J

Proposal Submission to

Recreational Fishery Advisory Board

by

The Virginia Institute of Marine Science
College of William and Mary

Genetic analysis of the distinctiveness of Cobia, *Rachycentron canadum*, from Chesapeake Bay

Proposed starting date: 1 January 2017
Proposed duration: 12months

Dr. Jan R. McDowell
Principal Investigator
Department of Fisheries Science Sciences

Dr. Hamish J. Small
Co-Principal Investigator
Department of Aquatic Health

Ms. Susanna Musick
Co-Principal Investigator
Advisory Services

Thomas J. Murray/Cht.
Mr. Thomas J. Murray
Associate Director for
Advisory Services

email approval attached
Dr. John E. Graves
Department Head
Department of Fisheries Science

Dr. Kimberly Reece
Department Head
Department Aquatic Animal Health Sciences

Ms. Constance Motley
Director, Sponsored Programs

Dr. Mark Luckenbach
Associate Dean of Research and
Advisory Services

Valerie Woodard

From: John E Graves
Sent: Monday, June 13, 2016 11:44 AM
To: Valerie Woodard
Subject: RE: Email Approval

Val:

Thanks for the email. I approve.

Cheers,

John

John Graves
Chancellor Professor of Marine Science & Chair, Department of Fisheries Science
P.O. Box 1346/1375 Greate Rd.
Gloucester Point, VA 23062
Phone 804.684.7352 Fax 804.684.7157

From: Valerie Woodard
Sent: Monday, June 13, 2016 11:40 AM
To: John E Graves
Subject: Email Approval

Hi John,
I have this proposal in my office for approval and review. Could you please email your approval for this submission.
Thanks
Val

Valerie D. Woodard | Sponsored Programs Administrator
Office of Sponsored Programs
804.684.7003 | 804.684.7614 | vwoodard@vims.edu
vims.edu | 1375 Greate Rd., Gloucester Pt., VA 23062

VIMS | WILLIAM & MARY
VIRGINIA INSTITUTE OF MARINE SCIENCE

VIRGINIA SALTWATER RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION*

NAME AND ADDRESS OF APPLICANT:	PROJECT LEADER (name, phone, e-mail):
Jan McDowell, Hamish Small, Susanna Musick Virginia Institute of Marine Science Rt. 1208 Greate Rd. Gloucester Point, VA 23062	Jan McDowell 804 684-7263 McDowell@vims.edu
PRIORITY AREA OF CONCERN:	**PROJECT LOCATION:**
Research and Data Collection	Virginia Institute of Marine Science Rt. 1208 Greate Rd Gloucester Point, VA 23062

DESCRIPTIVE TITLE OF PROJECT:

Genetic analysis of the distinctiveness of Cobia, *Rachycentron canadum*, from Chesapeake Bay

PROJECT SUMMARY:

Cobia, *Rachycentron canadum*, is a cosmopolitan coastal pelagic fish distributed throughout tropical and subtropical Atlantic, Indian, and western Pacific oceans. In spring and summer months, cobia in the western North Atlantic migrate from Florida northward with warming waters and aggregate in high-salinity estuaries, including the Chesapeake Bay, to spawn. Cobia are now considered the premiere Chesapeake Bay sport fish to many of the approximately 206,000 saltwater recreational fishing anglers in Virginia. Cobia are presently managed as two separate Gulf and Atlantic migratory groups by the South Atlantic Fishery Management Council (SAFMC) and the Gulf of Mexico Fishery Management Council (GMFMC) under the joint Coastal Migratory Pelagics Fishery Management Plan. Based on recent genetic and tagging data presented and reviewed at SEDAR 28, Amendment (20B), the management boundary for the Gulf and Atlantic cobia migratory groups was moved from the Florida Keys to the Florida/Georgia line. However, a later genetic study did not corroborate the observed differences between Gulf and Atlantic samples. The genetic data presented at SEDAR also indicated the presence of distinct inshore populations in Virginia and South Carolina that were different both from each other and from offshore aggregations in the Atlantic management group, suggesting that there may be multiple stocks within the Atlantic. We therefore seek to clarify the genetic population structure of cobia. To accomplish this work, we propose to use previously published microsatellite markers and sequencing of the mitochondrial control region to assess cobia stock structure.

EXPECTED BENEFITS:

Knowledge about the stock boundaries of cobia and the populations that migrate to and spawn within Virginia's coastal waters is critical information for management of this important recreational resource. This is especially important due to recent changes to management the management boundaries that have in turn reducted the Annual Catch Limit (ACL) allocation to the Atlantic Group (Figure 1). This information will be provided to the Virginia Marine Resources Commission for use in future stock assessment and management efforts to ensure appropriate management and preservation of healthy cobia stocks for Virginia's recreational fishermen.

COSTS:

VMRC Funding:	$ 89,681
Recipient Funding:	$ 38,048
Total Costs:	**$ 127,729**

Detailed budget must be included with proposal.

Title: Genetic analysis of the distinctiveness of Cobia, *Rachycentron canadum*, from Chesapeake Bay

Personnel	Time	Monthly	**Agency**	**VIMS**	**Total**
Faculty and Staff					
McDowell	1.00	$8,526	$8,526	$8,526	$17,052
Small	1.00	$5,423	$5,423	$0	$5,423
Musick	0.50	$5,692		$2,846	$2,846
Brightman	6.00	$3,121	$18,726	$0	$18,726
	-	$0	$0	$0	$0
	-	$0	$0	$0	$0
	-	$0	$0	$0	$0
Hourly					
	-	$0	$0	$0	$0
	-	$0	$0	$0	$0
Graduate Research Assistant					
	-	$0	$0	$0	$0
	-	$0	$0	$0	$0
Personnel, salaried			$32,675	$11,372	$44,047
Personnel, hourly			$0	$0	$0
Personnel, grad assist			$0	$0	$0
Fringe, 40% salaries;			$13,070	$4,549	$17,619
7.65% hourly			$0	$0	$0
Total Personnel			$45,745	$15,921	$61,666
Communications/Printing			$0	$0	$0
Supplies			$22,000	$0	$22,000
Consultant/Skilled Services			$3,000	$0	$3,000
Travel			$1,000	$0	$1,000
Subaward Agreements					
Name of Subaward Agency			$0	$0	$0
Name of Subaward Agency			$0	$0	$0
Tuition			$0	$0	$0
Vessels			$0	$0	$0
VIMS Communications/Publication Center			$0	$0	$0
Nutrient Analysis			$0	$0	$0
Seawater Research Lab			$0	$0	$0
Equipment			$0	$0	$0
SUBTOTAL: Direct Costs			$71,745	$15,921	$87,666
Facilities & Administrative Costs		**25.0%**	$17,936	$22,127	$40,063
TOTAL			**$89,681**	**$38,048**	**$127,729**

Project Summary

(1) Organization title: Virginia Institute of Marine Science, College of William and Mary

(2) Principal Investigators: Jan R. McDowell, Hamish J. Small and Susanna Musick

(3) Principal Investigator's Contact Information: VIMS, P.O. Box 1346, Gloucester Point, VA 23062; 804.684.7352; mcdowell@vims.edu

(4) Area of Interest: Research & Data Collection

(5) Project Title: Genetic analysis of the distinctiveness of Cobia, *Rachycentron canadum*, from Chesapeake Bay

(6) Project Duration: 12 months (January 2017 – Dec 2017)

(7) Project Summary:

Cobia, *Rachycentron canadum*, is a cosmopolitan coastal pelagic fish distributed throughout tropical and subtropical Atlantic, Indian, and western Pacific oceans. In spring and summer months, cobia in the western North Atlantic migrate from Florida northward with warming waters and aggregate in high-salinity estuaries, including the Chesapeake Bay, to spawn. Cobia are now considered a premiere Chesapeake Bay sport fish to many of the approximately 206,000 saltwater recreational fishing anglers in Virginia. Cobia are presently managed as two separate Gulf and Atlantic migratory groups by the South Atlantic Fishery Management Council (SAFMC) and the Gulf of Mexico Fishery Management Council (GMFMC) under the joint Coastal Migratory Pelagics Fishery Management Plan. Based on recent genetic and tagging data presented and reviewed at SEDAR 28, Amendment (20B), the management boundary for the Gulf and Atlantic cobia migratory groups was moved from the Florida Keys to the Florida/Georgia line. However, a later genetic study did not corroborate the observed differences between Gulf and Atlantic samples. The genetic data presented at SEDAR also indicated the presence of distinct inshore populations in Virginia and South Carolina that were different both from each other and from offshore aggregations in the Atlantic management group, suggesting that there may be multiple stocks within the Atlantic. We therefore seek to clarify the genetic population structure of cobia. To accomplish this work, we propose to use previously published microsatellite markers and sequencing of the mitochondrial control region to assess cobia stock structure.

(8) Expected Benefits:

Knowledge about the stock boundaries of cobia and the populations that migrate to and spawn within Virginia's coastal waters is critical information for management of this important recreational resource. This is especially important due to recent changes to management the management boundaries that have in turn reducted the Annual Catch Limit (ACL) allocation to the Atlantic Group (Figure 1). This information will be provided to the Virginia Marine Resources Commission for use in future stock

assessment and management efforts to ensure appropriate management and preservation of healthy cobia stocks for Virginia's recreational fishermen.

(9) Budget Information (fiscal year):

Total Funds Requested: 89,681
Cost-sharing: 38,048
Project Total: 127,729

Project Description

Need (State the problem or deficiency that the project will improve).
Background

Cobia, *Rachycentron canadum*, belonging to the monotypic family Rachycentridae (Actinopterygii: Perciformes) is a cosmopolitan coastal pelagic fish species distributed throughout tropical and subtropical Atlantic, Indian, and western Pacific oceans (Shaffer and Nakamura 1989). Cobia are a highly prized sport fish because of their size, fighting spirit, and meat quality. In spring and summer months cobia in the western North Atlantic migrate with warming waters from Florida northward and aggregate in high-salinity estuaries, including the Chesapeake Bay, to spawn (Shaffer and Nakamura 1989). Important recreational and commercial fisheries for this species exist from the Gulf of Mexico to Virginia, with the majority of cobia caught by recrational fishermen (Shaffer and Nakamura 1989, Franks et al. 1999).

Although cobia has historically been present and fished in Virginia waters throughout the summer months (Kirkley and Kerstetter 1997), it is only in the last decade that interest has increased and cobia are now considered the premiere Chesapeake Bay sport fish to many of the approximately 206,000 saltwater recreational fishing anglers in Virginia. Though the economic value of the recreational fishery to Virginia's economy has not been quantified recently, the rapid rise in the popularity of the fishery and the presence of a dedicated fleet of vessels targetting these fish suggest that the value is considerable.

Cobia are presently managed jointly in federal waters by the South Atlantic Fishery Management Council (SAFMC) and the Gulf of Mexico Fishery Management Council (GMFMC) under the joint Coastal Migratory Pelagics Fishery Management Plan that includes king mackeral, Spanish mackeral, and cobia. The species is also managed in collaboration with the Mid-Atlantic Fishery Management Council because of the migratory route of cobia along the Atlantic coast. Historically, cobia were managed as a single stock in state and federal waters from the Virginia/North Carolina border to Texas (GMFMC and SAFMC 1982). Amendment 8 (GMFMC and SAFMC 1997) extended the management area for cobia through New York. On the basis of differing age and growth rates, Amendment 18 established separate Gulf and Atlantic migratory groups of cobia (GMFMC and SAFMC 2011). Both groups were separated at the SAFMC/GMFMC boundary (FL Keys, see Fig. 1). In addition, in 2011 the cobia fishery started using the Annual Catch Limit (ACL) system in its management.

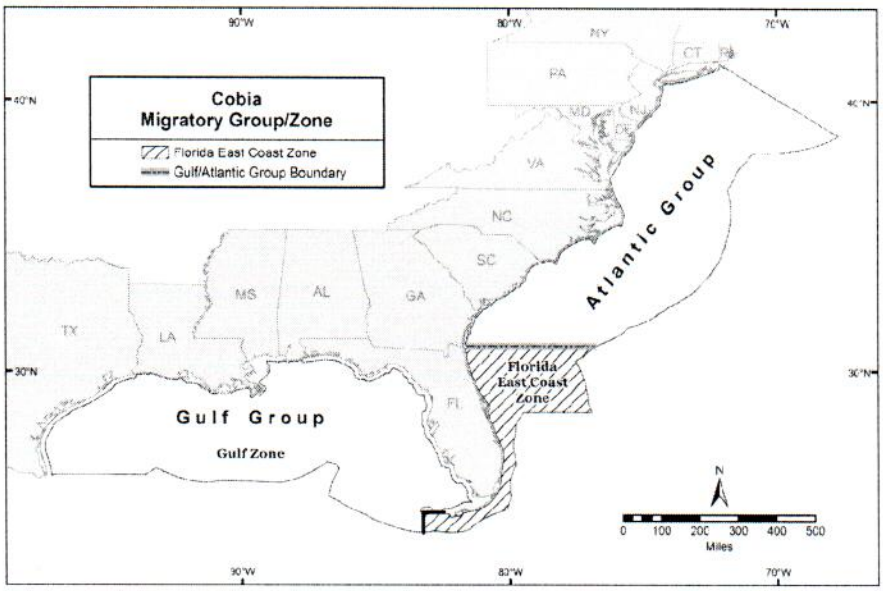

Fig. 1. Gulf and Atlantic cobia groups (from Amendment 20B).

However, during a recent stock assessment (data up to and including 2011) through the Southeast Data, Assesment, and Review program (SEDAR 28) new genetic (Darden, T. 2012, SEDAR28-DW01) and tagging (Perkinson, M. and Denson, M. 2012, SEDAR28-DW05) data was presented indicating that the biological boundary between the Gulf and Atlantic cobia groups was not in the Florida Keys, but further North of Brevard County Florida, with the northern deliniation set at the Florida/Georgia line for management purposes (see Fig. 1). However, this result has not been published in a peer-reviewed journal and a recent study by Gold et al. (2013) found no genetic differences between Gulf and Atlantic samples using an increased number of molecular markers (10 vs 28 loci). Amendment 20B (GMFMC and SAFMC 2014) subsequently set the southern boundary of the Atlantic Group at the Florida/Georgia line and in doing so drastically reduced the ACL for the Atlantic group from 1,445,687 pounds to 630,00 pounds (see Table. 1). Despite the most recent stock assessment of the Atlantic migratory group indicating that this is not overfished and that overfishing is not occurring (SEDAR 28), the reduced 2015 ACL for this group was exceeded, triggering accoutability measures to ensure overfishing does not occur the following year. This resulted in a closure of cobia retention in federal waters from June 20th 2016 (NOAA FB16-018) and stricter regulations (minimum size 40 inch TL, 1 fish per person, 2 fish boat limit, only one fish over 50 inch TL) introduced by Virginia Marine Resources Comission for state waters until Aug 31st 2016, after which retention of cobia will be prohibited.

Table 1. Annual Catch Limits (ACL) and landings for the Atlantic Group of cobia from 2016-2012. http://sero.nmfs.noaa.gov/sustainable_fisheries/acl_monitoring/

Year	ACL (pounds)	Total Reported (pounds)
2016	620,000	
2015	630,000	1,541,575
2014	1,445,687	1,179,031
2013	1,445,687	1,142,947
2012	1,445,687	1,058,583

The study presented by Darden, T. (2012) at SEDAR 28 (SEDAR28-DW01) described the genetic analysis of cobia samples from Virginia (n=76), North Carolina (n=248), South Carolina (n=891), Florida (178), Missisippi (n=6), and Texas (n=62) using 10 nuclear-encoded microsatellite loci. This study indicated that fish sampled in the Gulf of Mexico were a genetically homogenous group that continued around the Florida peninsula with a genetic break around northern Florida/southern Georgia. A second study presented at SEDAR 28 (Darden et al. 2012, SEDAR28-RD09) reported that the Atlantic Group of cobia appear to have a genetically homogeneous offshore component and genetically unique inshore components (Virginia and South Carolina) that are distict both from the offshore component and from each other. It was suggested that natal site fidelity was a possible explaination for the observed dfferences. However, this second study and the final publication (Darden et al. 2014) reflected far fewer samples from Virginia (n=35), presumeably due to a lack of relevant information (specific capture location, length and etc.) for some samples.

Few peer-reviewed published studies have investigated the population genetics of cobia from U.S. waters, and confusion exists over results and implications for cobia management. The results of the Darden (2012) study (presented at the SEDAR 28 meeting but still unpublished) are in disagreement with results published by Gold et al. (2013). Gold et al. used 28 nuclear encoded microsatellite loci to compare cobia samples from Virginia (n=35), Mississippi (n=52), Louisiana (n=14), and Taiwan (n=36). They also sequenced a 352 bp fragment of mitochondrial-encoded cytochrome *b* gene from a subset of fish (n=5) from each sample locality. They reported that cobia sampled from Virginia, Mississippi and Louisiana were genetically homogeneous, but did find significant genetic differences between samples from U.S. waters and those from Taiwan. In addition, confusion surrounds the nomenclature used in Gold et al (2014) and Darden et al. (2012, 2014) to describe/define where samples originated. Gold et al. reports that cobia were sampled in the summers of 2010 and 2011 from localities "*offshore of Virginia*" but provides no further details as to whether these cobia samples were from true offshore locations, or more likely from the Chesapeake Bay (nearshore). Likewise, Darden et al. reports that the Virginia cobia were sampled in 2008 from the vicinity Chesapeake Bay and are subsequently referred to as the "Virginia inshore" collection, with *inshore* being defined as *being captured landward of the barrier island, either along the coast, or in the estuary*, and offshore defined as *captured seaward of the barrier islands, mostly near wrecks or reefs*. Confusion therefore surrounds this labeling system as fish migrating towards the Chesapeake Bay past the offshore area of the Albemarle

Sound in North Carolina (where their North Carolina samples originated) would be termed "offshore" in that locality and then classified as "inshore" once the fish entered the Chesapeake Bay.

From 1995-2015, more than 200 recaptures have been reported (n=298) in the Virginia Game Fish Tagging Program, and 2015 led in annual recaptures (n=66). Cobia recaptures have been reported from 94 locations, ranging as far north as Moriches Inlet, Long Island, NY, and as far south as the Gulf of Mexico, off Mississippi, with the Latimer Shoal (RN 16 Buoy) in Virginia being the most popular recapture site (n=29 tags, 10% overall effort). Seasonally, recaptures were highest in July, and days at large ranged from one to 2,207 days with an average of 445 days.

Objective: (provide a concise statement of what is anticipated and the target date(s)
To effectively manage this important recreational resource it is necessary to understand stock structure. Whether a genetically distinct group of fish use Chesapeake Bay for spawning is unknown. The genetic evidence that was used to support movement of the biological boundary from the Florida Keys to the Florida/Georgia line also suggests that genetically distinct groups of fish use discrete spawning areas and stock structure in cobia may be complex. However, the recently published genetic study by Gold et al. (2013) concluded that cobia sampled from the Gulf and Atlantic were genetically homogeneous, confliciting with the results of the Darden (2012) study. We propose to clarify these issues by genetically analyzing cobia samples from the Atlantic Group with specific emphasis on comparing cobia sampled in Chesapeake Bay at the time of spawning across multiple years to those sampled from North Carolina and further south (South Carolina/Georgia). We also plan to compare these collections with a sample from the Gulf Group. Nuclear encoded microsatellite markers will be selected from the marker panels published by Darden et al. (2014) and Gold et al. (2013). We also plan to sequence the mitochondrial control region, as comparison of results based on nuclear and mitochondrial markers can help pinpoint spawning site fidelity/natal homing as the source of the observed genetic differences if unique mitochondrial haplotypes are observed in Chesapeake Bay across multiple years.

This data will provide resource managers the best available science in support of effective regulation and resource sustainability, and is also of significant interest to recreational fishermen across Virginia. This is especially relevant given the recent (2014) boundary shift, resultant reduction in ACL, and 2015 ACL overage which nearly caused the fishery to be closed to Virginia anglers in 2016. Target dates for completion of this research are one year from the proposed start date. These goals address management recommendations contained in the most recent stock assessment report for the southeast cobia stock (SEDAR 28). In particular, these address the Assessment Workshop Research Recommendation to *Better characterize the genetic structure of the stock and evaluate the possibility of local population structure*, and *Better characterize the migratory dynamics of the stock and the degree of fidelity to spawning areas.*

(III.) Expected results or benefits:

We propose a directed study of cobia from Chesapeake Bay to verify the results of an earlier South Carolina based genetic study that found significant differences between Gulf and Atlantic samples (Darden 2012). This study is in disagreement with a subsequent study (Gold et al. 2013), which did not find genetic evidence of differences between Gulf and Atlantic samples. We also propose to further explore the results of the Darden et al. (2014) study that concluded that cobia entering Chesapeake Bay comprise a distinct genetic unit as compared to samples collected both inshore and offshore in South Carolina. Previous studies were based on a very limited number of samples from Virginia (n=35, in both Darden et al., 2014 and Gold et al. 2013). In addition, the Darden et al. (2014) study used a limited number of molecular markers (n=10). Resolution of the differences between these studies is important for appropriate management of the species. If cobia from Chesapeake Bay are found to be distinct from those sampled in other locations (i.e. vs Gulf Group, and vs Atlantic Group offshore aggregations and other inshore locations), then localized depletion resulting from increasing fishing pressure may result in the loss of unique genetic variation and could ultimately result in localized collapse of the fishery. Under this scenario, alternate management recommendations may be appropriate to preserve this important spawning population and recreational fishing resource. Conversely, if cobia are comprised of a homogeneous stock within the Atlantic management area, the current management approach would seem appropriate.

We will address the following null hypotheses:

(1) There is no difference among cobia sampled from Chesapeake Bay (Atlantic Group) and those sampled in the Gulf of Mexico (Gulf Group).
(2) There is no genetic difference among cobia collected from Chesapeake Bay and those collected from other inshore/offshore aggregations from within the Atlantic Group cobia management area.
(3) There is no difference among samples of cobia collected in geographic areas within a season or across years in Chesapeake Bay.
(4) Results of nuclear and mitochondrial DNA analysis are concordant and provide no evidence of site fidelity.

(IV.) Approach

Sample Collections

In 2015 with the assistance of local recreational fishermen and charter boat captains, we collected 120+ cobia fin clip samples from fish caught within Chesapeake Bay during the summer months (June-August). 2016 collections are ongoing and we expect a similar number of samples, including samples from North Carolina. Members of the Virginia Gamefish Tagging Program (VGFTP) who are experienced cobia anglers have agreed to support future sampling efforts by collecting fin clip samples and recording relevant information. If funded, we will also collect samples in 2017. In addition, we will make every effort to collect samples fom cobia tournaments in Virginia. Gonads from fish landed at tournaments will be examined and subsampled for histological analysis to confirm that these fish are spawning in Chesapeake Bay. Gulf of Mexico collections are ongoing in the courtesy of Dr. David Portnoy from Texas A&M University. Overall, we plan to analyze samples of at least 50 cobia/year sampled from within Chesapeake Bay

from 2015-2017 during their presumed spawning season. We expect to analyze at least 200 Chesapeake Bay samples. In addition, we will collect at least 50 samples from the Gulf of Mexico and from offshore aggregations south of North Carolina. We expect the total number of samples analyzed to be approximately 400. These samples will be analyzed with a panel of 20-24 nuclear-encoded microsatellite markers and the mitochindrial control region as outlined below.

Microsatellite Analysis

Genomic DNA will be extracted from all samples using a Genomic DNA Tissue MiniPrep Kit (Zymo Research Corporation, Irvine, CA) following the manufacturer's protocol. Loci used in previous studies of cobia (Gold et al., 2013, Darden et al., 2014) will be optimized and multiplexed into multilocus panels using MULTIPLEX MANAGER 1.0 (Holleley and Geerts, 2009) using the Type-it Microsatellite PCR Kit (Qiagen). These loci will be supplemented with loci from other sources if necessary. To ensure consistency, 20% of the subset of samples will be re-analyzed from the point of DNA extraction through allele scoring and all allele scoring will be double blind. This will allow data to be checked for DNA contamination between samples, for loci that cannot be scored reliably, as well as for sample handling errors. This is especially important for microsatellite data as the wide range in allele sizes can make them susceptible to genotyping errors (see Morin et al. 2009 for a discussion). Once all data have been collected, MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) will be used to check for the presence of null alleles and evidence of scoring errors. The GENEPOP'007 software package (Rousset 2008) will be used to test for deviations of genotypic distributions from HWE expectations (F_{IS}, exact tests, Guo and Thompson 1992). To evaluate evidence for the presence of population structure, the ARLEQUIN software package (Excoffier and Lischer 2010) will be used to estimate Weir and Cockerhams' (1984) unbiased estimator of Wrights F-statistics. Significance will be assessed via permutations of the data. Exact tests of homogeneity in allele frequency distributions among all pairwise comparisons of collections will be carried out individually for each microsatellite locus and across all loci to identify pairs of collections that differ significantly. An analysis of molecular variance (AMOVA) will be carried out among alternate grouping of sample collections to maximize the amount of variance due to variation among groups of collections using the ARELQUIN software package. In addition, a spatial analysis of molecular variance (SAMOVA, Doupanloup et al. 2002, available at (http://cmpg.unibe.ch/software/samova/), which employs a simulated annealing approach to define groups that are geographically homogeneous and maximally differentiated from each other will be used. SAMOVA also results in the identification of genetic barriers between identified groups. Measures of allelic richness will be carried out within each geographic sample using the methods available in the FSTAT software package (Goudet 1995), and statistical significance of differences in allelic richness among geographic samples will be assessed using Wilcoxon signed rank tests. The Structure ver. 2.3.4 software package (Pritchard et al. 2000) will be used to estimate the most probable number of population clusters (K) following the methods of Evanno et al. 2005. The Structure software will also be used to look for evidence of admixture between identified clusters.

mtDNA analysis

Genomic DNA will be isolated as above. The mitochondrial control region locus will be amplified using specific primers that will be designed for this study based on the full mitochondrial genome of *R. canadum*, which is available in GenBank and the Taq PCR Core Kit (Qiagen). Amplification products will be cleaned using the QIAquick PCR Purification Kit (Qiagen) and sequenced using the BigDye Terminator ver. 3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.) at a 1:8 dilution. Sequenced samples will be electrophoresed on an ABI 3130xl Genetic Analyzer (Applied Biosystems), edited using the Sequencher 4.8 software package (Gene Codes Corp., Ann Arbor, Michigan, USA) and, aligned using the MAFFT algorithm (Katoh et al. 2005). Aligned sequences will be collapsed into haplotypes using FaBox (Vilesen, 2007). To characterize the levels of genetic variation, summary statistics including nucleon diversity, nucleotide diversity, number of polymorphic sites, base composition, and the number of transitions and transversions will be calculated in the Arlequin ver. 3.5 software packages (Excoffier and Lischer 2010). Relationships among haplotypes will be inferred by constructing haplotype networks using the network inference methods in the PopART package (http://popart.otago.ac.nz) and using the multivariate analyses (PCA, PCoA) available in the Adegenet package (Jombart, 2008). Relationships among sequences will also be inferred using standard phylogenetic methods (e.g. parsimony, maximum likelihood and Bayesian methods). The most probable model of nucleotide evolution will be estimated using jModelTest (Guindon & Gascuel 2003, Darriba et al., 2012). Levels of genetic differentiation among nursery areas will be estimated using ΦST and significance will be assessed based on 10,000 permutations of the data using the methods implemented in the Arlequin (Excoffier and Lischer, 2010). Hierarchical comparisons will be done using an Analysis of Molecular Variance (AMOVA) as implemented in ARELQUIN.

(V.) Location

All research will be carried out at the Virgnina Institute of Marine Science (VIMS). VIMS researchers submitting cobia research proposals in the June 2016 Virginia Saltwater Recreational Fishing Development Fund (VSRFDF) cycle will work cooperatively to capitalize on project dissemination, angler participation, and data and sample collection. The VIMS Marine Advisory Services Marine Recreation Specialist will serve as a central point of contact for stakeholders interested in the projects and coordinate information requests with each project's Principal Investigator.

Staff from the Virginia Institute of Marine Science will also work together to host a central, introductory stakeholder focus group workshop in early winter 2017. The workshop will be coordinated and facilitated by VIMS Marine Advisory Services' staff. The focus group will include cobia anglers, cobia charter captains, and top cobia taggers from the Virginia Game Fish Tagging Program. This workshop will provide an opportunity for all VIMS' staff working on VSRFDF projects to give an overview of their projects, data needs and field collection methods, and give an opportunity for anglers to give direct feedback. As many of the anglers in the stakeholder focus group will potentially be working on all of the projects, this workshop should also centralize outreach efforts and make it easier for anglers to contribute.

(VI.) Estimated Cost

Total Project Costs and Budget Narrative

See attached budget. The proposed budget reflects costs associated with extracting and stabilizing DNA from cobia sample collections, and generation and analysis of molecular makers.

Salaries: Salary support is requested for a technician to extract DNAs, assess their quality and generate molecular data. S.Musick will coordinate VGFTP angler participation, VGFTP tag and recapture analyses, and will be the liaison between scientists and anglers. J. McDowell and H. Small will participate in coordination of sample collection as needed and will be responsible for quality control of lab work, data analysis and reporting and publication of results.

Lab Supplies: The laboratory portion of the budget is based on the cost of DNA isolations, quality/quantity assessment of extracted DNAs, stabilization of DNA, DNA sequencing and amplification and sizing of microsatellite alleles. Included in this cost are consumables such as pipet tips, micro centrifuge tubes and gloves.

Travel: Travel costs are minimal and associated with local sample collection.

Consultant/Skilled Services: We have included $3,000 to charter boats for directed sampling effort.

Facilities and Administrative Costs

TOTAL

Facilities & Administrative Costs calculated at 25% of direct costs. The federally negotiated Facilities and Administrative rate for the Virginia Institute of Marine Science is 45.7% of the moditied total direct costs.

References

Darden T, Walker MJ, Brenkert K, Yost JR and Denson MR (2012) Population genetics of cobia *Rachycentron canadum*: Management implications along the Southeastern US coast. SEDAR28-RD09.

Darden T (2012) Cobia preliminary data analyses – US Atlantic and GOM genetic population structure. SEDAR28-DW01.

Darden TL, Walker MJ, Brenkert K, Yost JR, Denson, M.(2014) Population genetics of Cobia (*Rachycentron canadum*): implications for fishery management along the coast of the southeastern United States. Fishery Bulletin 112:24–35.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8), 772.

Dupanloup I, Schneider S, Excoffier L. (2002) A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11: 2571-2581.

Duberg J, Kirkley JE and Murray T (2006) Economic Contributions of Virginia's Commercial Seafood and Recreational Fishing Industries: A User's Manual for Assessing Economic Impacts. Virginia Institute of Marine Science, College of William and Mary.

Evanno G, Regnaut S, and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology. 14: 2611-2620.

Excoffier L, Lischer HEL (2010) Arlequin Suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10: 564-567.

Gold JR, Giresi M, Renshaw M, and Gwo J-C (2013) Population Genetic Comparisons among Cobia from Northern Gulf of Mexico, U.S. Western Atlantic, and Southeast Asia. North American Journal of Aquaculture 57:57-63.

Goudet J (1995) FSTAT (Version 1.2): A computer program to calculate Fstatistics. J. Hered. 86: 485-486.

Guindon S and Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology, 52, 696-704.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Holleley CE and Geerts PG (2009) Multiplex Manager 1.0: a crossplatform computer program that plans and optimizes multiplex PCR BioTechniques, Vol 46, No 7, pp 511-517.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403-1405. doi: 10.1093/bioinformatics/btn129.

Katoh K, Misawa K, Kuma K, and Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research. 30(14): 3059-3066.

Kirkley JE and Kerstetter DW (1997) "Saltwater angling and its economic importance to Virginia." Virginia Institute of Marine Science, Gloucester Point, Virginia.

Morin PA, Martien KK, Taylor BL (2009) Assessing statistical power of SNPs for

population structure and conservation studies. Mol. Ecol. Res. 9: 66-73.

Perkinson M and Denson M (2012) Evaluation of cobia movements and distribution using tagging data from the Gulf of Mexico and South Atlantic coast of the United States. SEDAR28-DW05.

Pritchard JK, Stephens M, and Donnelly PJ (2000) Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resour. 8:103-106.

SEDAR. 2013. SEDAR 28 – South Atlantic Cobia Stock Assessment Report. SEDAR, North Charleston SC. 412 pp. available online at: http://www.sefsc.noaa.gov/sedar/Sedar_Workshops.jsp?WorkshopNum=28

Shaffer RV, Nakamura EL (1989) Synopsis of biological data on the cobia *Rachycentron canadum*. Pisces: Rachycentridae. NOAA Tech. Rep. NMFS 82 (FAO Fisheries Synopsis153), 21 p.

Van Oosterhout C, Hutchinson WF, Wills DPM Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4: 535-538.

Villesen, P (2007) *FaBox: an online toolbox for fasta sequences*, Molecular Ecology Notes 7 (6), 965–968. doi:10.1111/j.1471-8286.2007.01821.x

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the analysis of population structure. *Evolution*, 38: 1358-1370.

Modifications to the Coastal Migratory Pelagics Zone Management. Final Amendment 20B to the Fishery Management Plan for the Coastal Migratory Pelagic Resources in the Gulf of Mexico and Atlantic Region. May 2014. 258p.