Proposal Submission to

Recreational Fishery Advisory Board

by

The Virginia Institute of Marine Science
College of William and Mary

Speckled trout, *Cynoscion nebulosus*, in Virginia: are genetic differences temporally stable?

Proposed starting date: 1 January 2014
Proposed duration: 12months

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VIRGINIA SALTWATER RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION

Please complete all fields. This page should be used as a coversheet for a detailed application.

NAME AND ADDRESS OF APPLICANT:
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Rt. 1208 Greate Rd.
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PROJECT LEADER (name, phone, email):
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DESCRIPTIVE TITLE OF EVENT:
Speckled trout, *Cynoscion nebulosus*, in Virginia: are genetic differences temporally stable?

PROJECT LOCATION:
Virginia Institute of Marine Science
Rt. 1208 Greate Rd.
Gloucester Point, VA 23062

BRIEF PROJECT SUMMARY: (include a detailed description of activity as an attachment)

Speckled trout, *Cynoscion nebulosus* (Cuvier, 1830), support an important recreational fishery in Chesapeake Bay. Speckled trout have been extensively studied throughout the Gulf of Mexico and off the Atlantic coast of Florida, with a few studies off the coasts of Georgia and South Carolina. These studies have shown that speckled trout undergo limited movement among estuaries. Some of these studies have used molecular markers to show that different estuaries harbor distinct genetic stocks. However, there have been no genetic studies of Virginia's speckled trout, thus there is no information available regarding genetic connectivity among locations either within Chesapeake Bay or between Chesapeake Bay and other locations. It is unknown if populations in Virginia are self-recruiting or to what extent recruitment relies on input from other geographic areas. The proposed research will extend our current study aimed at using genetic markers to assess the independence of Virginia's speckled trout populations. This information is crucial to appropriate management efforts and is of interest to recreational fishermen across Virginia. To accomplish this work, we propose to use the mitochondrial (mt) DNA control region and nuclear microsatellite markers to compare speckled trout collected from several Chesapeake Bay sub-estuaries across multiple years. The current study compares data among all study sites and also between individual sites, including locations north and south of Cape Hatteras. This proposal aims to expand the study to include analysis of samples collected in multiple years to assess the demographic independence of Virginia's speckled trout stock(s) and the temporal stability of our results.

***This form alone does not constitute a complete application, see application instructions or contact Alicia Nelson at 757-247-8155 or alicia.nelson@mrc.virginia.gov**

EXPECTED BENEFITS: (Describe how your project directly benefits the average Virginia recreational angler)

Knowledge about the demographic independence and stock boundaries of speckled trout populations in Virgnia is critical information for management of the speckled trout resource, and assessment of the temporal stability of genetic differences is a crucial component of this research. This information will be provided to the Virginia Marine Resources Commission for use in future stock assessment and management efforts. This will ensure appropriate management and preservation of the speckled trout resource for Virginia's recreational fishermen. Since many recreational anglers will be involved with the study, this will allow them access to the results of the analyses, which will strengthern the relationship between recreational anglers and scientists.

SUMMARY COSTS: (Please attach a detailed budget including all sources of recipient funding)

SUMMARY COSTS

Requested VMRC Funding:	**$ 62,791**
Recipient Funding:	**$ 33,656**
Total Costs:	**$ 96,447**

***This form alone does not constitute a complete application, see application instructions or contact Alicia Nelson at 757-247-8155 or alicia.nelson@mrc.virginia.gov**

Project Summary

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

(2) Principal Investigators: Jan R. McDowell, Susanna Musick, John E. Graves

(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: Speckled trout, *Cynoscion nebulosus*, in Virginia: are genetic differences temporally stable?

(6) Project Duration: 12 months (January 2015 – December 2015)

(7) Project Summary:

Speckled trout, *Cynoscion nebulosus* (Cuvier, 1830), support an important recreational fishery in Chesapeake Bay. Speckled trout have been extensively studied throughout the Gulf of Mexico and off the Atlantic coast of Florida, with a few studies off the coasts of Georgia and South Carolina. These studies have shown that speckled trout undergo limited movement among estuaries. Some of these studies have used molecular markers to show that different estuaries harbor distinct genetic stocks. However, there have been no genetic studies of Virginia's speckled trout, thus there is no information available regarding genetic connectivity among locations either within Chesapeake Bay or between Chesapeake Bay and other locations. It is unknown if populations in Virginia are self-recruiting or to what extent recruitment relies on input from other geographic areas. The proposed research will extend our current study aimed at using genetic markers to assess the independence of Virginia's speckled trout populations. This information is crucial to appropriate management efforts and is of interest to recreational fishermen across Virginia. To accomplish this work, we propose to use the mitochondrial (mt) DNA control region and nuclear microsatellite markers to compare speckled trout collected from several Chesapeake Bay sub-estuaries across multiple years. The current study compares data among all study sites and also between individual sites, including locations north and south of Cape Hatteras. This proposal aims to expand the study to include analysis of samples collected in multiple years to assess the demographic independence of Virginia's speckled trout stock(s) and the temporal stability of our results.

(8) Expected Benefits:

Knowledge about the demographic independence and stock boundaries of speckled trout populations in Virgnia is critical information for management of the speckled trout resource, and assessment of the temporal stability of genetic differences is a crucial component of this research. This information will be provided to the Virginia Marine Resources Commission for use in future stock assessment and management efforts. This will ensure appropriate management and preservation of the speckled trout resource for Virginia's recreational

fishermen. Since many recreational anglers will be involved with the study, this will allow them access to the results of the analyses, which will strengthern the relationship between recreational anglers and scientists.

(9) Budget Information (fiscal year):

Total Funds Requested: $62,791
Cost-sharing: $33,656
Project Total: $96,447

Project Description

Need

Speckled trout (*Cynoscion nebulosus*, Cuvier 1830), commonly known as spotted seatrout (Robbins and Ray 1986), is a member of the drum family, Sciaenidae, and is widely distributed in estuaries from Massachusetts to Mexico (Murphy et al. 2006). They are medium-sized fish with a maximum size of 40 inches and 17 pounds. Speckled trout reach a maximum age of at least 10 years in Chesapeake Bay (Ihde, 2000) and almost all speckled trout are mature by age 1 (Ihde, 2000, Jensen 2009).

Speckled trout support an important recreational fishery in Chesapeake Bay, with a recreational harvest estimated at 226,556 lbs in 2012 (NOAA MRIP data). In addition, a high percentage of fish caught recreationally are released (ASMFC, 2007). It is estimated that the recreational speckled trout fishery in Virginia generates $10,686,500 in sales and $6,224,800 in income (Duberg et al. 2006). Speckled trout are managed by the Atlantic States Marine Fisheries Commission (ASMFC) under an Omnibus Amendment to the interstate fishery management plan, which covers Maryland through eastern Florida and imposes a 12" TL minimum size limit on the fishery to limit the harvest of immature fish. Under this amendment, all states must implement harvest controls and may not adopt less protective management plans (ASMFC Omnibus Ammendment, 2011). No coastwide assessment of speckled trout has ever been conducted since it is widely recognized to be largely non-migratory with localized populations (ASMFC Omnibus Ammendment 2011).

At the southern end of its range, speckled trout are thought to exist in separate subpopulations within individual estuaries based on the limited movements observed based on conventional tagging studies (Moffett 1961, Iversen and Tabb 1962, Baker et al. 1986). These studies have concluded that speckled trout are composed of distinct stocks along Florida's Atlantic Coast and within the Gulf of Mexico (GOM) and they are considered to be largely nonmigratory throughout this region with little movement among estuaries (Music 1981, Overstreet 1983, Baker and Matlock 1993, Hendon et al. 2002, Murphy et al. 2006). The lack of migration in the southern rage of the species is consistent with results of genetic studies using a variety of markers. Weinstein and Yerger (1976) concluded that speckled trout from the Gulf coast of Florida comprise discreet populations based on allozyme electrophoresis. In the Gulf of Mexico, sequencing of the mitochondrial DNA (mtDNA) control region showed evidence of isolation by distance along the Texas shoreline (Anderson and Karel, 2009) and further analysis using both sequencing of the mtDNA control region and six microsatellite loci found evidence of multiple subpopulations (Anderson and Karel, 2010). This was corroborated by reports of differences in reproductive biology between females sampled from different estuaries in the Gulf of Mexico (Brown-Peterson et al. 2002).

Although it is commonly recognized that speckled trout are comprised of multiple localized stocks, there has been no genetic assessment of whether speckled trout from the different sub-estuaries of the Chesapeake Bay include more than a single stock. Further, there has been no genetic comparison of Virginia speckled trout with those taken from North Carolina. Currently speckled trout from Virginia and North Carolina are assumed to be a single stock in North

Carolina's assessments. This is interesting in light of the fact that samples of speckled trout taken from Virginia's Chesapeake Bay were found to be significantly different from those taken in Georgia and South Carolina based on very limited genetic data (two microsatellite loci, Wiley et al. 2003). **Preliminary data comparing samples collected in Virginia waters (n = 104) with a limited number of samples (n = 30) collected from South Carolina corroborate these these findings. Samples from the York, Ware, Elizabeth, and Fort Monroe areas were significantly different from those collected in South Carolina based on 9 microsatellite loci (McDowell, unpulbished data).**

In Chesapeake Bay, tagging studies have been conducted as part of the Virginia Game Fish Tagging Program (VGFTP) and most tagging effort for speckled trout has taken place in the Elizabeth River system (Musick and Gillingham 2011, 2012, 2013, 2014). This area is of further significance because tagging effort takes place here throughout the year, as speckled trout make use of warm water discharge areas to overwinter. Like other areas, the Elizabeth River has high site fidelity with the majority (90.1%) of speckled trout tagged there being recaptured within the same system (Musick 2011). Whether this pattern is the same for other Chesapeake Bay sub-estuaries is not known. **However, preliminary analysis based on a limited number of samples and 9 microsatellite loci suggests that samples taken from throughout the Elizabeth River (n = 22) are genetically differentiated from those taken from the Ware River (n = 23), York River (n= 40) and Fort Monroe (n = 19) and that Fort Monroe and the Ware River also show evidence of differentiation (McDowell unpublished data).** However, whether these differences are temporally stable needs to be evaluated since stochasic events such as high variability in reproductive success and shifting environmental conditions can cause fluctuations in allele frequencies over time.

Currently, North Carolina incorporates all speckled trout caught in Virginia into their assessments. This is due to the fact that the VGFTP statistics indicated that 15% of the total fish recaptured were recaptured in North Carolina. However, this statement was based on 37 of 246 fish, which were recaptured in North Carolina between 1995-2006. More recent data based on a much larger sample size, 54 of 1637 recaptures from 2007-2012, indicate that only 3.3% of fish tagged in Virginia were recaptured in North Carolina. It is unknown whether these fish reproduce in North Carolina or return to their natal estuaries to spawn. It is also unknown how many fish from North Carolina migrate to Virginia, although preliminary evidence suggests that around 7% of fish tagged in North Carolina are recaptured in Virginia, mostly in June-August (Tim Ellis, North Carolina State University, pers. comm.). However, it is anticipated that this percentage will be lower in the final analysis of North Carolina tag returns since the timing of the preliminary analysis may have biased the results (Tim Ellis, North Carolina State University, pers. comm.). It is interesting to note that the timing of these recaptures (June-August) seems to suggest that these fish may indeed be returning to natal spawning grounds in Virginia.

If Virginia and North Carolina speckled trout comprise a single contiguous genetic stock, then it is of concern that the portion of the stock in North Carolina is considered overfished. The 2009 North Carolina spotted seatrout stock assessment, which considered North Carolina and Virginia as a single unit stock and incorporated statistics from Virginia's fishery, indicated that the stock was overfished, with a rate of fishing mortality that was twice that allowable for a sustainable harvest (Jensen 2009). Furthermore, this study concluded that overfishing has been occurring

throughout the entire 18-year time series covered by the analysis (1991-2008; Jensen 2009). This was attributed to the increase in recreational fishing effort in recent years. North Carolina's recreational catch has averaged more than four times that of Virginia's over the period 1986-2009 (7,959,850 lbs vs 1,893,215 lbs; Table 9, ASMFC Omnibus Amendment 2011). If Virginia and North Carolina share a single stock, overfishing in North Carolina will very likely negatively impact Virginia's fishery. Additionally, if multiple independent stocks occur within the Chesapeake Bay watershed, as appears to be the case based on our preliminary genetic data, more localized management of the resource may be necessary to prevent localized depletion. Recent studies have shown that lack of knowledge about spatial structuring can lead to the risk of unintended overexploitation and localized depletions (Tuckey et al. 2007, Ying et al. 2011).

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 genetic stock structure and the level of connectivity among the different sub-estuaries in Chesapeake Bay so that appropriate management units can be delineated, and this should include an assessment of the temporal stability of these differences. This is especially critical given recent cold stun events, which have closed the recreational fishery in both Virginia and North Carolina. Although speckled trout have been extensively studied throughout the Gulf of Mexico and off the Atlantic coast of Florida, with limited studies off the coasts of Georgia and South Carolina, there have been no genetic studies of Virginia's speckled trout. As a result, there is no information available regarding genetic connectivity among locations either within Chesapeake Bay or between Chesapeake Bay and other locations. It is unknown if populations in Virginia are self-recruiting or to what extent recruitment relies on input from other geographic areas, such as North Carolina. The proposed research will continue to use the genetic markers optimized in the first year of this study to assess the independence of Virginia's speckled trout populations, with a focus on assessing the temporal stability of genetic differences. This information is crucial to appropriate management efforts and is of interest to recreational fishermen across Virginia.

To accomplish this work, we propose to continue to assay genetic variation using both the mitochondrial (mt) DNA control region and nuclear microsatellite markers. We will re-survey speckled trout collected from several Chesapeake Bay locations for a second year (outlined below) to compare data among these locations to assess the temporal stability of the genetic stock structure. We will also compare these collections to collections taken both north and south of Cape Hatteras, including a samples from South Carolina for a second year. The results of these analyses will be provided to the appropriate fisheries managers (VMRC) for incorporation into stock assessments and to more effectively manage this important recreational resource. Target dates for completion of this research are one year from the proposed start date as follows:

January-March	Begin coordination of sample collections with VGFTP volunteers and coordinate sample collection with North and South Carolina.
April-August	Sample collection (fin clips). Process samples: isolate DNA, begin generation of microsatellite and mtDNA sequence data.
April-November	Continue to process samples and collect microsatellite and mtDNA data.

November-December	Analyze and interpret data. Prepare final report and peer reviewed publication.

(III.) Expected results or benefits:

If speckled trout are composed of multiple, distinct temporally-stable genetic stocks within the Chesapeake Bay, localized depletion resulting from intense fishing pressure within an estuary may result in the loss of unique genetic variation. Alternately, if speckled trout within Chesapeake Bay represent a single stock, an increase in fishing pressure in one area may negatively impact other areas within Chesapeake Bay. Furthermore, if speckled trout in Chesapeake Bay comprise a single genetic stock with North Carolina, the high level of fishing pressure in North Carolina may negatively impact the Virginia fishery. Each of these scenarios has different implications for the stock. We propose to delineate the underlying genetic basis of stock structure in Chesapeake Bay and between Chesapeake Bay and North Carolina. We also plan to verify that speckled trout between Virginia and South Carolina represent distinct genetic stocks.

Our analysis will focus on two classes of rapidly evolving molecular markers; the mtDNA control region, and nuclear microsatellite loci. All fish will be sampled during the spawning season, which is when the stocks are most likely to be separate. We will address the following hypotheses:

(1) The is no genetic difference between samples of speckled trout collected from the same Chesapeake Bay sub-estuary **in different years**.
(2) There is no genetic difference between samples of speckled trout collected from among different Chesapeake Bay sub-estuaries.
(3) Genetic differences between speckled trout collected in Virginia and those collected in North Carolina are stable across multiple years.
(4) **Genetic differences between speckled trout collected in Virginia and those collected in South Carolina are stable across multiple years**.

(IV.) Approach

Sample Collections

Collections of at least 50 speckled trout will be obtained from locations within Chesapeake Bay including Mobjack Bay, York River, James River, Elizabeth River, Ware River, Eastern Shore (Chesapeake Bay side) and from the Honga River in Maryland. Members of the VGFTP who are experienced speckled trout anglers have already agreed to support this project by collecting fin clip samples. In addition, samples will also be collected in North Carolina north and south of Cape Hatteras (samples will be provided by Tim Ellis, NCSU) and from South Carolina (samples will be provided by South Carolina DNR). The majority of fin clips will be taken from fish during the peak spawning season (April-August, Ihde 2000) because if there are distinct stocks, they will be separated at the time of spawning. DNA will be isolated from tissue samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

Mitochondrial DNA analysis

The mtDNA control region will be amplified using previously described primers for speckled

trout (Anderson and Karel 2009; DLoop3: TCACCYTRRCTNCCAAAGC, F1: TCACCYTRRCTNCCAAAGC). PCR reactions will be carried out using Qiagen (Valencia, CA) reagents. Amplification products will be cleaned using the QIAquick PCR Purification Kit (Qiagen) and sequenced using the ABI PRISM Big Dye Terminator v 3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) at a 1:8 dilution. Sequencing reactions will be electrophoresed on an ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems, Forest City, CA). Sequences will subsequently be edited using the software Sequencher 4.8 (Gene Codes, Corp., Ann Arbor, MI) aligned using one of the algorithms available in MacVector 12 (MacVector, Inc., Cary, NC). Summary statistics such as nucleon diversity (h), nucleotide diversity (p), number of polymorphic sites (s), base composition, and the number of transitions and transversions will be calculated for each population in ARLEQUIN (Excoffier and Lischer, 2010). Genetic diversity within and among geographic samples will estimated using an analysis of molecular variance (AMOVA) implemented in ARLEQUIN with 10,000 permutations,. Genetic distances will calculated using a best fit model of nucleotide substitution as selected by jMODELTEST 0.1.1 (Guindon & Gascuel 2003; Posada 2008). Pairwise ΦST values will be calculated from control region sequence data in ARLEQUIN. In addition, Network v.4.510 (Fluxus-engineering.com) will be used to create minimum spanning networks from mtDNA sequence data, using the full median joining algorithm (Bandelt *et al.* 1999). Maximum parsimony (MP) analysis was used to remove unnecessary alternate connections (Polzin & Daneshmand 2003).

Microsatellite Analysis

Primers for 41 microsatellite loci have been developed for use in specked trout (Piller and Cordes 2011, Blandon et al. 2012) and many microsatellites developed for the closely related red drum, *Sciaenops ocellatus*, have also been shown to work well in speckled trout (Renshaw et al. 2009, 87 loci; Renshaw et al. 2012, 172 loci). A set of these previously developed are currently being evaluated for amplification consistency, variablilty and conformance to the expectations of Hardy-Weinburg equillibrium (HWE) using a subset of samples from three of the geographically most disparate areas to minimize ascertainment bias. **To date, we have optimized 20 variable microsatellite loci** and are using these markers to generate genetic profiles for our existing samples. We will use these same markers to generate genetic profiles for samples collected in 2015. To ensure consistency, 20% of the 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).

PCR reactions will be carried using Qiagen (Valencia, CA) reagents and fluorescently labeled primers. The resulting PCR products will be separated on an ABI 3130*xl* Prism Genetic Analyzer (Applied Biosystems, Foster City, CA) with a GeneScan 500-Liz size standard (Applied Biosystems, Foster City, CA). The chromatic peaks for each microsatellite locus will be scored using the GENEMARKER AFLP/Genotyping Software, ver. 1.75 (SoftGenetics, State College, PA). 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 of 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 samples will be carried for each microsatellite locus individually and across all loci combined to identify collections that are significantly different. 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 SAMOVA analysis (Doupanloup et al. 2002, available at http://cmpg.unibe.ch/software/samova/), which is similar to AMOVA but employs a simulated annealing approach to define groups of populations that are geographically homogeneous and maximally differentiated from each other will be conducted. 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 of the FSTAT software package (Goudet 1995) and statistically significance of difference in allelic richness among geographic samples will be assessed using Wilcoxon signed rank tests. Whether or not the distribution of genetic variation conforms to an isolation-by-distance model will evaluated using the IBD v1.52 software of Bohanak (2002). This test evaluates whether genetic distance increases with geographic distance and is used to infer limited dispersal ability. Evolutionary and phylogeographic hypotheses regarding alternative divergence models and timing of divergence between samples from different sites will be assessed using the software programs IMa2 (Hey and Nielsen 2004, Hey 2010) and Migrate 3.2.6 (Beerli and Felsenstein 2001). The Migrate software will also be used to evaluate historical effective population size (Ne).

(V.) Location

All research will be carried out at the Virginia Institute of Marine Science (VIMS).

(VI.) Estimated Cost

See attached budget. The proposed budget reflects costs associated with completing the collection of samples and the cost of using molecular markers to look for evidence of genetic stock structure and conducting estimates of genetic diversity.

Salaries: The co-principal investigators along with a technician will participate directly in this research. The genetic portion of the study is based on the labor-intensive nature of generating genetic data. It is also reflective of the time involved in analyzing and interpreting the data.

Lab Supplies: The laboratory portion of the budget is based on the average laboratory costs from the initial DNA isolations through sequencing of mitochondrial loci and amplifying and sizing microsatellite loci. These laboratory costs are based on the price of DNA isolation, PCR kits, cloning kits, sequencing supplies and custom labeled primers as well as consumables such as pipet tips, microcentrifuge tubes and gloves. It also includes supplies associated with running the ABI genetic analyzers such as HiDi formamide, 36 cm capillary arrays (microsatellites), 80 cm capillary arrays (DNA sequencing), GeneScan size standards (Liz 500), Pop 7 polymer, buffer, ABI 96 well plates, and sealing film. We have calculated these costs to be about $40.00/sample

Travel: Travel costs are primarily associated with sample collection.

Facilities and
Administrative Costs

TOTAL

Facilities & Administrative Costs calculated at 25% of direct costs. Approved rate is 44%

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Title: RFAB Speckled Trout, Cynoscion nebulosus, in Virginia: are genetic differences temporally stable?

Personnel	**Time**	**Monthly**	**Agency**	**VIMS**	**Total**
Faculty and Staff					
McDowell, J.	1.00	$7,300	$5,475	$1,825	$7,300
Musick, S.	1.00	$5,400	$0	$5,400	$5,400
Graves, J.	0.50	$13,528	$2,029	$4,735	$6,764
Technical staff	6.00	$3,063	$18,377	$0	$18,377
	-	$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			$25,881	$11,960	$37,841
Personnel, hourly			$0	$0	$0
Personnel, grad assist			$0	$0	$0
Fringe, 40% salaries;			$10,352	$4,784	$15,136
7.65% hourly			$0	$0	$0
Total Personnel			$36,233	$16,744	$52,977
Communications/Printing			$0	$0	$0
Supplies			$12,000	$0	$12,000
Consultant/Skilled Services			$0	$0	$0
Travel			$2,000	$0	$2,000
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			$50,233	$16,744	$66,977
Facilities & Administrative Costs		**25%**	$12,558	$16,912	$29,470
Match					
TOTAL			**$62,791**	**$33,656**	**$96,447**