Proposal Submission to

Recreational Fishery Advisory Board

by

The Virginia Institute of Marine Science
College of William and Mary

Genetic investigation into the distinctiveness of Tautog, *Tautoga onitis*, off the coast of Virginia

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

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VIRGINIA SALTWATER RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION*

NAME AND ADDRESS OF APPLICANT:

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PRIORITY AREA OF CONCERN:

Research and Data Collection

PROJECT LOCATION:

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

DESCRIPTIVE TITLE OF PROJECT:

Genetic investigation into the distinctiveness of Tautog, *Tautoga onitis*, off the coast of Virginia

PROJECT SUMMARY:

Tautog, *Tautoga onitis* (Linnaeus, 1758), support an important recreational fishery in Chesapeake Bay. Tautog, which aggregate around structured habitat (wrecks and rocks, bridge pilings etc.), are slow to mature and have a low reproductive rate relative to other teleosts. Like other reef fishes, tautog is extremely vulnerable to overexploitation. Despite the fact that their movements are limited based on available VGFTP tagging data, tautog are assessed as a single coastwide stock due to the lack of knowledge about appropriate management units. It is unknown if populations in Virginia are self-recruiting, or to what extent recruitment relies on input from other geographic areas. Given that the fishing mortality is above the target reference point, understanding the stock structure is a critical first step towards delineating appropriate management actions. The proposed research will use genetic markers to assess the independence of Virginia's tautog populations. To accomplish this work, we propose to use the mitochondrial (mt) DNA control region and nuclear microsatellite markers to analyze tautog collected from three imporant recreational angling locations in Virginia (CBBT, Chesapeake Light Tower Reef, and Triangle Reef), and compare this data to that obtained from sampling locations north and south of Virginia to identify potential stock boundaries.

EXPECTED BENEFITS:

Knowledge about the demographic independence and stock boundaries of tautog populations in Virginia is critical information for management of this important recreational resource. 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 tautog stocks for Virginia's recreational fishermen.

COSTS:

VMRC Funding:	$76,031
Recipient Funding:	$24,451
Total Costs:	**$100.482**

Detailed budget must be included with proposal.

***This form alone does not constitute a complete application, see application instructions or contact Rob O'Reilly at 757-247-2247or rob.o'reilly@mrc.virginia.gov**

Project Summary

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

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

(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 investigation into the distinctiveness of Tautog, *Tautoga onitis*, off the coast of Virginia

(6) Project Duration: 12 months (January 2014 – December 2014)

(7) Project Summary:

Tautog, *Tautoga onitis* (Linnaeus, 1758), support an important recreational fishery in Chesapeake Bay. Tautog, which aggregate around structured habitat (wrecks and rocks, bridge pilings etc.), are slow to mature and have a low reproductive rate relative to other teleosts. Like other reef fishes, tautog is extremely vulnerable to overexploitation. Despite the fact that their movements are limited based on available Virginia Gamefish Tagging Program (VGFTP) tagging data, tautog are assessed as a single coastwide stock due to the lack of knowledge about appropriate management units. It is unknown if populations in Virginia are self-recruiting, or to what extent recruitment relies on input from other geographic areas. Given that the fishing mortality is above the target reference point, understanding the stock structure is a critical first step towards delineating appropriate management actions. The proposed research will use genetic markers to assess the independence of Virginia's tautog populations. To accomplish this work, we propose to use the mitochondrial (mt) DNA control region and nuclear microsatellite markers to analyze tautog collected from three imporant recreational angling locations in Virginia (Chesapeake Bay Bridge Tunnel (CBBT), Chesapeake Light Tower Reef, and Tringle Reef), and compare this data to that obtained from sampling locations north and south of Virginia to identify potential stock boundaries.

(8) Expected Benefits:
Knowledge about the demographic independence and stock boundaries of tautog populations in Virgnia is critical information for management of this important recreational resource. 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 tautog stocks for Virginia's recreational fishermen.

(9) Budget Information (fiscal year):

Total Funds Requested: 76.031

Cost-sharing: 23,451

Project Total: 100,482

Project Description

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

Tautog, *Tautoga onitis* (Linnaeus, 1758), is a member of the Labridae, a family of lipped fishes commonly known as wrasses. Tautog is generally a coastal species found from the outer coast of Nova Scotia to South Carolina (Collette and Klein-MacPhee, 2002) but is most common from Chesapeake Bay to Cape Cod. Tautog are generally associated with structured habitats such as hard bottom areas around jetties, bridges, reefs and ship wrecks, all of which represent patchy habitat in Virginia (Hostetter and Munroe, 1993).

Tautog support an important recreational fishery in Chesapeake Bay, with a recreational harvest estimated at 479,462 lbs in 2010, and 173, 871 lbs in 2011 (Personal communication from the National Marine Fisheries Service, Fisheries Statistics Division June 11, 2013). It was estimated that over 99.73% of the tautog landed in Virginia were landed by the recreational fishery in 2010 (ASMFC 2011). The recreational tautog fishery in Virginia is a valuable resource, generating 9.7 million dollars in sales and 5.6 million dollars in income yearly (Duberg et al. 2006). Tautog are managed by the Atlantic States Marine Fisheries Commission (ASMFC) and the Fishery Management Plan (FMP) applies to all Atlantic coast states from Massachusetts to North Carolina.

Tagging studies indicate that tautog undergo limited movements, with an onshore offshore pattern of migration but no evidence of either long-range or north-south migration (Cooper 1966, Lynch 1991, Olla et al. 1980). This suggests that it is likely that distinct stocks exist along the Atlantic coast. In Virginia, a study using ultrasonic tags detected limited movement between adjacent inshore sites but did not detect the seasonal offshore movements to overwintering areas seen in more northerly populations (Arendt at al., 2001). This is consistent with results of conventional tagging from the Virginia Gamefish Tagging Program (VGFTP), which showed that, of 563 tagged and recaptured tautog, 476 (85%) were recaptured at the site of release after 0-1214 days at large and these recaptures occurred in all seasons (Arendt et al. 2001). Only 5% of returns during this time period indicated movement between Chesapeake Bay and nearby coastal waters and these movements were relatively short (8-97 km, Arendt et al. 2001). A review of the VGFTP data between 1995-2005 indicated that only 3 out of 1,410 tag returns were recaptured outside Virginia (Hoenig and Lucy, 2004, Tuckey et al, 2007). Similarly, in 2011 and 2012 combined, a single fish tagged in Chesapeake Bay was recaptured in Ocean City, Maryland after 386 days at large. All other recaptures were within Virginia coastal waters (Susanna Musick, VGFTP, personal communication). This tagging data seems to support the hypothesis that there are multiple tautog stocks.

To date, there has been only a single study that used molecular markers to investigate stock structure in tautog. In this study, Orbacz and Gaffney (2000) used restriction length polymorphism (RFLP) analysis of two mitochondrial DNA regions; NADH

dehydrogenase subunit 2 (ND2) and control region. Each of these regions was assayed with a single restriction endonuclease. Denaturing gradient gel electrophoresis (DGGE) was used to assay three additional DNA regions; the nuclear lactate dehydrogenase (LDH) intron and two further mitochondrial regions (cytochrome oxidase subunit I and cytochrome *b*). Samples were obtained from each of three sampling locations: Narragansett Bay, Rhode Island, Delaware Bay, Delaware and Chesapeake Bay, Virginia. This study found no evidence of population structure, however, the sample sizes used were low (24 samples/location) and the methods had low power to resolve differences as compared to highly polymorphic loci, such as nuclear microsatellite loci, that are commonly used today in similar studies.

Although ASMFC initially split the management of tautog into nothern (MA, RI, CT, NY, NJ) and southern (DE, MD, VA, NC) management zones, tautog are currently assessed as a single coastwide stock due to lack of basic data (ASMFC 2002). The most recent coastwide assessment concluded that the stock is overfished and that overfishing is continuing to occur (ASFMC 2011). Current recreational regulations in Virginia require fish to be a minimum size of 16" TL with a posession limit of three fish and a closed season from May1-September 19 (Virginia Marine Resources Commission "Pertaining to Tautog" Regulation 4VAC 20-960-10**).** These regulations are more restrictive than the 2010 regulations which required a 14" TL minimum size, a 4 fish posession limit, and a season that was closed May 1-June 24. Despite continued management efforts, the stock does not show signs of recovery from its overfished status. This may, in part, be due to the underlying stock strucuture. If tautog are found to be comprised of multiple stocks as tagging studies appear to suggest, regional assessments would be more appropriate. Managing several distinct stocks as a single unit can lead to localized depletion, loss of genetic variation and lower recruitment. Alternately, it can also lead to overly restrictive regulations on the fishery (Tuckey et al. 2007). For this reason, the 2010 ASMFC assessment team listed exploring the possibility of local and regional genetic differences in support of region-specific management strategies as a research priority (ASMFC 2010). We propose to develop high resolution genetic markers and use these markers to test the null hypothesis that tautog comprise a single coastwide stock.

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 so that appropriate management units can be delineated. To date, there has been only a single low-resolution genetic study of tautog. As a result, there is no information available regarding genetic connectivity among locations either within Chesapeake Bay or between Chesapeake Bay and adjacent locations. It is unknown if populations in Virginia are self-recruiting or to what extent recruitment relies on input from other geographc areas. The proposed research will use genetic markers to assess the independence of Virginia's coastal tautog 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 assay variation of the mitochondrial (mt) DNA control region and develop and apply nuclear microsatellite markers to survey tautog

collected from several locations off the coast of Virginia. We will compare data among these locations to look for evidence of stock structure. We will also compare these collections to smaller-scale collections from sampling locations both north and south of Virginia to assess whether north-south geographical stock boundaries exist. The results of these analyses will be provided to the Virginia Marine Resources Commission 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.

(III.) Expected results or benefits:

If tautog are composed of multiple distinct genetic stocks, localized depletion resulting from intense fishing pressure at single locations may result in the loss of unique genetic variation and localized collapse of the fishery. Alternately, if tautog are comprised of a single coastwide stock, an increase in fishing pressure in other states may negatively impact Virginia's fishery due to lower recruitment. Each of these scenarios has different implications for the stock. We propose to delineate the underlying genetic basis of stock structure within Virginia's waters. We also plan to compare these samples to samples collected both North and South of Virginia.

Our analysis will focus on the rapidly evolving molecular markers; the mtDNA control region, and nuclear microsatellite loci. We will address the following null hypotheses:

(1) There is no genetic difference between samples of tautog collected from among different sampling locations off the coast of Virginia.
(2) There is no genetic difference between tautog collected from Virginia and adjacent regions (Maryland and North Carolina).

(IV.) Approach

Sample Collections

Collections of at least 50 tautog fin clip samples/location will be obtained in copperation from recreational fishermen (VGFTP and local angling clubs) from three important rectreational angling locations representing an inshore-offshore gradient. Locations will include the CBBT complex, the Chesapeake Light Tower Reef, and the Triangle Reef area. These samples will be compared to those taken north of Virginia (Maryland and New Jersey). If available, samples will also be collected from the North Carolina fishery.

Mitochondrial DNA analysis

DNA will be isolated from tissue samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA). The mtDNA control region will be amplified using previously described primers for tautog (see Orbacz and Gaffney 2000). 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; Excoffier *et al*. 1992) 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

Marker Development:
There are no microsatellite markers published for the tautog or for any closely related species, therefore, de novo markers need to be developed. We will use a shotgun sequencing approach for microsatellite marker discovery. Briefly genomic DNA will be extracted from tautog tissue collected from a fresh specimen following a standard phenol-chloroform extraction protocol (Sambrook and Russell 2001) and rehydrated in 1.0 ml of TE buffer (pH 8.0). Approximately 2 µg of extracted DNA will then purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) following the manufacturer's protocol and eluted in 60 µl of sterile dH_2O. A fragment library will be constructed and sequenced on a 318 sequencing chip using an Ion Torrent sequencer (Ion Torrent Systems, Inc., Guilford, CT), which will generate sequence data for analyses. The resulting sequences will be groomed using the FASTQ groomer (v 1.0.4, Blankenberg et al., 2010) in the Galaxy platform and the quality will be checked using the FASTQC software (Babraham Bionformatics, Cambridge UK). Sequences will then be quality filtered to remove low-quality sequences using the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). After processing, sequences will be converted to FASTA format using the FASTq to FASTA converter in Galaxy (Blankenberg et. al., 2010) and exported. Exported sequence files will be screened for the presence of microsatellites using the MSATCOMMANDER 1.0.8 software (Faircloth 2008) and primers will be designed using PRIMER3 (Rozen and Skaletsky 2000). This approach to markers development has been used to successfully generate hundreds to thousands of potential microsatellite loci in other fish species (McDowell, unpublished data).

Once designed, primers will be used to amplify targeted loci using standard PCR protocols, and the resulting amplification products will be electrophoresed against a size standard on submerged horizontal agarose gels to assure that products amplify successfully and are of the expected size. Markers found to reliably amplify a product of the expected size will be evaluated for a subset of samples (n = 16) to assess amplification consistency, levels of polymorphism and conformance to the expectations of Hardy-Weinburg Equilibrium (HWE) as follows: DNA will be extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. 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 scored, 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 (exact tests, Guo and Thompson 1992) .

When markers have been optimized, all sample collections will be processed as above. To ensure consistency, 20% of the subset of samples used to evaluate the newly developed markers 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 has 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. As above, 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 samples will be carried out individually for each microsatellite locus and across all loci to identity pairs of samples 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 SAMOVA analysis (Dupanloup 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. 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 in the using 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 as a function of 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 Virgnina Institute of Marine Science (VIMS)

(VI.) Estimated Cost

Total Project Costs and Budget Narrative

See attached budget. The proposed budget reflects costs associated with completing sample collection, the creation of molecular markers specific to tautog, screening these new markers for reliability and variability, and applying these markers to multiple sample collections to look for genetic evidence of stock structure.

Salaries: The co-principal investigator will participate directly in this research, developing molecular markers, collecting samples and data and analyzing the data. The genetic portion of the study is based on the labor-intensive nature of developing microsatellite DNA libraries, generating sequence data and designing and optimizing appropriate primer-pairs to amplify the loci. It is also reflective of the time involved in analyzing the samples collected with these newly developed markers, analyzing the data and interpreting the results.

Lab Supplies: The laboratory portion of the budget is based on the average cost of generating a microsatellite DNA library from the initial DNA isolations through sequencing of potential loci and marker development. 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, 80 cm capillary arrays, GeneScan size standards (Liz 500), Pop 7 polymer, buffer, ABI 96 well plates, and sealing film. We have calculated the costs of all the supplies at abut $40/sample once the molecular markers have been developed. The proposed 250 samples over would cost an estimated 10,000. This does not include the cost of marker development, which is estimated at $4,000 for the necessary supplies.

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%.

References

Arendt MD, Lucy JA, Munroe TA (2001) Seasonal occurrence and site utilization patterns of adult tautog, *Tautoga onitis* (Labridae), at manmade and natural structures in lower Chesapeake Bay. Fishery Bulletin 99: 519-527.

Atlantic States Marine Fisheries Commission (ASMFC) (2002) Review of the Atlantic States Marine Fisheries Commission fishery management plan for tautog (*Tautoga onitis*). Report prepared by H. Stirratt (ASMFC) and the Tautog Plan Review Team (P. Caruso, D.Simpson, F. Steimle, and N. La.

ASMFC (2010) Review Of The Atlantic States Marine Fisheries Commission Fishery Management Plan For TAUTOG Prepared by: Tautog Plan Review Team Board Accepted August 2011.

ASMFC (2011) Tautog Assessment Update Summary. ASMFC Stock Assessment Subcommittee (P. Caruso, J. Cimino, J. McNamee, J. Brust, G. Nesslage, R. Wong)

Bandelt H-J, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16, 37–48.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98: 4563-4568.

Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A. Galaxy Team (2010) Manipulation of FASTQ data with Galaxy. Bioinformatics. 5;26(14):1783-5.

Bohonak AJ (2002) IBD (Isolation By Distance): A program for analyses of isolation by distance. Journal of Heredity 93: 153-154.

Collette BB, Klein-MacPhee G. (2002) Bigelow and Schroeder's 'Fishes of the Gulf of Maine' (3rd ed.). Washington (DC): Smithsonian Institution Press. 748 pp.

Cooper, R. A. (1966) Migration and population estimation of the tautog, *Tautoga onitis* (Linnaeus), from Rhode Island. Fish. Soc. 95:239–247. Trans. Am.

Duberg J., Kirkley JE, 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.

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

Excoffier L, Smouse, PE, Quattro, JM (1992) Anaylisis of Molecular Variance Inferred From Metric Distances Among Haplotypes- Application to Human Mitochondrrial- DNA Restriction Data. Genetics 131: 479-491.

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

Faircloth, BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources 8:92-94. doi:10.1111/j.1471-8286.2007.01884.x.

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

Guindon S, 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.

Guo, SW and Thompson, EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48 (2): 361-72.

Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura and D. persimilis.* Genetics 167: 747-760.

Hey J. (2010) Isolation with Migration Models for More Than Two Populations. Mol. Biol. Evol. 27: 905-20.

Hoenig, JM, Lucy J (2004) Project RF03-15: Determining stock status of tautog in Virginia waters using data from Virginia's fishery. Final Report to the Virginia Marine Resources Commission, Project RF03-15. Newport News, Virginia.

Hostetter EB, Munroe TA (1993) Age, growth, and reproduction of tautog *Tautoga onitis* (Labridae: Perciformes) from coastal waters of Virginia. Fish. Bull. 91:45–64.

Lynch TR (1991) Sportfish population survey in Rhode Island marine waters-Tautog studies. Performance Report. R.I. Div. Fish and Wildlife Project No. F-54-R-1.

Morin PA, Leduc RG, Archer FI, Martien KK, Huebinger R, Bickham JW, Taylor BL (2009) Significant deviations from Hardy-Weinberg equilibrium caused by low levels of microsatellite genotype errors. Molecular Ecology Resources 9(2): 498-504.

Olla BL, Studholme AL, Bejda AJ, Samet C. (1980) Role of Temperature in Triggering Migratory Behavior of the Adult Tautog *Tautog onitis* Under Laboratory Conditions. Marine Biology 59: 23-30.

Orbacz EA, Gaffney PM (2000) Genetic structure of tautog (*Tautoga onitis*) populations assayed by RFLP and DGGE analysis of mitochondrial and nuclear genes. Fishery Bulletin 98:336-344.

Polzin T, Daneshmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. Operations Research Letters, 31, 12–20.

Posada D (2008) jMODELTEST: Phylogenetic model averaging. Molecular Biology and Evolution, 25, 1253-1256.

Rozen S, Skaletsky HJ. (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, NJ, pp 365-386.

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

Sambrook J and Russell DW (2001) Molecular Cloning: A Laboratory Manual. ColdSpring Harbor Laboratory Press, Cold Spring Harbor, New York.

Tuckey T, Yochum N, Hoenig J, Lucy J, Cimino J (2007) Evaluating Localized vs. Large-scale Management: The Example of Tautog in Virginia. Fisheries: 32 21-28.

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.

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

RFAB Tautog Project

Personnel	**Time**	**Monthly**	**Agency**	**VIMS**	**Total**
Faculty and Staff					
McDowell, Jan	2.00	$6,396	$6,396	$6,396	$12,792
Small, Hamish	6.00	$4,270	$25,622	$0	$25,622
Technical support	-	$0	$0	$0	$0
	-	$0	$0	$0	$0
	-	$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			$32,018	$6,396	$38,414
Personnel, hourly			$0	$0	$0
Personnel, grad assist			$0	$0	$0
Fringe, 40% salaries;			$12,807	$2,558	$15,365
7.65% hourly			$0	$0	$0
Total Personnel			$44,825	$8,954	$53,779
Communications/Printing			$0	$0	$0
Supplies			$14,000	$0	$14,000
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		$60,825	$8,954	$69,779
Facilities & Administrative Costs	**25%**	$15,206	$15,497	$30,703
TOTAL		**$76,031**	**$24,451**	**$100,482**