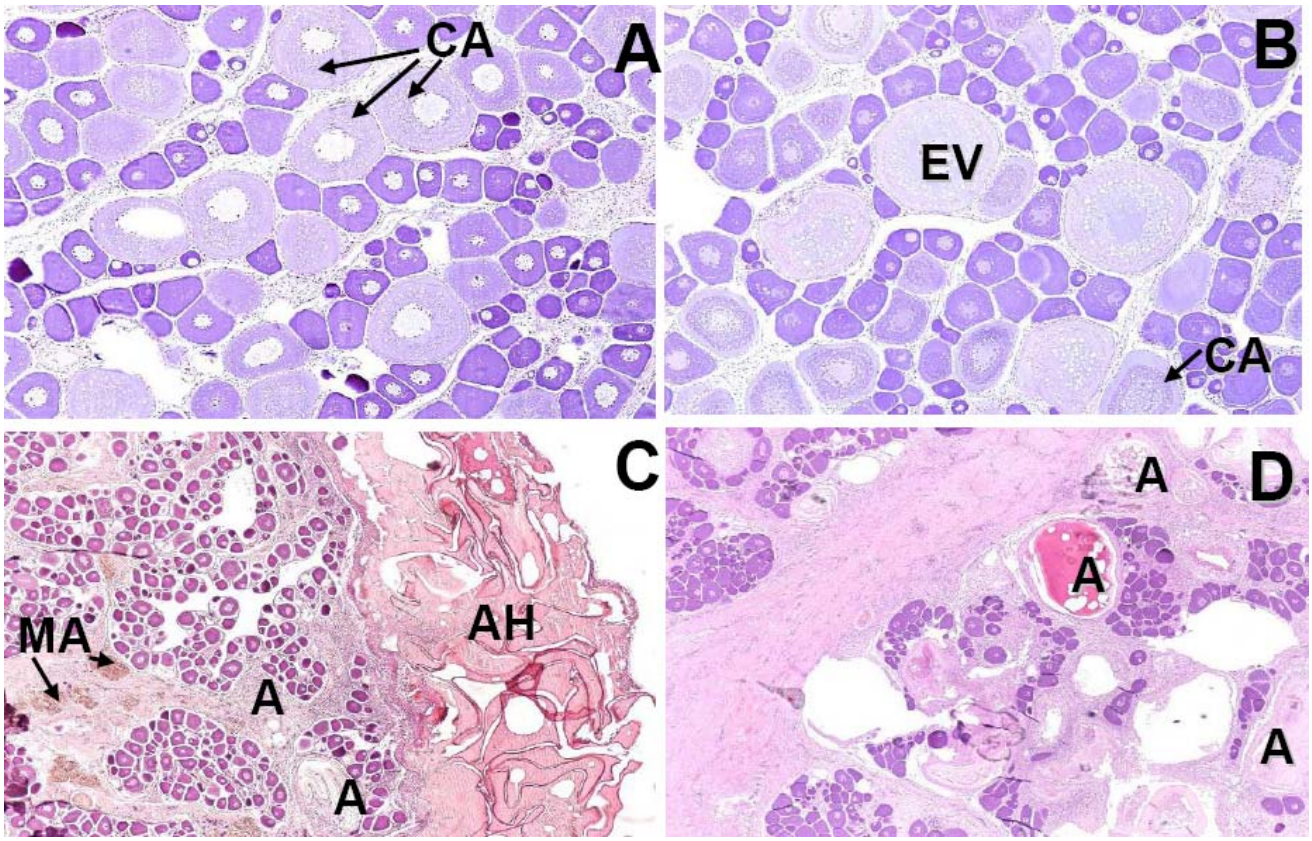

Figure 13. Histological photographs of ovaries from blue marlin captured in the northern Gulf of Mexico. A. Ovary in the early Developing phase, captured in July 2006. The most advance stage of oocyte development is cortical alveoli. 100X. B. Ovary in the Developing phase, captured in July 2005. This individual had the most advanced oocyte stage of any blue marlin examined. 100X. C. Ovary in the Regressing phase captured 1 September 2007. Areas of atretic hydrated oocytes suggest this female was unable to release all hydrated oocytes during the spawning season. 40X. D. Ovary in the Regressing phase captured in July 2006. The presence of atretic vitellogenic oocytes suggests this female spawned earlier in 2006. 40X. Key: A—atretic oocyte; AH—atretic hydrated oocytes; CA—cortical alveolar oocyte; EV—early vitellogenic oocyte; MA—macrophage aggregates.

A female in the developing phase captured in July 2005 (265.4 cm LJFL, 214.7 kg) had a large ovary (8.26 kg) that was almost entirely filled with a parasite infection (Figure 14). The GSI of this fish was high (4.0) due to the large number of parasites; this fish was not included in the monthly GSI calculations for this reason. The parasite has been tentatively identified as a didymozoid, previously reported by Russian scientists in blue marlin from the Indian Ocean (A. Bullard, USM, pers. comm.). However, this represents the first report of the parasite from the United States as well as the first histological sections of the parasite from the gonad of a blue marlin.

The relatively high percentages of mature blue marlin showing no ovarian development during May, June and July suggests some individuals may skip spawning seasons. The phenomena of skip spawning has been documented in Gulf of Mexico groupers (Collins et al. 2002; Fitzhugh et al. 2006) as well as Atlantic cod (Rideout et al. 2005; Jorgensen et al. 2006), and may occur more frequently in large species than originally suspected. The possibility that blue marlin use this reproductive strategy needs additional investigation, and may suggest an explanation for the large numbers of reproductively inactive blue marlin captured in the northeastern GOM.

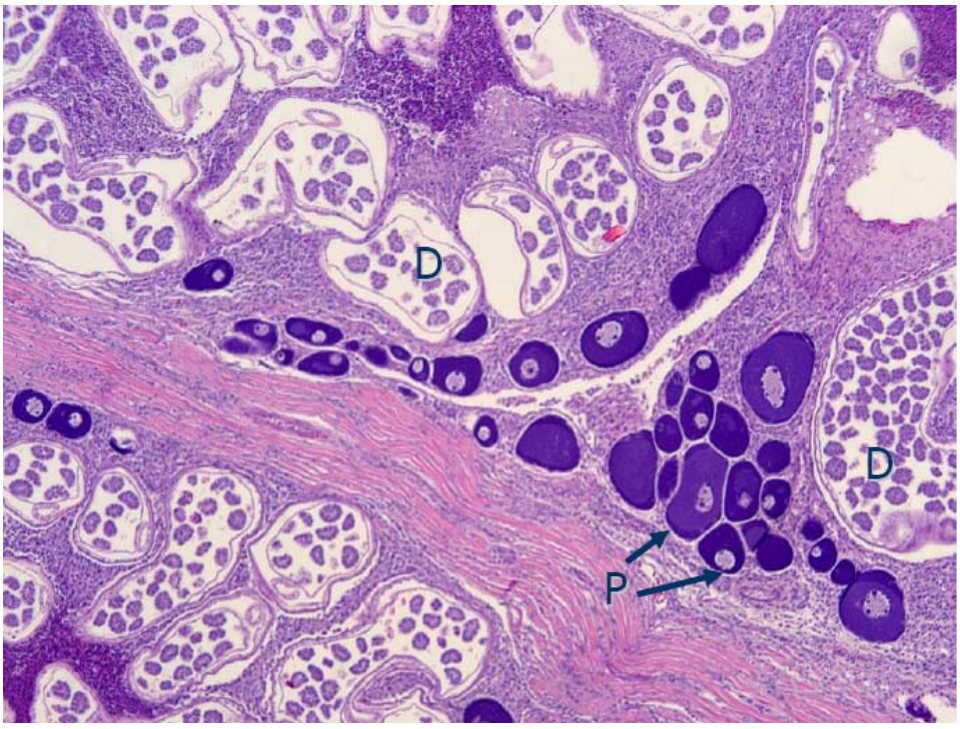

Figure 14. Histological section of an ovary from a blue marlin showing a massive didymozoid parasite infection. D—didymozoid parasite; P—primary oocytes

Histological examination of ovarian tissue indicates that blue marlin captured in the northeastern Gulf of Mexico from May through September are not spawning. However, ovarian recrudescence was occurring in the majority of blue marlin captured in May, June and July during the course of this study, although no non-atretic oocytes more advanced than early vitellogenesis were seen. Thus, while blue marlin from this section of the GOM do exhibit some

ovarian growth and maturation, it appears that spawning is not likely to occur in GOM waters east of the Mississippi River during summer. In contrast, blue marlin have been reported to spawn from May through August from a number of locations based on histological inspections (Bahamas, Yeo 1978; Bermuda, Luckhurst et al. 2006; Caribbean, Erdman 1968; Hawaii, Hopper 1990). The lack of spawning capable and actively spawning blue marlin in our collections during the reported reproductive season despite a sizeable sample size is surprising.

Collection of larval blue marlin and other istiophorids from the north central GOM

2000 – 2005 collections in *Sargassum*

Between June and August, 2000 – 2005, multiple collections in *Sargassum* habitat in the north-central Gulf produced a total of 17,142 larval fishes. Of this number of larval fish, 43 istiophorids were collected and only one was identified as a blue marlin.

Additional collections in *Sargassum* habitat that was associated with the Loop Current were taken from June through August 2003 – 2004. A total of 16,363 larval fishes were taken during these collections, representing 60 istiophorid billfishes and 3 blue marlin. In total, 4 blue marlin larvae were taken during the five years of sampling *Sargassum* habitat, suggesting *Sargassum* may not be an important nursery habitat for larval blue marlin, particularly when it is not associated with the Loop Current.

2005 collections in Loop Current

Only transects along the Loop Current (transects 2 – 8 in Figure 6) produced billfish larvae. A total of 8,234 larvae were collected during this cruise; 244 were istiophorid larvae and 39 were identified as blue marlin. Table 3 lists the 48 collections taken during the cruise, with latitude, longitude, temperature, and salinity. Additionally, the volume of water filtered during each collection is shown, along with total istiophorid larvae collected and the number of blue marlin larvae from each collection. The first 6 collections (BUM01 – BUM06) were taken in the Loop Current eddy (transect 1 in Figure 6), and no billfish larvae were captured in this location. Collections BUM07 - BUM12 were taken on transect 2 and produced 21 billfish larvae, 3 of which were blue marlin (collection BUM 09). Transect 3 (BUM13 - BUM18; 37 billfish larvae, 11 blue marlin), transect 4 (BUM19 – BUM24; 69 billfish larvae, 8 blue marlin) and transect 5 (BUM25 – BUM29; 69 billfish larvae, 15 blue marlin) were the most productive transects, with BUM25 alone containing 42 billfish larvae and 12 blue marlin. Transect 6 (BUM30 – BUM35; 8 billfish larvae) and transect 7 (BUM36 – BUM40; 7 billfish larvae) collections produced no blue marlin. Transect 8 (BUM41 – BUM48) collections contained 32 billfish larvae and 2 blue marlin (BUM47). Blue marlin larvae were collected at a temperature and salinity range of 29.7 – 31.9^{o}C and 35.1 – 36.2 ppt, respectively.

2006 collections in DeSoto Canyon

A week of intensive sampling in the DeSoto Canyon area of the northcentral Gulf of Mexico during August 2006 produced 4,132 larval fishes, including 19 istiophorids. However, none of the billfishes were identified as blue marlin. These results, in combination with the 2000 – 2005 *Sargassum* samples and the 2006 samples from the Loop Current, suggest larval billfish only occur in the ncGOM in areas associated with the Loop Current. In 6 years of sampling a total of 29,508 fish larvae, only one blue marlin larvae was not associated with the Loop Current. Other

istiophorids, such as sailfish, were occasionally captured in non-Loop Current samples and do not appear to to be as limited in their larval distribution in the ncGOM as do blue marlin.

Table 3. Summary of collection and abundance data for larval istiophorids and *Makaira nigricans* taken in near-surface neuston net collections from the Loop Current boundary, 6-11 August 2005.

Collection	Date	Latitude	Longitude	Temp. °C	Sal. ppt.	Vol. Filtered (m^3)	Istiophoridae		*M. nigricans*	
							No. Larvae	No. Larvae per 100 m^3	No. Larvae	No. Larvae per 100 m^3
BUM01	08/07/05	28 20.38	90 02.14	30.8	32.8	216	0	0.00	0	0.00
BUM02	"	28 18.82	90 00.88	30.6	32.9	170	1	0.59	0	0.00
BUM03	"	28 17.03	90 00.14	30.4	33.4	157	0	0.00	0	0.00
BUM04	"	28 15.53	89 58.99	30.3	34.8	167	0	0.00	0	0.00
BUM05	"	28 14.09	89 57.77	30.1	36.2	133	0	0.00	0	0.00
BUM06	"	28 12.23	89 56.10	30.2	36.3	179	0	0.00	0	0.00
BUM07	08/08/05	24 18.18	87 26.60	30.1	35.8	176	0	0.00	0	0.00
BUM08	"	24 18.16	87 27.49	30.3	35.9	147	2	1.36	0	0.00
BUM09	"	24 18.15	87 31.59	30.9	35.9	165	5	1.21	**3**	**3.03**
BUM10	"	24 18.04	87 22.58	30.8	36.0	168	3	1.79	0	0.00
BUM11	"	24 18.09	87 20.50	31.4	35.9	187	1	0.53	0	0.00
BUM12	"	24 18.02	97 17.26	31.5	35.5	155	10	6.45	0	0.00
BUM13	"	24 09.02	87 31.86	31.3	35.6	167	0	0.00	0	0.00
BUM14	"	24 09.02	87 33.60	30.9	36.2	149	5	3.35	**2**	**1.34**
BUM15	"	24 09.87	87 38.66	30.7	36.0	164	15	9.16	**7**	**4.27**
BUM16	"	24 09.09	87 29.76	30.7	35.9	185	12	6.48	**1**	**0.54**
BUM17	"	24 09.43	87 26.71	30.6	35.6	150	5	3.34	**1**	**0.67**
BUM18	"	24 09.09	87 23.68	30.0	35.3	164	0	0.00	0	0.00
BUM19	08/09/05	24 59.04	87 23.87	29.7	36.0	152	25	16.40	**4**	**2.62**
BUM20	"	24 59.11	87 26.60	30.2	36.0	154	33	21.40	**3**	**1.95**
BUM21	"	24 59.05	87 31.30	30.7	36.2	153	0	0.00	0	0.00
BUM22	"	24 59.60	87 21.77	31.2	36.0	151	0	0.00	0	0.00
BUM23	"	24 58.99	87 18.33	31.4	36.2	163	2	1.23	0	0.00
BUM24	"	24 59.06	87 15.01	31.9	35.1	176	9	5.12	**1**	**0.57**
BUM25	"	24 03.13	87 22.32	31.2	36.0	171	42	24.53	**12**	**7.01**
BUM26	08/09/05	25 04.52	87 24.48	31.2	35.9	207	19	9.16	**1**	**0.48**
BUM27	"	25 04.14	87 29.06	31.3	36.0	190	4	2.10	**1**	**0.53**

BUM28	"	25 04.07	87 20.30	30.4	36.0	200	0	0.00	0	0.00
BUM29	"	25 03.94	87 14.91	30.2	35.6	169	4	2.37	**1**	**0.59**
BUM30	08/10/05	25 00.09	84 58.57	30.1	36.0	154	4	2.60	0	0.00
BUM31	"	25 00.02	84 55.99	30.4	36.8	164	4	2.44	0	0.00
BUM32	"	25 00.13	84 51.34	30.4	36.0	146	0	0.00	0	0.00
BUM33	"	24 59.65	85 02.02	30.6	36.0	180	0	0.00	0	0.00
BUM34	"	25 00.02	85 04.03	30.8	35.8	205	0	0.00	0	0.00
BUM35	"	25 00.06	85 07.43	31.2	36.0	207	0	0.00	0	0.00
BUM36	"	25 05.19	85 04.68	30.9	36.0	194	1	0.51	0	0.00
BUM37	"	25 04.88	84.57.66	31.6	35.5	201	6	2.99	0	0.00
BUM38	"	25 05.26	84.53.57	31.7	35.6	205	0	0.00	0	0.00
BUM39	"	25 05.03	85 06.70	30.7	35.3	205	0	0.00	0	0.00
BUM40	08/11/05	25 05.17	85 13.07	30.5	36.5	184	0	0.00	0	0.00
BUM41	"	26 19.92	85 41.17	30.1	36.0	128	0	0.00	0	0.00
BUM42	"	26 18.45	85 45.31	31.0	36.0	168	1	0.60	0	0.00
BUM43	"	26 17.54	85 49.47	30.7	35.9	148	0	0.00	0	0.00
BUM44	"	26 16.16	85 53.50	31.2	36.0	159	0	0.00	0	0.00
BUM45	"	26 14.43	84 57.50	31.1	36.0	160	0	0.00	0	0.00
BUM46	"	26 13.69	86 01.67	31.4	35.8	186	10	5.37	0	0.00
BUM47	"	26 12.57	86 05.83	30.1	35.5	164	21	12.82	**2**	**1.22**
BUM48	"	26 10.98	86 08.81	30.7	36.0	133	0	0.00	0	0.00

Identification of larval blue marlin and other istiophorids using morphometric and molecular techniques

Morphometric identification

Initial morphometric identifications were done on istiophhorid larvae captured in association with *Sargassum* as part of another project during 2000 – 2004. Of the 16 larvae submitted for genetic analysis from these collections, only six specimens provided DNA of a quality that could provide larval identifications. This was likely because these specimens were several years old. Of these six fish, two were white marlin, three were sailfish, and one was a blue marlin. All three sailfish and the blue marlin were correctly identified morphologically using characteristic lower jaw pigment patterns.

The second batch of istiophorids (n=48) that were genetically analyzed were collected as part of the current study, and apparently provided DNA of a higher quality; identifications were determined for 38 of these specimens. Nine blue marlin larvae and 10 sailfish larvae were identified based on lower jaw pigment, and the correct identification of all specimens was genetically verified. Based on these results, we were confident that our morphological identifications were accurate since genetic analysis confirmed all our morphological

identifications. A blue marlin larva, confirmed by morphological and molecular identification, is shown in Figure 15.

Figure 15. Blue marlin larvae, 2.9 mm BL captured in the Loop Current on 8 August 2005.

Because only ~ 40% of pre-flexion blue marlin larvae and ~ 60% of sailfish larvae can be confidently identified using lower jaw pigment (Luthy et al. 2005), all larvae with intermediate lower jaw pigment (n=62) were genetically analyzed. It was not deemed necessary to genetically analyze an additional 14 larvae that possessed the characteristic lower jaw pigment of pre-flexion blue marlin larvae due to results from the two previous batches of molecular analysis. Of these 62 larvae with intermediate pigment patterns, 15 were blue marlin, 41 were sailfish, and six were white marlin. Thus, a total of 39 blue marlin larvae were identified from the northcentral Gulf of Mexico during the course of this project, and blue marlin larvae were not as abundant as sailfish larvae in the region.

It is noteworthy that that we collected white marlin larvae during August in the Loop Current, because Luthy et al. (2005) use June as a cutoff point in a dichotomous key to distinguish white marlin from blue marlin and sailfish collected in the Straits of Florida and the Bahamas. It is assumed in the key that larvae caught later than June are not white marlin, since the spawning season of white marlin in the Florida Current has been reported to be March through June (Richards and Luthy 2006). However, the collection of several larval white marlin in August 2005 in the Loop Current suggests the spawning season of white marlin in this region may be longer than previously reported.

Molecular identification

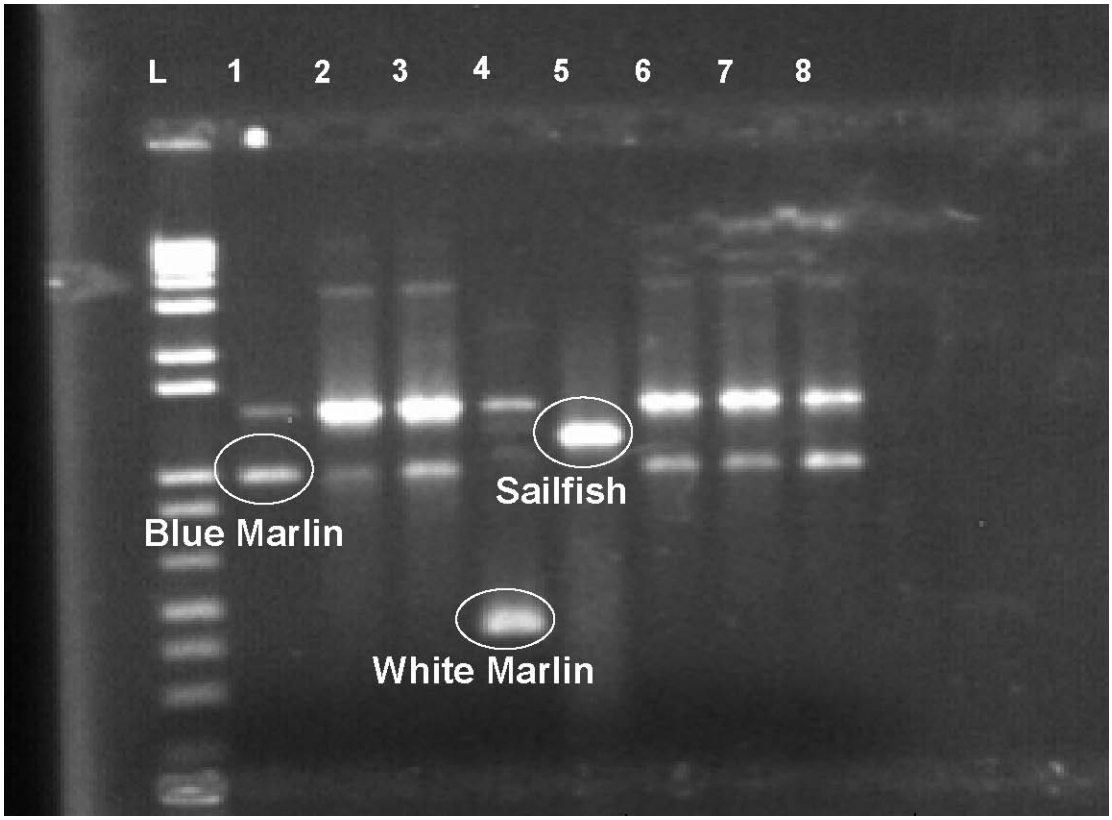

Figure 16. Gel showing specific identification of istiophorid billfish larvae amplified by primers and digested with restriction endonucleases. Unknown samples were identified as blue marlin.

Of the 48 larvae examined in 2006, 31 were identified as sailfish and 13 were identified as blue marlin. Two other larvae were tentatively identified, one as blue marlin and one as sailfish, although these identifications were ambiguous. Due to the presence of degraded DNA, three larvae remained unidentified. Twenty-one larvae were identified using the restriction site method (Figure 16), 17 were identified using the multiplex method and sequencing of the control region was used to identify 9 of the larvae.

Of the 82 samples sent in 2007, 68 were unambiguously identified. The extremely small size of the larvae collected meant that control region sequences were used to identify the majority of the samples; 62 of the 68 identified larvae were sequenced using this protocol while 6 others were identified using the multiplex method. Of the 68 larvae identified, 45 were sailfish, 15 were blue marlin and 6 were white marlin. Two additional larvae were tentatively identified, one as blue marlin and one as sailfish although unambiguous identification was not possible due to the poor quality of the sequences.

Estimate age and growth rates of larval blue marlin

The larval blue marlin otoliths were exceptionally difficult to age; all growth increments were never visible in a single plane of view under the microscope (Figure 17). Furthermore, daily otolith growth increments were often not well defined and the innermost couple of increments

were frequently not visible. Measurements taken from the primordium to each daily growth increment for otoliths that had distinct innermost increments made it possible to account for these increments in otoliths for which they were not visible.

A total of 43 blue marlin larvae were collected during the course of this project. Whole otoliths were recovered from 37 of these fish and used for ageing; the otoliths shattered during removal from the other 6 larvae and were thus discarded. Blue marlin larvae ranged in age and length from 4 - 12 days, and 2.5 - 5.2 mm body length (BL), respectively (Table 4). The majority of the larvae captured were $\leq$ 7 days old.

Most of the aged specimens (n=34) were collected in August 2005 from the Loop Current, and growth rates were estimated using these larvae. Based on the growth equation (Figure 18), the mean growth rate of 4-12 day-old-larvae was 0.25 mm/day. This is slower than growth rates reported by Sponaugle et al. (2005). These authors examined growth rates of blue marlin larvae collected at six locations in the Straits of Florida and Exuma Sound. Based on their growth equations, the mean growth rate of 4-12 day-old-larvae ranged from 0.40 to 0.81 mm per day. It should be noted that our growth rates were strongly influenced by smaller larvae; only two of the 34 larvae were >7 mm.

Table 4. Larval blue marlin length and age data.

Fish I. D.	Collection	Date	Body Length (mm)	Age (days)
2	19-13-1*	6/05/04	4.2	10
B66	"	"	3.0	NA
B74	19-10-1*	"	3.1	6
B77	25-05**	6/27/05	2.7	5
B3	BUM 09	8/08/05	2.8	5
B4	"	"	2.9	5
B49	"	"	2.9	6
B82	BUM 14	"	3.1	7
B9	"	"	2.6	5
B6	BUM 15	"	2.9	5
B8	"	"	2.9	5
B50	"	"	3.0	4
B51	"	"	2.8	4
B52	"	"	2.6	5
B85	"	"	2.7	5
B86	"	"	2.6	6
B58	BUM 16	"	2.9	5
B88	BUM 17	"	2.9	NA
B19	BUM 19	8/09/05	3.2	8
B23	"	"	2.8	5
B93	"	"	2.7	6
B94	"	"	2.6	4
B56	BUM 20	"	3.1	NA

B57	“	"	2.6	5
B14	“	"	2.9	NA
B55	BUM 24	“	2.6	4
B60	BUM 25	“	3.7	NA
B61	“	"	2.9	7
B62	“	"	3.0	7
B63	“	"	3.2	7
B64	“	"	2.9	6
B27	“	"	3.0	5
B30	“	"	5.2	12
B108	“	"	2.9	5
B109	“	"	3.3	6
B110	“	"	3.1	6
B111	“	“	2.5	5
B113	“	"	3.4	7
B38	BUM 26	“	3.1	6
B43	BUM 27	“	3.0	6
B36	BUM 29	“	2.3	NA
B54	BUM 47	8/11/05	2.6	5
B11	“	"	3.2	7

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* = Blue marlin specimens found in Loop Current collections at *Sargassum*.

** = Blue marlin specimen found in northern GOM collection at *Sargassum*

BUM = Blue marlin specimens found in Loop Current during billfish cruise

NA = Not aged

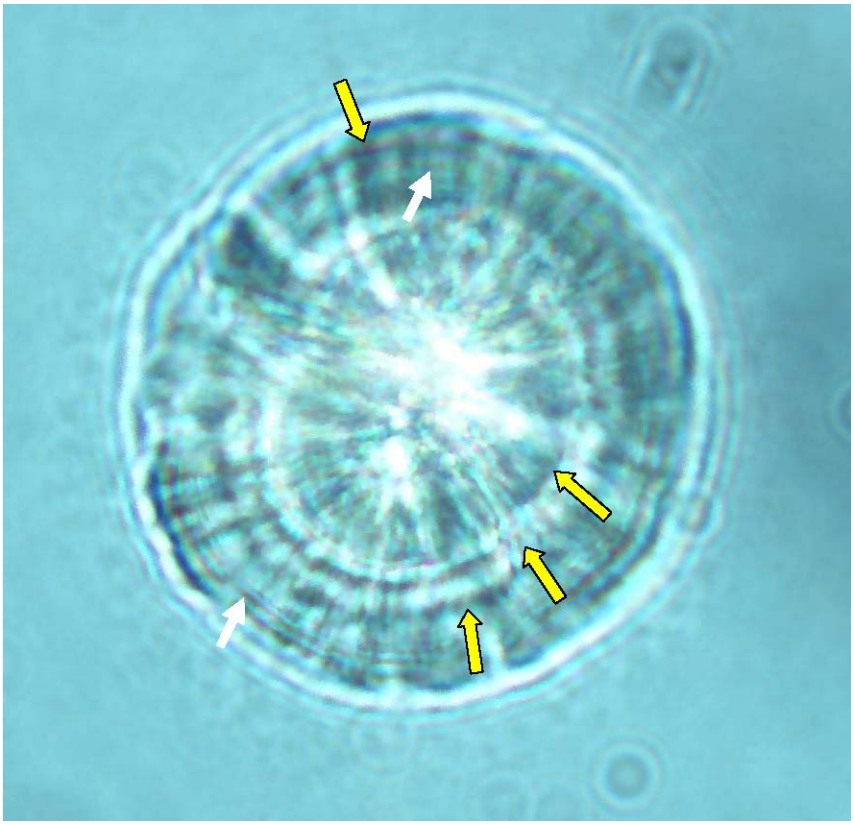

Figure 17. Otolith of a 5 day old blue marlin larvae showing four distinct bands (yellow arrows). The innermost ring is not visible in this plane of focus. Subdaily rings are indicated by the white arrows.

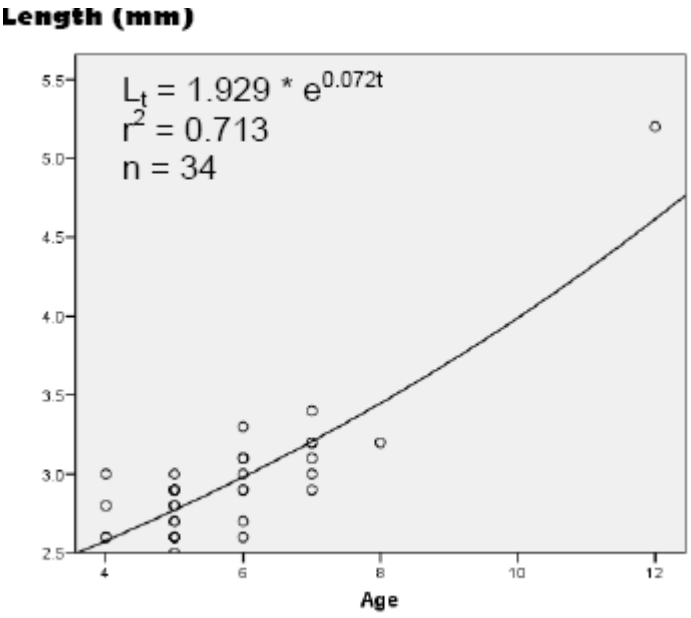

Figure 18. Age-length relationship for blue marlin larvae collected from the Loop Current in August 2005.

Identify potential blue marlin spawning and nursery areas

Initial tests of the larval transport model with a single, 7 day old blue marlin larva captured in the loop current in the central Gulf of Mexico during August 2005 suggests this fish was spawned off the Yucatan peninsula (Figure 19). Variation in the path of this model was minimal along 10 simulations run with sub-grid scale turbulence, providing strong evidence that this larva was spawned along the upper continental slope near the entrance to the Gulf, not in the central Gulf as previously thought.

When the locations and dates of all blue marlin larvae captured during the course of this project (n=38) were input into the model, there is strong evidence that blue marlin appear to be spawning off the Yucatan Peninsula in Mexico (Figure 20). In all but two cases, all 16 passes (applying sub-grid turbulence for each pass) of the model for each capture point resulted in predicted spawning along the shelf break and upper slope of the Yucatan Peninsula. The exceptions include one larva captured in the central GOM (Point A in Figure 20) and one larva captured on the shelf off the Mississippi Coast (Point B in Figure 20). There were actually two larvae captured in the central GOM near Point A at the edge of the Loop Current. In the figure the symbols are overlapping. The southern of these two capture locations tracked back to the upper slope region of the Yucatan Peninsula. When the northern of the capture locations was moved 0.15^{o} south, it too tracked back to the Yucatan Peninsula. Modeling the edge of the Loop Current with such precision was a remarkable achievement.

There are two potential explanations for the predicted spawning location of Point B in Figure 20. The transport model does not predict the current flow as accurately crossing the slope and shelf break locations as in the open GOM. Thus, the calculated shelf spawning location may be an artifact, particularly since no spawning-capable adult fish were captured along the shelf off Mississippi during the course of this project. It is also possible that some blue marlin spawn in the northwestern Gulf and this larva was transported from a shelf or shelf-break area to the capture location. Numerous small blue marlin larvae have been captured in the northwestern GOM during June and July (J. Rooker, Texas A&M University, pers. comm.; http://www.tamug.edu/pelagic/billfish_life_ecology.html); the spawning location of these fish is currently unknown.

Overall, a significant finding of this project is the consistent prediction of a blue marlin spawning area along the shelf break and upper slope of the Yucatan peninsula. The proximity of the Loop Current to this location ensures transport of planktonic eggs and larvae into the central Gulf of Mexico. The Loop Current is characterized by convergences, upwellings and strong flow (current speed characteristically 50 cm/s) along its outer boundary. Planktonic organisms, including larvae of Caribbean and southern GOM origins, can become entrained and transported into the northern GOM by the Loop Current (Johnson et al. 1992, Gasca et al. 2001), as predicted by the transport model. There has been speculation that cyclonic eddies in Hawaii are important areas of Pacific blue marlin (*M. mazara*) reproduction due to the capture of larvae in these eddies but not in adjacent, non-eddy sites (Seki et al. 2002). These eddies formed off the west coast of the big island along the shelf/slope break and followed the island topography, thus providing similar oceanographic features to those observed off the Yucatan Peninsula. Based on available

data and simulations, it appears that blue marlin spawn along the shelf/slope area, often in conjunction with currents.

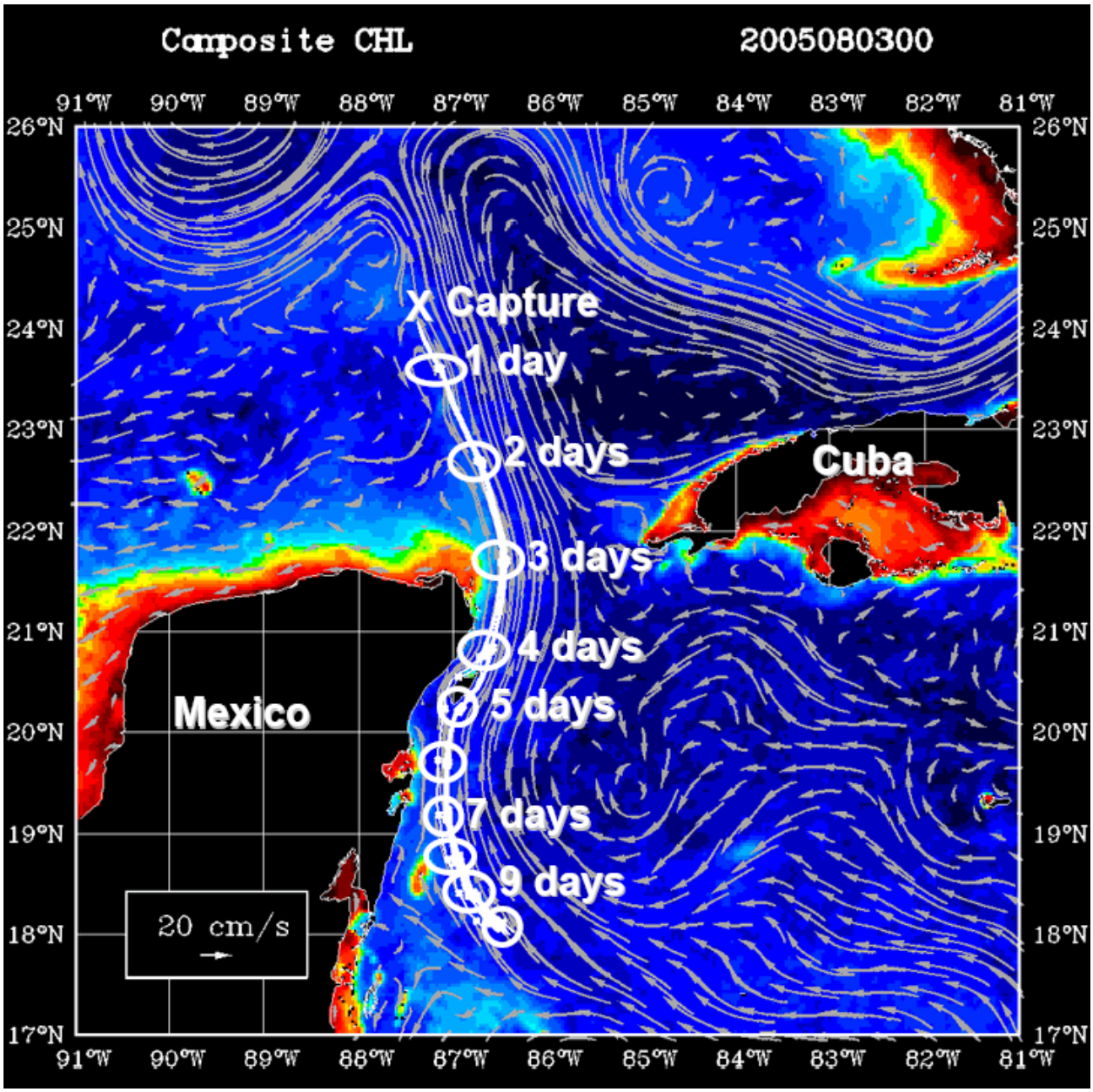

Figure 19. Model of larval transport of a single blue marlin larva captured along the Loop Current in the north central Gulf of Mexico in August 2005. The current and temperature profiles represent conditions at time of larval sampling.. Each circle indicates estimated spawning location for estimated age based o the capture location. This fish was estimated to be 7 days old.

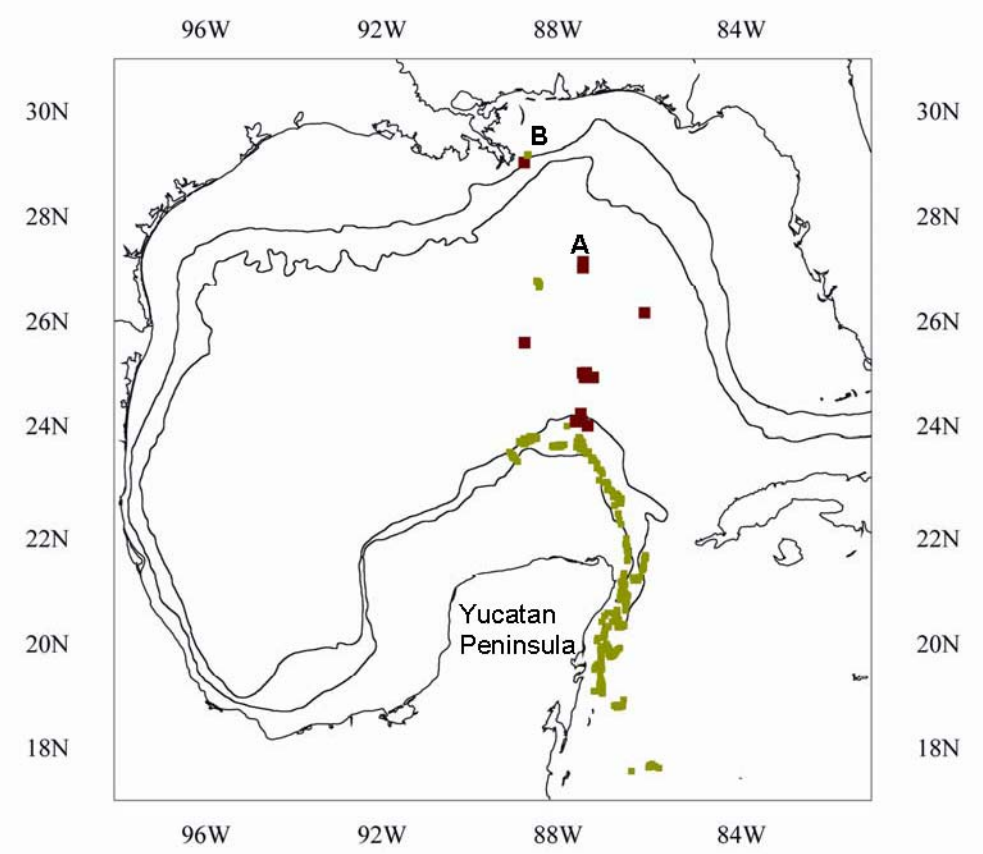

Figure 20. Predicted blue marlin spawning locations (green squares) based on capture locations (red squares) and age of fish. Only 2 of the 38 larvae did not track back to a spawning location along the Yucatan Peninsula; point A in the northcentral Gulf of Mexico and point B along the shelf/slope break off Mississippi.

The fecundity and spawning frequency of blue marlin was not able to be evaluated since no spawning capable or actively spawning fish were captured during the course of this project. All other project objectives were met, although no-cost extensions were necessary to complete the project due to the impact of Hurricane Katrina on the Gulf Coast Research Laboratory. Destruction of laboratory space and equipment resulted in major delays in ichthyoplankton sorting, identification and ageing. Luckily, the only sample lost in Hurricane Katrina was an ovarian sample from an actively spawning blue marlin captured in Louisiana. However, since this was the only sample of a potentially actively spawning fish obtained during the project, the loss was unfortunate.

Unanticipated difficulties in obtaining molecular identification of some istiophorid larvae due to sample degradation required an alternative protocol using multiplex primers developed by J. Magnussen and M. Shivji at Nova Southeastern University. Additionally, the small size of many of the samples required using the entire body posterior of the head, rather than the eyeball, to achieve reliable results for molecular analysis. Finally, difficulties in reading daily rings on blue marlin otoliths required investigations of techniques to enhance the appearance of the rings. One promising, but time consuming, technique of embedding whole larvae in resin and sectioning

until the otolith was visible did not improve the visibility of the rings and was subsequently abandoned.

Examination of female adult blue marlin suggests the species does not spawn in the northern Gulf of Mexico east of the Mississippi River. The lack of larvae in the same section of the GOM, with the exception of those found at the Loop Current, supports this speculation. However, there is strong anecdotal evidence suggesting that blue marlin do spawn in some areas of the northern GOM during the summer. Evidence of previous spawning, in the form of hydrated, but atretic, oocytes in females with regressing ovaries was seen in July, August and early September, indicating spawning occurred several weeks prior to capture. While blue marlin are a highly migratory species, it seems doubtful that these fish spawned in the Caribbean or Straits of Florida and immediately returned to the GOM. Satellite tagging data supports the premise that blue marlin remain in the northern Gulf of Mexico during June and July (J. Rooker, Texas A&M University, Galveston, pers. comm.). Additionally, one female blue marlin with hydrated oocytes was captured in Louisiana west of the Mississippi River in July 2005 (J. Yurt, Louisiana Department of Fish and Wildlife, pers. comm.), and numerous small blue marlin larvae have been captured in the northwestern GOM during June and July (J. Rooker, Texas A&M University, Galveston, pers. comm.; http://www.tamug.edu/pelagic/billfish_life_ecology.htm). Unfortunately, ovarian tissue of blue marlin from the northwestern GOM has not been examined, and thus the reproductive status of adults in the area is undocumented. The available data suggest blue marlin may indeed spawn in the northern GOM west of the Mississippi River; additional coordinated collections of both adults and larvae from this region are necessary to provide definitive data.

Data from the larval transport models strongly suggest blue marlin are spawning in Mexican waters off the Yucutan peninsula. However, there have been no collections of adult fish or ichthyoplankton from these waters. Thus, coordinated collections of both adults and larvae from the Yucutan peninsula during June through August are necessary to obtain a better understanding of the spawning dynamics of blue marlin in this portion of the Gulf of Mexico.

The abundance of blue marlin in the northcentral Gulf of Mexico during the spawning season despite being reproductively inactive requires additional investigation. Satellite tagging of these fish would provide information on their movements, and may document if they move between potential spawning grounds both around the Yucutan Peninsula and in the northwestern GOM and probably feeding grounds in the northcentral GOM. Tagging data would also provide information on where fish from the GOM overwinter, if the same individuals return to the GOM each year, and if some females in the population do not spawn every year, as suggested by histological results.

Conclusions

All original project goals and objectives were met with the exception of estimating fecundity and spawning frequency of blue marlin. We were unable to complete this objective due to the lack of spawning capable or actively spawning blue marlin in the area; fish with fully grown vitellogenic oocytes are necessary for these estimates, and none of the marlin examined during the course of this project had fully grown vitellogenic oocytes. However, data from this project has provided a wealth of new information on blue marlin in the northcentral GOM, including a better

understanding of adult reproductive potential and larval identification and ageing. These data were combined to provide insights into spawning areas for blue marlin in the northern GOM through larval transport models, which provided the surprising result of an apparent blue marlin spawning "hot spot" in Mexican waters off the Yucutan peninsula.

Acknowledgments

The authors thank NOAA/NMFS/Gulf States Marine Fisheries Commission for funding this research. Derek Snodgrass (NMFS, Miami) provided archived data and gonad samples from blue marlin collected from 2000 – 2004. Anna Avrigian (NMFS, Panama City) was instrumental in coordinating the collection of gonad samples from billfish tournaments during 2005 – 2007 through her network of port samplers. We thank Anna Avrigian, Craig Martin (NOAA/NMFS) and Joe Yurt (NOAA/NMFS) for providing both samples and data from these tournaments. Cheryl Crowder (LSU Veterinary Pathology) produced high quality histological sections for analysis. Ash Bullard (USM) identified the parasites and took the photograph of the parasitized gonad. We thank Don Johnson, Richard Waller, Paul Grammer, James Ballard, Arthur Karels and the captains and crew of the R/V Tommy Munro who participated in the two ichthyoplankton cruises. The majority of the ichthyoplankton sorting was done by Mae Blake; her perseverance throughout this task in less than ideal post-Katrina conditions is greatly appreciated. James Ballard assisted with the morphological identification of blue marlin larvae. Assistance in the laboratory for molecular identification was provided by Emily Chandler. We thank Eric Hoffmayer, Read Hendon and Kelly Lucas for their expertise in making maps. Finally, we are indebted to Dr. Dong-Shan Ko (Naval Research Laboratory, Stennis Space Center) for producing the larval transport models, and to Dr. Donald Johnson for his interpretation of these models.

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