VIRGINIA SALTWATER RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION*

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NAME AND ADDRESS OF APPLICANT:	PROJECT LEADER (name, phone, e-mail):
Virginia Institute of Marine Science P.O. Box 1346, 1375 Greate Rd. Gloucester Point, VA 23062	Jan McDowell (804) 684-7263 McDowell@vims.edu John Graves (804) 684-7352 Graves@vims.edu
PRIORITY AREA OF CONCERN:	**PROJECT LOCATION:**
Research	Primary sample collection in Virginia with comparative samples in North Carolina, South Carolina and the Gulf of Mexico.

DESCRIPTIVE TITLE OF PROJECT:

A genetic-based investigation of blueline tilefish (*Caulolatilus microps*) and snowy grouper (*Epinephelus niveatus)* stock structure

PROJECT SUMMARY:

We propose to examine population structuring within the snowy grouper and blueline tilefish, two species targeted in the Virginia deep-drop recreational fishery. Our analysis will focus on rapidly evolving molecular markers, mitochondrial DNA (mtDNA) control region and nuclear microsatellite loci. We will assess whether or not there are genetic differences between samples collected among geographic locations for both species. We will also assess temporal variation among samples taken across years in Virginia.

EXPECTED BENEFITS:

The snowy grouper and blueline tilefish are extremely vulnerable to overfishing because of they are long-lived, slow growing and live in localized aggregations as adults that make them easy to target. There have been no genetic studies conducted for either of these species and it is unknown if populations in Virginia are self-recruiting or if and to what extent they rely on input from other geographic areas. Obtaining a baseline measure of genetic diversity among fish sampled in Virginia will allow monitoring of stocks for the loss genetic diversity that has been documented in other species with similar life history characteristics. This information is crucial to appropriate management efforts.

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COSTS:

		Year 1
VMRC Funding:	~~150,893~~	$ 74,155
Recipient Funding:	~~27,764~~	$ 13,645
Total Costs:	~~**178,657**~~	$ 87,800

Detailed budget must be included with proposal.

Updated 11/12/08

***This form alone does not constitute a complete application, see application instructions or contact Sonya Davis at 757-247-8155 or sonya.davis@mrc.virginia.gov**

				YEAR 1					YEAR 2			SUMMARY		
A.	**Personnel**	Time	Monthly	**Agency**	**VIMS**	**Total**	Time	Monthly	**Agency**	**VIMS**	**Total**	**Agency**	**VIMS**	**TOTAL**
	McDowell, J	1.00	$5,959	$5,959	$0	$5,959	1.00	$6,257	$6,257	$0	$6,257	$12,216	$0	**$12,216**
	Graves, J.	0.50	$12,460	$6,230	$0	$6,230	0.50	$13,083	$6,542	$0	$6,542	$12,772	$0	**$12,772**
	Hill, K	6.00	$2,888	$17,328	$0	$17,328	6.00	$3,032	$18,194	$0	$18,194	$35,522	$0	**$35,522**
		-	$0	$0	$0	$0	-	$0	$0	$0	$0	$0	$0	**$0**
		-	$0	$0	$0	$0	-	$0	$0	$0	$0	$0	$0	**$0**
		-	$0	$0	$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	$0	$0	**$0**
		-	$0	$0	$0	$0	-	$0	$0	$0	$0	$0	$0	**$0**
	Personnel, salaried			$29,517	$0	$29,517			$30,993	$0	$30,993	$60,510	$0	**$60,510**
	Personnel, hourly			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
	Fringe, 40% salaries;			$11,807	$0	$11,807			$12,397	$0	$12,397	$24,204	$0	**$24,204**
	7.65% hourly			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
	Total Personnel			$41,324	$0	$41,324			$43,390	$0	$43,390	$84,714	$0	**$84,714**
B.	**Communications/Printing**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
C.	**Supplies** (sequencing kits, primers, lab supplies, etc.)			$15,000	$0	$15,000			$15,000	$0	$15,000	$30,000	$0	**$30,000**
D.	**Travel** (to field sites)			$3,000	$0	$3,000			$3,000	$0	$3,000	$6,000	$0	**$6,000**
E.	**Contractual Services**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
				$0	$0	$0			$0	$0	$0	$0	$0	**$0**
				$0	$0	$0			$0	$0	$0	$0	$0	**$0**
F.	**Tuition**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
G.	**Vessels**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
H.	**Publication Center**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
I.	**Nutrient Analysis**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
J.	**Equipment**			$0	$0	$0			$0	$0	$0	$0	$0	**$0**
	SUBTOTAL: Direct Costs			$59,324	$0	$59,324			$61,390	$0	$61,390	$120,714	$0	**$120,714**
K.	**Facilities & Administrative Costs**	**25%**		$14,831	$13,645	$28,476			$15,348	$14,119	$29,467	$30,179	$27,764	**$57,943**
	TOTAL			**$74,155**	**$13,645**	**$87,800**			**$76,738**	**$14,119**	**$90,857**	**$150,893**	**$27,764**	**$178,657**

Notes on Budget Items

A. Salaries include annual **5%** increase.
Fringes are based on average costs: **40%**

E. Only take IDC on first $25,000 of each contract.

J. Equipment -- items more than $5,000, no IDC. If the equipment is under $5,000 include it in the supply line item.

K. F&A calculated on MTDC (modified total direct cost): personnel, supplies, travel, and first $25,000 of each subcontract, etc.; excludes service centers, tuition and equipment.
The current federally negotiated F&A rate is **48%**

Proposal Submission to

Recreational Fishery Advisory Board

by

The Virginia Institute of Marine Science
College of William and Mary

A genetic-based investigation of blueline tilefish (*Caulolatilus microps*) and snowy gouper (*Epinephelus niveatus)* stock structure along the US east coast

Proposed starting date: 1 January 2013
Proposed duration: 24 months

Dr. Jan R. McDowel
Principal Investigator
Department of Fisheries Science

Dr. John E. Graves
Co-Principal Investigator
Department of Fisheries Science

Ms. Margaret Fonner
Director, Sponsored Programs

Dr. Roger L. Mann
Director for Research and
Advisory Services

Project Description

(I.) Identification of Problem:

Until recently, North Carolina was believed to be the northern extent of the range of both the blueline tilefish (*Caulolatilus microps*) and snowy grouper (*Epinephelus niveatus)*. These species, which are often caught together in the same location and during the same trip, have recently been discovered in Virginia waters and have become the focus of a deep-drop fishery, resulting in several world records. In addition to being target species of the Virginia deep-drop fishery, blueline tilefish and snowy grouper are caught in commercial fisheries.

Blueline tilefish, *Caulolatilus microps*, also known as Gray tilefish (Goode & Bean, 1878), is a bottom dweller found at depths of 240-780 feet from Virginia to the Campeche Banks of Mexico, in the same habitat as groupers and snappers. North Carolina was previously considered to be the northern extent of the range of blueline tilefish (Dooley1978), however populations have been discovered in Virginia over the past several years, extending the known range. Like other species of tilefish, blueline tilefish are long-lived and slow-growing with an estimated lifespan of 15 years. Blueline tilefish reach maturity at around age three and are sexually dimorphic, with males reaching a larger maximum size than females (Harris, 2004). A study off the southeastern coast of the United States (North and South Carolina) found that blueline tilefish are batch spawners. Spawning occurs in the evening from February-October with a peak in May. This study also found a positive relationship between size and fecundity in females (Harris et al. 2004).

The life-history parameters of blueline tilefish make them particularly vulnerable to overfishing. A recent productivity susceptibility analysis (PSA), which estimates risk of human impact, i.e. whether or not a fishery is ecologically sustainable, rated the blueline tilefish as 'most vulnerable' to overfishing (PSA 3.4, MRAG Americas 2009). This is based on productivity of the stock, which determines the rate at which it can recover from potential depletion and on the susceptibility to overfishing. Recent studies of blueline tilefish off the Carolinas (Herbert 2004, Rudershausen et al. 2009) indicate that the age structure and sex ratio of blueline tilefish in that area have undergone significant changes over a short period of time due to overfishing. Currently Virginia has regulations regarding the harvest of tilefish with a recreational limit of seven (http://mrc.virginia.gov/regulations/fr1120.shtm) and a commercial limit of 300 pounds (http://mrc.virginia.gov/Notices/pn_multi0112.shtm).

The snowy grouper (*Epinephelus niveatus)* is a commercially important deepwater species with a distribution ranging from North Carolina to Brazil, including the Gulf of Mexico and the Caribbean (Smith 1971, Huntsman and Dixon 1976, Matheson and Huntsman 1984, Moore and Labisky, 1984), with populations recently discovered off Virginia. As adults, snowy grouper are predominantly found on the upper continental slope at depths of 116-259 meters, while juveniles are generally found at shallower depths (Moore and Labinsky 1984, Parker and Ross, 1986).

Snowy grouper are protogynous hermaphrodites and females begin to mature at around 3 years with 100% maturity by age seven (Wyanski et al. 2000). Transformation from female to male can begin as early as age seven, no males under age seven have been found (Kowal, 2010). It is not known if all females eventually transition to males. Eggs and larvae are pelagic (Moore and Labinsky 1984, Parker and Mays 1998) and larvae have only been reported in the Florida Keys. The pelagic larval duration is unknown. Nursery grounds are hypothesized to be in shallower depths along the shelf (Moore and Labinsky 1984).

Snowy grouper are very long-lived with an estimated lifespan of 21- 29 years based on ageing of otoliths from samples taken off the Carolinas (Wyanski et al. 2000), although ageing of fish caught off Brazil resulted in an estimated lifespan to up to 59 years (Costa et al. 2011). There is some evidence that snowy grouper, like other epinephaline serranids, form spawning aggregations (Wyanski et al. 2000). This combination of life history traits, along with its patchy distribution makes the snow grouper extremely vulnerable to over-exploitation (Colmn et al. 2000, Morris et al. 2000). Wyanski et al (2000) examined 599 sexually mature individuals from the 1970s, 1980s and 1990s to look for evidence of over-exploitation and found that the number of males in the population had substantially decreased from 22.9% to 1.9% in the time period examined, a hallmark of over-exploitation in grouper species. A recent PSA rated the blueline tilefish as a high risk for overexploitation and overfished (PSA 3.5, MRAG Americas 2009). Currently Virginia has regulations regarding the harvest of snowy with a recreational limit of one fish and and a commercial limit of 175 pounds (http://mrc.virginia.gov/regulations/fr1120.shtm).

Genetic monitoring, which uses molecular markers to follow changes in populations over time, is an increasingly important component of conservation efforts because of the wide range of information that can be obtained from a single sample. Monitoring can include identification of genetic stocks, mixed stock analysis, genetic tagging (capture-recapture) of individuals, changes in population genetic parameters such as loss of alleles, shifts in allele frequencies and effective population size, and can also include assessment of historical demography for comparison with current estimates (see Luikart et al. 2003 and Schwartz et al. 2007 for reviews). Genetic monitoring becomes increasingly important as species become exploited. The establishment of a genetic baseline before exploitation and ability to monitor blueline tilefish and snowy grouper in the future is hindered by an almost complete lack of genetic information.

The life-history parameters of both the blueline tilefish and snowy grouper make them susceptible to rapid depletion (Coleman, 2000). They are long-lived and slow-growing species. In addition, they live in localized aggregations as adults, which make them easy to target. Release mortality can be very high for both species due to barotrauma. This has been a problem in other states, leading to strict limitations on the fishery; under current South Atlantic Fishery Management Council (SAFMC) regulations, harvest and retention of snowy grouper and blueline tilefish is prohibited beyond 240 ft., however Virginia falls outside the management range. No genetic studies have been conducted for either of these species, thus there is no information available regarding genetic connectivity among

locations (i.e., it is unknown if stocks in Virginia are self-recruiting or if and to what extent they rely on input from other geographic areas). Additionally, obtaining a baseline measure of genetic diversity among fish sampled in Virginia will allow monitoring of stocks for the loss genetic diversity that has been documented in other species with similar life history characteristics. This information is crucial to developing appropriate management efforts to preserve the fishery for recreational fishermen in Virginia.

(II.) Objective:

To accomplish this work, we propose to assay genetic variation based on both the mitochondrial DNA (mtDNA) control region and nuclear microsatellite markers to survey stock structure of blueline tilefish and snowy grouper collected from across the range of both species. Samples of blueline tilefish and snowy grouper will be collected from Virginia, North Carolina, Florida and the Gulf of Mexico. Sampling will take place over two years for both species to assess temporal stability of genetic profiles at each collection location.

DNA will be isolated from tissue samples and the mtDNA control region will be amplified and sequenced. In addition, a suite of nuclear markers will be screened for appropriate levels of genetic variation using a subset of samples from at least two sampling locations. All collections will be screened using those markers that are found to be variable. The mtDNA and nuclear data will be analyzed to evaluate temporal and spatial components of genetic variance. We anticipate this work will take two years for the collection of samples, development of molecular markers, and analysis of results.

(III.) Expected results or benefits:

The expected outcomes of this study are two well-characterized and optimized sets of molecular markers, one for use in studies of the blueline tilefish and another for use in studies of the snowy grouper. These markers will be used to examine samples of from along the U.S. east coast to test whether they comprise separate genetic stocks. If these species are found to have significant genetically based stock structure, localized depletion resulting from fishing pressure may result in the loss of unique genetic variation. We will generate baseline information on population genetic parameters that will be important in the management of these species. This information is critical prior to exploitation and to the ongoing conservation of the species. The data obtained will be made available to resources managers at state, regional, and national levels for use in stock assessments and the development of management plans. This will take the form of a peer-reviewed publication, and seminars given to the scientific community through conference presentations, which will benefit both the public and fishery managers.

(IV.) Approach:

We propose to examine genetic variability and population structuring within the snowy grouper and blueline tilefish. Our analysis will focus on rapidly evolving molecular markers, namely the mtDNA control region and nuclear microsatellite loci. We will use these markers to address the following null hypotheses:

(1) There is no genetic difference among samples of snowy grouper collected from different geographic locations.

(2) There is no genetic difference between temporal samples of snowy grouper collected from the same location.
(3) There is no genetic difference among samples of blueline tilefish collected from different geographic locations.
(4) There is no genetic difference between temporal samples of snowy grouper collected from the same location.

Sample Collection (blueline tilefish and snowy grouper):
To address these hypotheses, at collections of 50 individuals each of snowy grouper and blueline tilefish will be obtained from each of four locations along the US east coast (Virginia, North Carolina, Florida, Gulf of Mexico) during 2013 and 2014. We have arranged with colleagues both in Virginia and in other collection areas to obtain samples. Tissue samples will be placed in 95% ethanol at the point of collection for storage and transport to VIMS for DNA extraction.

MtDNA Analysis (blueline tilefish and snowy grouper): Genomic DNA will be extracted from all samples using the DNeasy Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The control region will be amplified using conserved primers and 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 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 the best fit model of nucletide substitution as selected by jMODELTEST 0.1.1 (Guindon & Gascuel 2003; Posada 2008). Pairwise Φ_{ST} 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 library development (blueline tilefish only): There are no existing microsatellite markers available for the blueline tilefish or for any closely related species, therefore, de novo markers need to be developed. The protocol to generate the microsatellite enriched genomic library for blueline tilefish will primarily follow the procedures of Glenn and Schable (2005) and McDowell et al. (2002). Briefly, genomic DNA will be extracted from blueline tilefish heart tissue collected from a fresh specimen following a standard phenol-chloroform extraction protocol (Sambrook & Russell 2001)

and rehydrated in 1.0 ml of TE buffer (pH 8.0). Approximately 2 µg of extracted DNA will then be digested to an average size of 500-1200 bp and DNA fragments <100 bp will be removed from the digestion reaction using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) following the manufacturer's protocol and eluted in 60 µl of EB buffer.

The digested DNA will be ligated to SuperSNX24 double stranded linkers (Glenn & Schable 2005) and enrichment will be performed on the ligated DNA fragments using a mixture of di and tetra-nucleotide repeat motifs that have had biotin added to their 3' ends for subsequent magnetic capture. Hybridizations of DNA to probes will be carried out following the methods outlined in McDowell et al., 2002. Hybridized DNA-probe mixtures will then be added to Streptavidin MagneSphere Paramagnetic Particles (Promega, Madison, WI) and incubated at 43º C to capture microsatellite enriched DNA following Kijas et al. (1994). The resulting microsatellite-enriched DNA will then be ligated into plasmids and transformed into competent cells using the TOPO TA Cloning Kit and TOP 10 chemically competent cells (Invitrogen, Carlsbad, CA). Cells will be grown on LB ampicillin plates to select for cells with plasmids containing inserts.

The clones generated from the microsatellite-enriched library will be sequenced using ABI PRISM Big Dye Terminator v 3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) at 1:8 dilution, and sequenced 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), and microsatellite motifs will be identified using Repeat Masker (available at http://ftp.genome.washington.edu/RM/RepeatMasker.html). Primers will be developed from flanking regions using PRIMER 3 (Rozen and Skaletsky, 2000) and MacVector 10 (MacVector, Inc., Cary, NC).

Screening of microsatellite loci (blueline tilefish and snowy grouper): For blueline tilefish, newly developed markers will be screened for variability. For snowy grouper, microsatellite markers developed for closely related species (Renshaw et al., 2009) will be screened. Protocols for the two species will be identical from this point forward.

Once designed, primers for both species 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 Virginia and Gulf of Mexico samples (n = 30 each) to assess amplification consistency and levels of polymorphism 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, vers. 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) and 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 accessed via permutations of the data.

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 markers have been optimized, samples of both species from all collection locations will be analyzed as follows. Briefly, DNA will be extracted and 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, vers. 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. 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) and 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. Measures of allelic richness will be carried out using the FSTAT software (Goudet 1995) and 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).

(V.) Location:
All research will be carried out at the Virginia Institute of Marine Science in Gloucester Point, Virginia.

(VI.) Estimated Cost:
See attached budget. The proposed budget reflects costs associated with completing sample collection, the creation of molecular markers specific to blueline tilefish and snowy grouper, screening these new markers for reliability and variability, and looking for genetic evidence of 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 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 the markers developed in the course of this study.

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 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, microtubes 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 $60/sample once the molecular markers have been developed. The proposed 400 samples over the two years of the study would cost an estimated 24000. This does not include the cost of marker development which is estimated at $6,000 for the necessary supplies.

Travel: Travel costs are primarily associated with sample collection, which will entail travel to docks where the fish are landed.

References:

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