

Virginia Fishery Resource Grant Program 2019 – Final Report

**A Genetic Assessment of the Channeled Whelk Resource Targeted
by Virginia Commercial Fishermen**

Project #: FRG 2019-02

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Introduction:

Channeled whelk (*Busycoptypus canniculatus* Linnaeus, 1758) are predatory marine gastropods found in intertidal regions of the continental slope along the U.S. Atlantic coast from Cape Cod, MA to Cape Canaveral, FL (Figure 1) (Davis & Sisson 1988; Edwards & Harasewych 1988). For the past 5 decades, channeled whelk have provided economic benefits to directed fisheries in the North and mid-Atlantic region. Beginning in the mid 1980's an unregulated commercial fishery developed in New England and by the 1990s, landings from post-production processed whelk meat had reached an all-time high of 1.4 million pounds at \$1.80 per pound, generating approximately \$2.5 M in ex-vessel revenue. In 2010, the total economic value of processed channeled whelk from Virginia's conch-pot fishery, including supplies from other mid-Atlantic states, was estimated at \$15-20 million dollars (Manion et al. 2000; Fisher 2014). In 2016, Virginia's conch industry estimated landings at 1.2 million pounds of shellstock valued at \$2.8 M (R. Robins data). The conch fishery has developed into an important source of supplementary income for Virginia's commercial fishers.

Currently, regulations for the channeled whelk commercial fishery are based on those of the knobbed whelk (*Busycon carica* Gmelin, 1791), a closely related species that occupies the same geographic range. The channeled whelk resource is managed at the state level in the mid-Atlantic region and minimum landing size (MLS) varies by state. The current MLS is 5" in New Jersey, 6" in Maryland and Delaware, and 5.5" in Virginia; other states with channeled whelk fisheries are unregulated (Figure 2). In the early 1990s, Virginia began issuing experimental fishing permits in recognition of knowledge gaps surrounding stock structure and reproductive parameters of channeled whelk. Despite these efforts, channeled whelk life history data remains limited.

Throughout its range, the age and growth estimates for channeled whelk, assessed with statolith recordings and von Bertalanffy growth curves, can vary regionally (Fisher & Rudders 2017; Nelson et al. 2018). On average channeled whelk mature between ages 5-7, with the oldest recorded age estimated at 14 years. Females are known to be larger than males on average and do not reach sexual maturity until 5.8 – 6.2" shell length (SL), as compared to 4.7 – 5.3" SL in males (Figure 2) (Fisher & Rudders 2017). Based on shell width, a measurement used for determining MLS, age at a MLS of 2.75" shell width (SW) in Buzzards Bay, Massachusetts was calculated at 7.5 years for males and 6.3 years for females (Peemoeller & Stevens 2013). At this MLS, females are estimated to be entering the fishery approximately 2 years before they are sexually mature (Peemoeller & Stevens 2013). Research conducted in the mid-Atlantic region found females harvested at the MLS of 5.5" SL in Virginia had a low probability (1-15%) of being mature, making them extremely vulnerable to overexploitation (Fisher & Rudders 2017). In 2018 the first stock assessment for channeled whelk was performed in Massachusetts, which found that channeled whelk populations in Nantucket Sound are likely overfished and overfishing is occurring (Nelson et al. 2018). There is rising concern about the potential for population collapse caused by recruitment overfishing, the removal of females before they have a chance to reproduce. Recruitment overfishing can lead to stock collapse, with increased risk in areas with a highly variable environment.

It is critical to explore the spatial interactions among subpopulations when developing a sustainable fishery (Ying et al. 2011). Ignoring such spatial dynamics can lead to biased estimations of population parameters and stock status, and thus inaccurate management targets and harvest levels. For example, if several demographically independent stocks are managed as a single stock, some stocks may be extirpated. Alternately, management of a single panmictic

stock, as multiple distinct units may lead to overly restrictive management of the resource (Tuckey et al. 2007). The lack of understanding of the spatial scale of connectivity has hindered the ability to effectively manage the whelk resource, making stock assessment for this species difficult and impacting the ability to determine the appropriate management scale.

The purpose of this project was to provide genetic information that can be used by management agencies to develop a fisheries management plan for channeled whelk, or improve coordination between state regulators. The main objective of this research project was to investigate whether a single or multiple populations of channeled whelk are being exploited as a first step to ensuring sustainable management of the valuable whelk resource. The genetic assessment, combined with existing data on life history characteristics, can be used by state agencies to create appropriate management strategies that will help ensure continued successful commercial harvest of channeled whelk in the mid-Atlantic.

Methods:

Tissue sample collection was focused on the mid-Atlantic region, however to put the mid-Atlantic region into context, sample collection also took place throughout the entire geographic range of channeled whelk (Figure 1 & Table 1). This grant provided funding to obtain samples from NC and MA, while other samples were provided by Bernie's Conchs, LLC or by state agencies. Site locations were chosen due to their proximity to commercial fishing regions. Channeled whelk were measured and sexed, and a small piece of foot muscle was placed into 95% ethanol and sent to the Fisheries Genetics Lab at the Virginia Institute of Marine Science (VIMS) (Figures 3 & 4).

Generation and analysis of genetic data were contracted to Virginia Institute of Marine Science and data are part of the Master's research of Sam Askin (J. McDowell, advisor). A brief outline of genetic lab work is listed herein (Figure 5). DNA was isolated from tissue samples using the Macherey-Nagel NucleoSpin Tissue DNA Extraction Kit according to the published procedure (Machery Nagle, Düren, Germany). There were no published microsatellite markers for channeled whelk or for any closely related species, therefore *de novo* markers were developed. Testing and optimization of microsatellite loci (markers) occurred from July 1 to August 22, 2019. Of the 62 primer pairs that were ordered, 20 microsatellite markers showed a clear amplification product and were subsequently tested across multiple whelk samples. Five of these microsatellite markers amplified consistently across samples and had multiple alleles, while the remainder (15 markers) were either monomorphic or failed to amplify across a panel of test samples from across the geographic range of channeled whelk. To address this issue, we changed approach and used high throughput genotyping-by-sequencing to discover single nucleotide polymorphism (SNP) markers.

A genotyping-by-sequencing method, DArTseq, was completed at the Diversity Arrays Technology facility in Canberra, Australia. This process combines complexity reduction methods to target low copy sequences with high throughput sequencing allowing for the identification of thousands of SNPs (DArT PL, Canberra, Australia). DArTseq ensures high call rates and reproducibility, with reliability in calling heterozygotes. DNA extractions from 282 channeled whelk samples were evaluated to ensure high quality and quantity DNA. Out of the 282 channeled whelk DNA extractions, 239 had a sufficient amount of high molecular weight DNA (20µl of an aqueous solution of DNA at 50 – 100 ng per µl) to perform sequencing (Table 1). DArTseq yielded 27,344 SNPs that passed standard Illumina quality filtering procedures.

Additional filtering was performed on the SNP data provided by DArT PL in R version 3.3.1 (R Core Team, 2020) using the program package “dartR” (Gruber et al. 2019). SNPs were removed if locus genotype calls were missing in $\geq 10\%$ of samples, average read depths fell outside of the default settings, average reproducibility between technical replicates fell below $< 95\%$, multiple SNPs were present within a fragment, if minor allele frequencies were $< 5\%$, if individual genotype calls were missing data for $\geq 20\%$ of loci, and if the loci were not in conformance with the expectations of Hardy-Weinberg Equilibrium (HWE). A total of 5,328 SNPs were retained for statistical analyses. Preliminary analysis included principal component analyses (PCA) created in R version 3.3.1 (R Core Team, 2020) using the program package “adeigenet” (Jombart et al. 2020), to visualize the genetic similarities or dissimilarities in data and identify genetic clusters. An unbiased estimator of Wrights F-statistics (F_{ST}) was calculated between pairs of sampled populations to evaluate the presence of population structure. This was completed in R version 3.3.1 (R Core Team, 2020) using the program package “StAMPP” (Pembleton 2020). The significance of F_{ST} values were calculated using 10,000 bootstraps to generate confidence intervals and p-values and confidence intervals were set at 99%.

Results:

This study aimed to determine the level of genetic differentiation among 239 channeled whelk samples collected from 10 resource areas from Massachusetts to South Carolina, with fine-scaled sampling in the mid-Atlantic region. A total of 5,328 SNPs were used to estimate genetic diversity and delineate population structure among resource areas along the US Atlantic coast. A preliminary analysis of the data revealed estimates of genetic divergence, F_{ST} , ranging from 0.002 – 0.432, with the largest values (read as most divergent) observed between Buzzard Bay, MA and Charleston, SC and the smallest values (least divergent) observed between Light Tower, VA and Hog Island, VA (Table 2). F_{ST} values were highest (0.106 – 0.432) when comparing Pamlico Sound, NC, Wilmington, NC, and Charleston, SC to all other resource areas, with elevated levels of genetic divergence observed across Cape Hatteras and Cape Fear on the coast of North Carolina, which are known biogeographic barriers (Table 2). All but two pairwise comparisons were significant with p-values at < 0.001 . After a correction for multiple tests comparison, the pairwise F_{ST} values between Hog Island, VA and Chincoteague, VA and between Hog Island, VA and Light Tower, VA were not significant (Table 2).

Plots were created using principal component analyses (PCA) to visualize the genetic differentiation among whelk samples. The axes represent the first two components and the percent variation of the data explained by each component is displayed on the axes, as calculated from the eigenvalues. When comparing all ten resource areas, the first component explains 24.63% of the variation (Figure 6). The southern-most resource areas (NCPS, NCW, SC) were separated from one another and from the remaining resource areas (MA, RI, OC, CT, ES, LT, VB) (Figure 6). This finding, paired with the high F_{ST} values among NCPS, NCW, SC indicates that the southern resource areas are highly differentiated both from each other and from resource areas north of Cape Hatteras, NC (Table 2). The high level of differentiation among sample collections from the southern-most resource areas cause the remaining seven resource areas to be tightly clustered in the PCA. To better visualize the data, the southern-most resource areas were excluded in a second PCA of the 7 resource areas located north of Pamlico, Sound, NC i.e. north of the Cape Hatteras biogeographic barrier (Figure 7). The northern most resource areas (MA and RI) were separated from one another and from the mid-Atlantic resource areas (OC, CT, ES, LT, VB) (Figure 7). F_{ST} values of pairwise comparisons of MA and RI to samples from all other resource areas ranged

from 0.060 – 0.097, which were an order of magnitude higher than F_{ST} values seen among samples in the mid-Atlantic (0.002 – 0.015) (Table 2). The resource area in VB had a wider spread in the PCA than all other resource areas in the mid-Atlantic, indicating a higher level of genetic diversity (Figure 7) and F_{ST} values were elevated for mid-Atlantic pairwise comparisons involving VB (0.009 – 0.015) as compared to other mid-Atlantic comparisons (0.002-0.004) (Table 2).

Conclusions:

Long-term sustainability of the channeled whelk commercial fishery may be threatened due to over harvest and removal of whelk before they are sexually mature. Management is inconsistent, with adjacent states having different harvest regulations or unregulated harvest (e.g. North Carolina). An understanding of the population genetic structure of this species will allow better understanding of the impacts of current fisheries management regulations. New management strategies are needed for more sustainable commercial harvest in the mid-Atlantic region.

As a general rule in the marine environment common F_{ST} values showing population differentiation range from 0.01-0.02 in marine invertebrates (Mladenov et al. 1997). F_{ST} values for this study spanned several orders of magnitude (0.002 – 0.432), representing widely varying levels of divergence between sampling areas. Several patterns appeared when examining the F_{ST} values and PCAs. Within the mid-Atlantic region, F_{ST} values were an order of magnitude higher in comparisons involving VB than comparisons among other mid-Atlantic resource areas, suggesting that the mouth of Chesapeake Bay may be a barrier to dispersal for channeled whelk. In New England, samples from MA and RI samples were significantly different from each other, indicating that they represent separate stocks. Comparison of samples from New England, (MA and RI) to samples from the mid-Atlantic resulted in F_{ST} values that were an order of magnitude higher than F_{ST} values between samples within the mid-Atlantic region, and MA and RI samples clustered separately from mid-Atlantic samples in the PCA. This indicates a lack of connectivity between New England mid-Atlantic resource areas (i.e. separate stocks), although conclusions are limited by the lack of samples between RI and OC. On a wider geographic scale pairwise comparisons of samples from southern resource areas (NCPS, NCW, SC) with samples from areas to the north resulted in F_{ST} values that were two orders of magnitude higher than comparisons within the mid-Atlantic region, with F_{ST} values increasing as biogeographic barriers were crossed, indicating that Cape Hatteras and Cape Fear both represent significant barriers to dispersal of channeled whelk. Samples from the three southern resource areas were also significantly different from each other. These preliminary findings indicate substantial stock structure along the US Atlantic coast, and underscore the need for new management strategies for the channeled whelk fishery.

Based on the results from this study, it is recommended that a channeled whelk working group including representatives of the channeled whelk resource from Massachusetts to north of Cape Hatteras be formed to further assess the state of the channeled whelk resource. It would be beneficial to conduct meetings with scientists, commercial fishers, and management agencies to facilitate conversations about the current regulations and how state-by-state management may or may not align with findings from this study. There is a strong need to reconsider the degree to which whelk are being managed. There are clearly multiple biological stocks isolated within a state, and potentially shared stocks between states. Regional management could be a possible change for this fishery, however more information will be needed before making a decision.

This project was a preliminary look into the population genetic structure of channeled whelk. A more comprehensive look at this resource is needed, and we suggest adding more

samples though out the geographic range of channeled whelk. Additional sampling should include a genetic comparison among offshore and inshore assemblages and should also focus on sampling gaps between New York and New Jersey and the inshore side of North Carolina near Pamlico Sound. The analysis of these additional samples would allow more informed decision making for better management of this resource. Results from this study will be published on the FRG website and will also be incorporated into the thesis of VIMS Master's student Sam Askin, with additional submission to a peer-reviewed publication in a scientific journal. These results will be presented at industry and academic fisheries meetings in addition to being presented to state and regional management agencies in the form of advisory council briefings.

Acknowledgements:

This work was performed through the collaboration between industry, VIMS, and state management agencies. Rick Robins is an industry leader and the PI for the FRG project and served as the primary industry collaborator by assisting with collection of channeled whelk from various whelk harvesters and through providing expertise in the commercial whelk fishery in Virginia. Bob Fisher is a commercial fisheries specialist with the Marine Advisory Program at VIMS. He was responsible for guiding the appropriate sampling of the whelk resource and for ensuring that the information generated as part of this proposal was disseminated to the appropriate management agencies. Jan McDowell, an Associate Research Professor at VIMS, is the major advisor of graduate student Samantha Askin. She was responsible for supervising the genetics component of the project. Samantha Askin, a Master's student at VIMS, was responsible for generating the genetic data and conducting the statistical analysis on channeled whelk collected as part of this proposal. Sampling was achieved through the help of commercial whelk fishers from Virginia and North Carolina as well as from South Carolina Department of Natural Resources and Massachusetts Division of Marine Fisheries. The funding for this project aided in the successful collaboration between various state agencies, industry, and fisheries research scientist to provide a preliminary look into the population genetic structure of the channeled whelk resource.

Tables and Figures:

Table 1. Locations channeled whelk were sampled, the year channeled whelk were sampled at each location, the number of individuals sampled at each location, and the number of samples selected for DArTseq.

Location	Collection Year	# of samples collected	# of samples selected for DArTseq
Buzzard Bay, MA	2019	30	21
Rhode Island	2018	12	12
Ocean City, MD	2018	34	34
Chicotague, VA	2018	25	20
Hog Island, VA	2018	32	14
Light Tower, VA	2018	25	22
Sand Bridge, VA	2018	34	34
Pamlico Sound, NC	2018	30	28
Wilmington, NC	2019	30	30
Charleston, SC	2015-2018	30	24
	TOTAL	282	239

Table 2. Wrights F-statistics (F_{ST}) for all 10 resource areas sampled. F_{ST} values are on the lower diagonal of the table, with non-significant F_{ST} values are denoted in bold. P-values are located on the upper diagonal of the table.

Pairwise F_{ST} Values										
0.100 - 0.400										
0.050 - 0.099										
0.009 - 0.049										
0.002 - 0.0089										
Pairwise F_{ST}	MA	RI	OC	CT	ES	LT	VB	NCPS	NCW	SC
MA	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RI	0.051	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
OC	0.091	0.060	NA	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CT	0.094	0.064	0.004	NA	0.029	<0.001	<0.001	<0.001	<0.001	<0.001
ES	0.097	0.065	0.002	0.002	NA	0.023	<0.001	<0.001	<0.001	<0.001
LT	0.093	0.064	0.004	0.004	0.002	NA	<0.001	<0.001	<0.001	<0.001
VB	0.096	0.070	0.015	0.014	0.013	0.009	NA	<0.001	<0.001	<0.001
NCPS	0.188	0.157	0.0125	0.114	0.108	0.106	0.089	NA	<0.001	<0.001
NCW	0.359	0.328	0.332	0.306	0.296	0.302	0.292	0.173	NA	<0.001
SC	0.432	0.400	0.402	0.377	0.367	0.373	0.360	0.254	0.050	NA

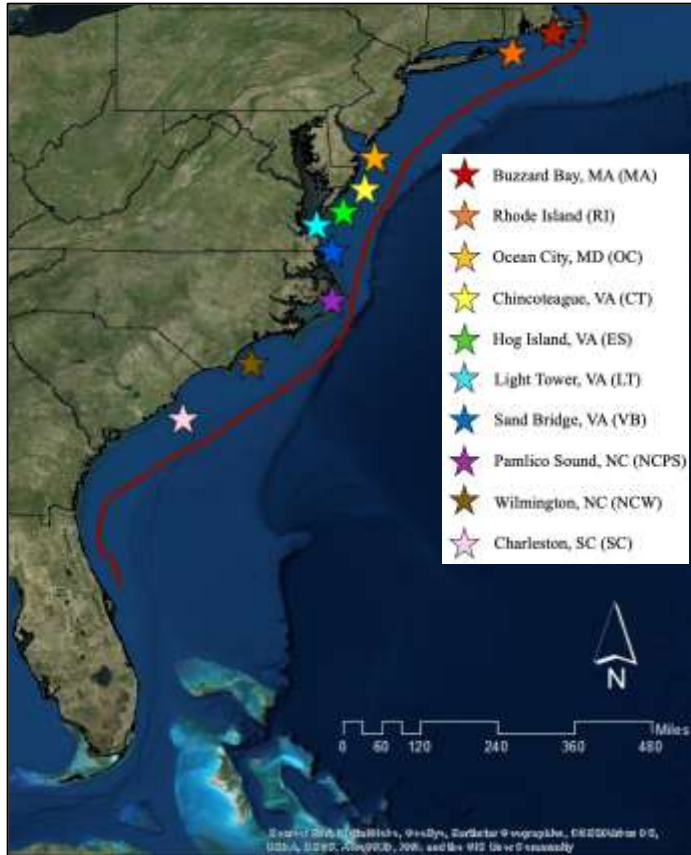


Figure 1. The geographic range of channeled whelk represented by the red line, and the sampling locations and abbreviations used for analyses.

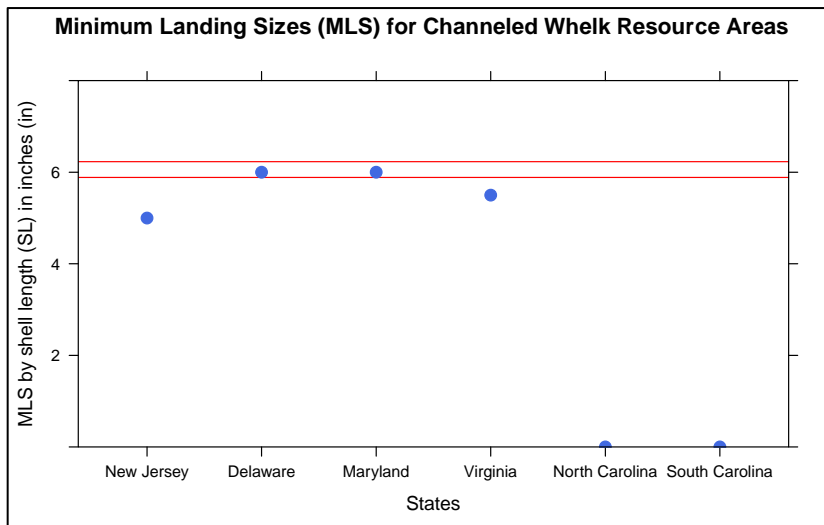


Figure 2. Minimum landing sizes for channeled whelk resource areas, with the range of female channeled whelk maturity denoted by the red lines.



Figure 3. An example of sample collection work station.



Figure 4. Sample collection of channeled whelk foot tissue from individuals collected at a whelk processing plant.



Figure 5. A workflow for the methodology. Images provided by: Ian.umces.edu and Illumina.com.

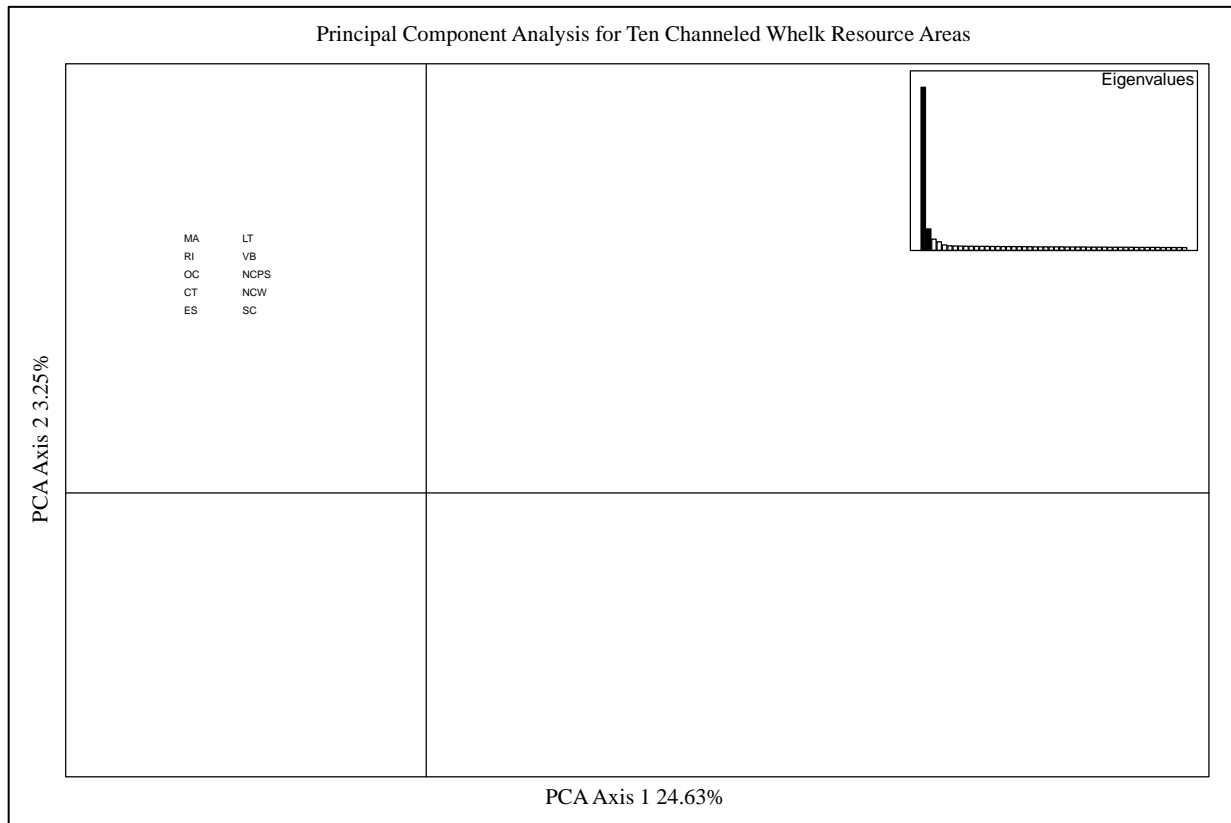


Figure 6. PCA plotting the relative positioning for 239 channeled whelk using the first two principal component scores. The grouping of individuals were created using a genetic distance matrix calculated from 5,328 SNPs, and ellipses surround individuals grouped by resource areas. Resource area abbreviations correspond to those listed in Figure 1. The eigenvalues calculated for this plot are highlighted in the top right corner. Axis 1 and 2 explained 24.63% and 3.35% of the variation in the data respectively.

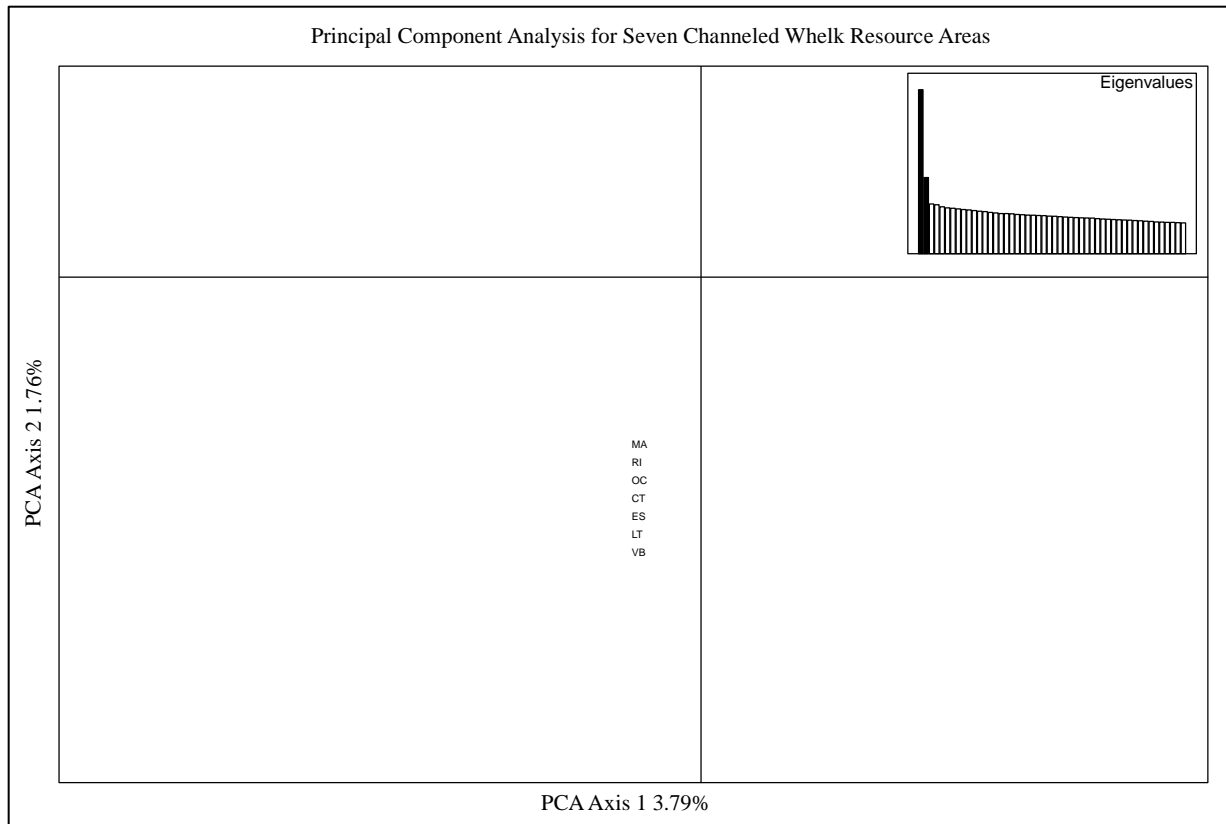


Figure 7. PCA plotting the relative positioning for 239 channeled whelk using the first two principal component scores. The grouping of individuals were created using a genetic distance matrix calculated from 5,328 SNPs, and ellipses surround individuals grouped by resource areas. Resource area abbreviations correspond to those listed in Figure 1. The eigenvalues calculated for this plot are highlighted in the top right corner. Axis 1 and 2 explained 3.79% and 1.76% of the variation in the data respectively.

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