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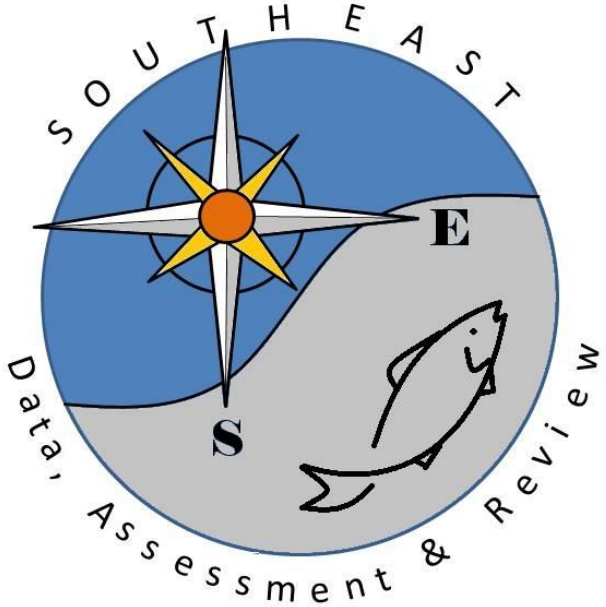

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Use of otolith microchemistry to improve fisheries-independent indices of recruitment for gag (*Mycteroperca microlepis*): linking estuarine nurseries to nearshore reefs in the eastern Gulf of Mexico

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1 INTRODUCTION

1.1 Background

Reef-fish resources along the Gulf Coast of Florida support valuable commercial and recreational fisheries. Recent studies indicate that many exploited reef fishes, including gag (*Mycteroperca microlepis*), are currently experiencing overfishing, due in part to increased fishing effort during recent decades. Gag are especially susceptible to the effects of overfishing due to unique life-history characteristics such as depth and habitat preferences, longevity, slow maturation, and the tendency to form spawning aggregations (Coleman et al., 1996, 1999). Because of these life-history characteristics, traditional management practices such as restrictive size and bag limits have proved to be problematic in managing reef fisheries due in part to the high probability of discard mortality for undersized individuals (Bartholomew and Bohnsack, 2005) as well as the tendency to harvest proportionally more males from the protogynous population. Accordingly, overfishing of exploited reef fishes may be manifested in a variety of population-level responses, including declining abundances, reduced sizes, and skewed sex ratios (Coleman et al., 1996; Ault et al., 2005a, 2005b).

Recent assessments of the status of Gulf of Mexico stocks of gag suggest the fishery, which is concentrated over the West Florida Shelf, is currently overfished (SEDAR, 2006, 2007, 2009; Worm et al., 2009). The ratio of males to females in this monoandric protogynous hermaphrodite has also dramatically declined (Coleman et al., 1996; McGovern et al., 1998), raising additional concerns about sperm-limitation, inbreeding, genetic fitness, and impending collapse of the population (Chapman et al., 1999; Alonzo and Mangel, 2004, 2005; Brooks et al., 2008). The impact of size-selective fishing pressure on a species that is spatially segregated according to size and that spawns in aggregations makes this species especially vulnerable to overfishing (Bannerot et al., 1987; Coleman et al., 1996, 2000; Armsworth, 2001; Alonzo and Mangel, 2004, 2005; Heppell et al., 2006).

Previous work indicates the primary spawning habitat for gag along the West Florida Shelf occurs along high relief hard bottom shelf-edge (70–90 m) habitat from January through April (Coleman et al., 1996; Koenig et al., 2000; Fitzhugh et al., 2005). After hatching, larval gag inhabit the offshore pelagic environment for a period of 29–52 days before settling in nearshore high salinity seagrass habitats (Fitzhugh et al., 2005; Switzer et al., 2012). Recruitment of juvenile gag to nursery habitat is highly variable, but appears to be cyclical with strong year classes evident every two to four years (Fitzhugh et al., 2005; Johnson and Koenig, 2005; Switzer et al., 2012). Switzer et al. (2012) noted disparate trends in juvenile gag abundance in southern estuaries during certain years when compared to more northern estuaries, most notably in 2005 when juvenile gag abundance increased markedly in Charlotte Harbor but not in the other Gulf coast estuaries, and in 2011 when there was an apparent recruitment failure of gag in all estuarine systems except Charlotte Harbor (FWC/FWRI unpublished data).

1.2 Rationale

The assessment and management of commercial and recreational fisheries has historically relied heavily on fisheries-dependent data, although limitations and biases inherent in these data are a source of uncertainty in current stock assessments. Recent reviews of reef

fish stock assessments have highlighted the need for increased fisheries-independent surveys of reef fish resources. When available, fisheries-independent indices of abundance are especially valuable to the assessment of fisheries resources because available data generally: (1) are based on a statistically-valid, stratified-random sampling design; (2) incorporate standardized sampling methodologies; and (3) are collected over long-term periods of time with precisely defined amounts of effort. Fisheries-independent data also have potential utility in forecasting future fisheries production from juvenile recruitment data (Johnson and Koenig, 2005). To that end, several researchers have recently worked towards developing indices of juvenile recruitment for gag in the Gulf of Mexico based upon available long-term data sets (Johnson and Koenig, 2005; Koenig and Coleman, 1998; Casey et al., 2007).

These data are undoubtedly invaluable in the assessment of gag in the Gulf of Mexico; nevertheless, the question of how to most appropriately combine data from different estuarine systems in calculating a Gulf-wide index of recruitment must be resolved before these data can be used to their fullest potential. There is a current need for a new approach that can account for: (1) differences in the relative contribution each estuarine system makes toward replenishing nearshore and offshore gag populations; and (2) processes that contribute to the high degree of interannual variability (Johnson and Koenig, 2005; Casey et al., 2007; Switzer et al., 2012). Ideally, indices of recruitment for juvenile gag would be constructed by weighting data based upon a quantitative measure of the nursery function of each estuarine system as defined by the relative contribution to nearshore populations for each specific year class. This approach requires a careful examination of the connectivity between estuarine and nearshore gag populations, a process that will likely improve the utility of fisheries-independent data in support of the assessment of gag stocks by improving the correlation between indices of juvenile recruitment and subsequent recruitment to the fishery.

1.3 Otolith Microchemistry

The analysis of the chemical composition of fish otoliths is a well established tool used to answer a variety of questions in fisheries biology by: (1) defining the separation of fish stocks; (2) tracking ontogenetic migrations of fishes; and (3) determining the origin of adult populations (see reviews by Campana, 1999; Thresher, 1999; Thorrold and Hare, 2002; Thorrold et al., 2002). Trace elements dissolved in the ambient water mass are incorporated into the otoliths of fishes during growth. The presence and relative proportions of these elements define a distinct, permanent microchemical signature that varies among fishes exposed to different water masses and environmental conditions (Campana and Neilson, 1985; Campana et al., 1995). This has the effect of marking the otoliths with natural tags that provide a record of habitat history. Previous work examining gag nursery habitats along the west coast of Florida has demonstrated that otolith microchemisty is capable of discriminating among different geographic locations (Hanson et al., 2004). Previous work has also identified inter-annual variability in otolith microchemical signatures (Gillanders, 2002; Hanson et al., 2004; Patterson et al., 2004).

1.4 Aims

The purpose of this study was to elucidate habitat connectivity, migration pathways, and recruitment processes for eastern Gulf of Mexico populations of gag with the ultimate goal

of facilitating improvement to the existing methods of stock assessment. This was accomplished by examining the otolith microchemistry of juvenile and subadult gag along the west coast of Florida to: (1) distinguish juveniles inhabiting different estuarine nursery habitats; (2) identify the nearshore nurseries that replenish populations of gag on nearshore reefs; and (3) gauge the relative contribution of different estuaries to subsequent year class strengths of gag. These commercially and recreationally important fish are of major ecological and economic value in Florida and rely heavily on estuarine nursery grounds along Florida's western coastline. The results of this study will facilitate the construction of more realistic models of regional recruitment, thus increasing the predictive capability of these models to ultimately guide management decisions. This work should facilitate prediction of annual year-class strength based on fishery independent monitoring of juveniles inhabiting estuarine nursery habitats. Given the highly-variable nature of recruitment of juvenile gag along the Gulf coast of Florida (Johnson and Koenig, 2005; Casey et al., 2007; Switzer et al., 2012), it is essential to develop better linkages between juvenile abundance within presumed estuarine nurseries and the abundance of subadult and adult gag in nearshore waters so that managers can more effectively predict the influence of exceptionally-strong year classes on future fisheries productivity.

2 METHODS

2.1 Specimen Collection

FWRI maintains a multispecies, multiple gear, habitat-based monitoring effort which encompass most of the west Florida shelf from Pensacola south to Fort Myers, covering habitats ranging from shallow estuarine systems to neritic waters 110 m deep. Juveniles inhabiting polyhaline seagrass beds (Koenig and Coleman, 1998; Casey et al., 2007) within six Gulf coast estuaries (Figure 1) were collected as part of FWRI's fishery-independent monitoring (FIM) surveys of estuarine-dependent reef fishes. Two gear types were employed by these surveys: (1) a 183-m haul seine (38-mm stretch mesh) targeting offshore shoals (defined as an area >100 m from the nearest shoreline with $\geq$50% seagrass coverage, and with depths ranging from 0.5–1.0 m at the wing to 1.5–2.5 m at the bag); and (2) a 6.1-m otter trawl (38-mm stretch mesh with a 3.2-mm liner) targeting areas with depths ranging from 1.0–7.6 m and $\geq$50% seagrass coverage. Polyhaline seagrass surveys were conducted monthly from May–November, corresponding to the timing of peak abundance of juvenile reef fishes within estuarine waters. Monthly sampling within each estuary occurred at a series of sites that were randomly selected from a universe of all potential locations meeting the above criteria, and were allocated among pre-defined spatial strata to assure broad geographic coverage of sampling effort within each system.

Subadults inhabiting low-relief, soft-bottom habitats in nearshore waters were collected as part of FWRI's recently expanded Southeast Monitoring and Assessment Program (SEAMAP) groundfish surveys. This effort deployed standard 12.8-m SEAMAP shrimp trawls of 30 min duration within three aggregated shrimp fishery statistical reporting zones (Florida Panhandle, Big Bend, Mid-Peninsular Florida). A depth-stratified random sampling design was employed and waters 10–110 m deep were sampled with two replicates (1 day, 1 night) taken within each stratum. Subadults occupying high-relief and/or hard-bottom habitats were taken by a recently initiated FWRI effort to sample managed

reef fishes within the Mid-Peninsular Florida reporting zone. This work used baited chevron traps (1.76m x 1.52m x 0.61m; 28cm throat diameter; 3.81cm vinyl-clad mesh) soaked $\geq$90 min in a depth-stratified random sampling design targeting waters 10–110 m in depth. Though restricted to summer months, this effort sampled $\sim$ 160 sites annually, each with 3–4 replicates. These collections were augmented by FWRI with the addition of new fishery-independent reef fish surveys of aggregated reporting zones, which began in 2010. These surveys employed a spatially stratified sampling design using commercial electric and bandit reels with $\sim$ 30 min of vertical line effort. Each vertical fishing rig was equipped with a 100–200 lb test mainline connected to a two-hook terminal rig (lower hook: 11/0 circle hook; upper hook: 8/0 circle hook) constructed using 80 lb test leader material, with $\leq$4 fished simultaneously.

2.2 Trace Elemental Assays

2.2.1 Sample Preparation

Whole fish collected during FIM surveys were kept on ice for transport and frozen until the otoliths were removed by dissection, usually within 30 days. The right sagittal otolith was arbitrarily selected for analysis, when both were available and unbroken, since differences in chemical composition within individual fish (left vs. right otoliths) was not a concern (Campana et al., 2000). All work involving cleaning and drying of otoliths and laboratory equipment was performed under class-100 clean room conditions within a laminar flood clean hood using trace-metal grade reagents and Milli-Q filtered (18 M$\Omega \cdot$ cm) water. Only non-metallic, HNO_3 acid-washed instruments and glass slides were used for sample preparation. Any remaining extraneous tissue was manually removed before otoliths were triple rinsed with Milli-Q water, soaked in 36% ultrapure H_2O_2 for 3 min to remove any adhering particles, and triple rinsed again before being dried for 24 h, and stored in acid-washed polyethylene embedding capsules. Otoliths were subsequently mounted on petrographic glass slides using Crystalbond™ 509 and cut into 1 mm wide transverse sections using a low speed diamond wafering saw (Buehler Isomet®) equipped with dual blades with Milli-Q water used as the lubricant. Thin sections were then hand polished using a sequential series of lapping paper (220, 800, and 3μm grit) until the core and growth rings of each otolith were clearly visible. After polishing, up to 25 otolith thin sections were mounted on a single acid-washed petrographic slide, sonicated in Milli-Q water for 5 min to remove surface contaminants, then triple rinsed and dried for 24 h.

2.2.2 Instrumentation & Methodology

A Photon-Machines' Analyte 193 nm excimer UV laser ablation system (LA) was used to sample the outermost growth bands along the dorsal and ventral edges of saggital otolith thin sections from age-0 juvenile stage fishes. Subadults were sampled in a similar manner, but ablations were targeted inside the first annular growth ring corresponding to the "juvenile core". A sequence of replicate (n=3) 83 μm diameter laser ablation circular spot scans of 60 s duration were made using Photon-Machines' Chromium software to control targeted sampling. Live monitoring by video light microscopy combined with computer-controlled stage travel in the LA allowed precise targeting of spot scans and confirmation that ablation samples were located appropriately. Sample material vaporized by the ablation process was carried by a mixture of He transport and Ar makeup gas to the plasma

torch of an Agilent Technologies 7500CX quadrupole inductively coupled plasma–mass spectrometer (ICP-MS). Data acquisition performed by this instrument employed Agilent Technologies' ChemStation software operating in time-resolved analysis mode. Background data (i.e., gas blanks) were collected for 90 s before each spot scan was initiated. NIST-612 silicate glasses (Pearce et al., 1997) were used as external calibration reference material and ablated with n=2 replicates in brackets before and after $\sim$ every fifth otolith thin section was sampled. Matrix-matched MACS-3 microanalytical carbonate standard material (Koenig and Wilson, 2007) was ablated in brackets before and after each slide containing multiple thin sections was assayed to estimate experiment-wide levels of precision as percent relative standard deviations (%RSD). Just prior to data collection, the instrumentation was tuned while ablating NIST-612 reference material in order to: (1) maximize analytical sensitivity for increased precision and lower limits of detection; (2) minimize interferences due to formation of oxides and doubly-charged ions in the plasma torch; and (3) minimize mass-specific fractionation of analytes. A summary of instrument operating conditions used during data collection is provided in Table 1. All laboratory facilities and instrumentation were located on the campus of the College of Marine Science, University of South Florida in St. Petersburg, FL, USA.

2.2.3 Data Collection & Processing

Data for 26 target masses were acquired in ChemStation and exported as standardized count rates (ions$\cdot$s^{-1}) to an ASCII data file for: Li^{7}, Na^{23}, Mg^{24}, P^{31}, Ca^{43}, Sc^{45}, V^{51}, Cr^{53}, Mn^{55}, Fe^{57}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{64}, Cu^{65}, Ge^{72}, Rb^{85}, Sr^{88}, Y^{89}, Cd^{114}, Sn^{118}, Ba^{137}, Au^{197}, Pb^{208}, Th^{232}, and U^{238}. These analytes were screened for their ability to allow discrimination among gag estuarine nursery habitats as they: (1) are reported to occur above detection limits in the otoliths of marine fishes; (2) are not physiologically regulated, so their presence and abundances are indicative of the environmental conditions to which the fish were exposed; and (3) minimize potential interferences due to isobaric spectral overlap, sample matrix effects, and the presence of molecular ions. Ca^{43} was used as the internal standard in subsequent data reduction to account for mass bias and instrument drift since, as an indigenous component of otoliths, its concentration in the aragonitic carbonate matrix can be determined stoichiometrically (i.e., 40%).

The time series of raw count rate data were processed offline using purpose-built functions implemented in the Fathom Toolbox for MATLAB (FTM) (Jones, 2012; 2013). ASCII data in Perkin-Elmer Elan "XL" format were imported into the MATLAB workspace where the transient signal data were visualized using a custom graphical user interface. The quality of the signal representing each spot scan was visually assessed and the data were parsed into separate signal and background components, with portions of the signal displaying peaks likely associated with surface contaminants excluded from further processing. Grubb's test for outliers (with $\alpha = 0.05$) was used to identify mass-specific spikes in the transient signal of each spot scan, which were replaced with mean values when present. Raw ion counts-per-second (cps) data for each otolith spot scan were converted to mean analyte concentration (ppm) using algorithms implemented in FTM that precisely follow established methods of geochemical data reduction (i.e., Longerich et al., 1996; 1997; Halter et al., 2002; Henrich et al., 2003; Jackson, 2008; Jones, 2012, 2013). Corrections for mass-specific drift in the sensitivity of the ICP-MS were applied using: (1) linear interpolation when regressions of acquisition time vs. the yield (i.e., cps$\cdot$ppm^{-1}) from spot scans of the NIST-612 reference material resulted in coefficient of determination (R^2)

values ≥ 0.55; and (2) nearest neighbor interpolation when otherwise. Limits of detection (LOD) were taken as $3 \cdot$SD(background levels) and analyte concentrations $<$ LOD were set as ppm $= 0$. Outliers among replicate spot scans of the same otolith were identified using a multivariate measure of outlyingness (Breiman and Cutler, 2003) based on analyte concentrations (ppm). Replicates with outlyingness values > 10 were excluded before reducing the data to mean ppm and LOD values for each otolith thin section for subsequent statistical analysis.

2.3 Multivariate Analysis of Otolith Microchemistry

2.3.1 Statistical Analysis & Hypothesis Testing

The microchemistry data obtained from assaying age-0 juveniles from the 2009 year class were examined to detect the presence of significant spatial variation in mean otolith elemental concentrations among six estuarine nursery habitats. The null hypothesis that no difference in otolith microchemistry existed among estuaries was evaluated using NP-MANOVA (Anderson, 2001). This is a non-parametric (i.e., permutation-based) variant of MANOVA appropriate for analyzing otolith microchemistry data which, even after transformation (e.g., Lara et al., 2008; Mercier et al., 2011) often fail to meet the underlying assumptions of any one distributional model. A Euclidean distance matrix, based on mean otolith concentrations of 25 elements, served as multiple, quantitative explanatory variables in a one-way MANOVA, with estuary serving as a categorical response variable.

2.3.2 Classification by Random Forest

A random forest (RF) is a derivative of the family of classification and regression tree (CART) methods used to model and predict the relationships between a set of multiple predictor variables and a single response variable (Breiman, 2001a). It provides an algorithmic approach employing recursion, resampling, and randomization to discover and model these relationships (Breiman, 2001b), eliminating the need to make *a priori* assumptions regarding the functional form of the predictor–response relationship. This approach offers substantial improvements over traditional data modeling in terms of accuracy, speed, and interpretation (De'ath and Fabricius, 2000; Breiman, 2001b; Elith et al., 2006; Prasad et al., 2006; Peters et al., 2007; Oppel et al., 2009; Mercier et al., 2011) while providing the ability to model complex, non-linear relationships involving interactions and noisy data (Moore et al., 1991).

For classification, the RF algorithm requires a categorical response variable specifying group membership of the observations in a corresponding set of quantitative predictor variables. An ensemble of binary decision trees making up a forest are grown, each starting from a root node containing a different bootstrap training sample derived from the full set of observations. Data in the root node are recursively bifurcated into progressively smaller, more homogeneous subsets that form the nodes (branches) of the tree. At each node, a random subset of predictor variables is searched and the one that minimizes the sum-of-squared errors among the remaining observations, in terms of the categorical response variable, is used to split the data. Trees are grown to their full extent until terminal nodes contain observations that can no longer be split into more homogeneous subunits. Since pruning (node removal) is not applied to tress built by RF, the highly subjective cross-validation procedure required by conventional CART is not required

(Hastie et al., 2009). Once a tree is fully grown, fitted values of the categorical variable returned by each of its terminal nodes are aggregated across the entire ensemble of trees using a simple majority rules voting scheme, producing the final predicted responses of the forest.

As each tree is built, $\sim$ one-third of the total observations are excluded from the bootstrapped data comprising its root node (i.e., the out-of-bag observations). Since these are not used to grow the current tree, they serve as a natural test set for internal cross-validation and calculation of the out-of-bag error rates. This provides an unbiased estimate of the generalization error of the RF model, which eliminates the need to partition the data into separate training and test sets (Breiman, 2001a; Peters et al., 2007). Since RFs are not susceptible to over-fitting (Breiman, 2001a; Perdiguero-Alonso et al., 2008), hundreds (or thousands) of trees are typically built when constructing an ensemble to minimize and stabilize the model's generalization error. Prediction accuracy is maximized in RF by creating a diverse ensemble of minimally correlated trees built from bootstrapped resampling of observations that are split by randomized subsets of predictors (Breiman, 2001a; Berk, 2006).

The RF method is user-friendly as it is easy to parameterize and implement and the output is readily interpretable. Two basic user-supplied parameters are required to construct a RF: (1) $\mathrm{N_{tree}}$, the number of trees to grow; and (2) $\mathrm{M_{try,}}$ the number of predictors to search at each split (node). However, robust solutions can be obtained using the default values of $N_{\mathrm{tree}} = 1000$ and $M_{\mathrm{try}} =$ square root of the number of predictors (Breiman, 2001a; Breiman and Cutler, 2003; Cutler et al., 2007). A relative measure of proximity (similarity) among all pairs of observations is internally calculated during the RF fitting process as the proportion of times each pair shared a terminal node. These are returned as a square, symmetric similarity matrix that, when converted to dissimilarities, provide Euclidean distances in high dimensional space. These can be used to visualize the fitted values with traditional multivariate ordination methods, such as principal coordinates analysis (PCoA).

All data manipulations and statistical analyses were performed in this study using the Fathom Toolbox for MATLAB (Jones, 2013).

3 RESULTS & DISCUSSION

3.1 Otolith Microchemical Nursery Signatures

Multivariate analysis of mean otolith elemental concentration for age-0 juvenile gag from the 2009 year class revealed the existence of highly significant spatial variability among estuarine nursery habitats located along the western coast of Florida (NP-MANOVA: $F = 5.6$, $df = 5$, $n = 100$ fish, $p < 0.001$). *Post-hoc* multiple pair-wise tests indicated significant differences existed among most pairs of estuaries ($p \leq 0.05$), with the exception of: (1) the Big Bend Region, which only differed from Charlotte Harbor; and (2) St. Joseph Sound, which was not significantly different from Tampa Bay (Table 2). Since significant differences were present in the juvenile data, a random forest (RF) was constructed using the otolith concentration data to create a nonlinear, tree-based classifier and visualize the differences in nursery signatures among estuaries. Figure 2 depicts the degree of separation of estuaries based on differences of 25 elements present in the otoliths of juveniles,

while Figure 3 illustrates the directional gradients of the 8 most important elements contributing to spatial variation. These figures indicate that otolith microchemical nursery signatures along Florida's western coast are a complex composite of trace elements from both naturally occurring (e.g., Na, Sr, and Y) and anthropogenically derived (e.g., Fe, Co, and Ni) sources. Relatively high levels of Sr and Y, both representative of the level of fresh water input, terrestrial runoff, and terrigenous sedimentation estuarine waters were exposed to, distinguished fish from Charlotte Harbor. Increased concentrations of elements associated with pollutants (i.e., Fe, Co, and Ni) characterized Tampa Bay while decreased amounts discriminated St. Joseph Sound.

The RF's internal cross-validation procedure indicated the overall reclassification-to-estuary success rate was 80%, with estuary-specific classification rates ranging from 0–97% (Table 3). The bulk of mis-classifications resulted from confusions of fish from the Big Bend Region with those from St. Joseph Sound and of St. Andrews Bay samples with those from Apalachicola Bay. In both cases, mis-classifications occurred between estuaries located adjacent to each other rather than among more extreme locations within the sampling domain (Figure 1). The proportional chance criterion (Morrison, 1969; McGarigal et al., 2000; White and Ruttenberg, 2007) was used to evaluate the overall reclassification-to-estuary success rate of the RF classifier by comparing its performance to that expected by chance. Each of the $n = 100$ juveniles were classified using a null model based on random assignment, while maintaining the original within-estuary sample sizes. The permuted distribution of success rates generated from $i = 1000$ sets of random allocations by the null model (not shown) indicated the overall reclassification-to-estuary success rate observed by the random forest (RF) model (i.e., 80%) was significantly better than the 21% success rate expected by chance ($p < 0.001$).

3.2 Mixture Proportions of Offshore Subadults

Subadult stages of gag obtained during offshore collection surveys were aged according to annular growth rings observed in the transverse thin sections of their saggital otoliths. Fish determined to be ages 1, 2, and 3 from collections conducted during 2010, 2011, and 2012, respectively, were assigned to the 2009 year class. The otolith elemental concentration data collected from LA-ICP-MS assays of the juvenile cores of these specimens represent the estuarine nursery signatures established prior to the onset of their ontogenetic migrations to offshore waters. The random forest (RF) classifier previously constructed using data from age-0/2009 juveniles was applied to the otolith elemental concentration data of 2009 year class subadults of unknown origin to determine the putative estuarine nursery habitats previously exploited by these fishes. The normalized votes generated during the fitting process from $n = 1000$ trees comprising the RF were used in a maximum likelihood estimation (MLE) based approach (Millar, 1987; Millar, 1990; White and Ruttenberg, 2007) to provide marginal posterior probabilities that subadults inhabiting offshore waters originated from each of the six estuaries.

A summary of the posterior probabilities estimated for $n = 36$ subadult gag (ages 1–3) from the 2009 year class are presented in Figure 4, which indicates populations in Charlotte Harbor and St. Joseph Sound contributed the most to the year class strength of these fishes. Mean posterior probabilities were used to estimate the mixing proportions (Θ) of the offshore population; i.e., the proportion of subadults (mixture group) that previously resided in each estuarine nursery habitat (baseline groups). These results, present-

ed in Table 5, represent a relative measure of the contribution each estuary made towards replenishment of the subadult population and provides a weighting factor that can be applied to annual abundance and recruitment indices used in the stock assessment process.

Since the MLE-based approach incorporates the uncertainty of the classifier used to estimate the mixture proportions (Θ), the normalized votes obtained during classification of the subadult otolith microchemistry data were subjected to principal coordinate analysis (PCoA) to visualize the relative affinities individuals displayed for each of the six estuaries (Figure 5 and Figure 6). The resulting ordination diagram depicts two distinct classes of fishes with affinities for: (1) Charlotte Harbor, Tampa Bay, and Apalachicola; and (2) St. Joseph Sound, St. Andrew's Bay, and the Big Bend Region (see Figure 1). This dichotomy may represent a large-scale latitudinal gradient underlying the environmental conditions responsible for the differences observed in the age-0 juvenile data, with fishes from Tampa Bay and Charlotte Harbor representing a southern region and samples from St. Joseph Sound and northward comprising a northern region. Alternatively, samples displaying Charlotte Harbor/Tampa Bay/Apalachicola type nursery signatures may originate from estuaries characterized as having more enclosed, protected bays with more anthropogenic influences and higher levels of tannins or turbidity. In contrast, otoliths exhibiting signatures indicative of the St. Andrew's Bay/Big Bend Region/St. Andrews group may have migrated from nurseries within more open coastlines and under greater influence from oceanic waters.

Table 1. Summary of typical instrument operating conditions used when conducting LA-ICP-MS assays of gag otolith thin sections; some parameters concerning the plasma and ion lenses were adjusted as part of the normal daily tuning regime.

	Agilent 7500CX Quadrupole:	
Plasma	RF Power	1200 W
	RF Matching	1.8 V
	Sample Depth	6 mm
	Torch Horizontal	0.4 mm
	Torch Vertical	-0.3 mm
Ion Lenses	Extraction 1	1.2 V
	Extraction 2	-125 V
	Omega Bias	-18 V
	Omega Lens	0.6 V
	Cell Entrance	-30 V
	Quadrupole Focus	3 V
	Cell Exit	-30 V
Octopole	RF	170 V
	Bias	-6 V
Quadrupole	Axis Gain	0.9
	Axis Offset	0
Detector	Analog HV	1
	Pulse HV	1
Data Acquisition	Detector	Analog/Digital
	Mode	Time-resolved
	Samples per Peak	1
	Dwell Time	10 ms
	PhotonMachines Analyte.193:	
Laser Ablation	Type	Excimer UV 193 nm
	Energy Setpoint	7.0 mJ
	Power Attenuation	86%
	Pulse Frequency	5 Hz
	Spot Size	83 um
	Travel Speed	10 um/s
	Sampling Time	60 s
	Washout Time	90 s
	He Carrier Gas	0.6 lpm
	Ar Plasma Gas	0.8 lpm

Table 2. Summary of *post-hoc* multiple pair-wise tests used to examine differences in mean otolith elemental concentrations among age-0/2009 juvenile gag collected from pairs of estuaries along the western coast of Florida; t = t-statistic, p = randomized probability using $i = 1000$ permutation iterations; * = indicate p-values significant at the $\alpha = 0.05$ level. Abbreviations of sites are as follows: SAM = St. Andrews Bay, APM = Apalachicola Bay, BBM = Big Bend Region, HIM = St. Joseph Sound, TBM = Tampa Bay, and CHM = Charlotte Harbor); locations of sites are depicted in Figure 1.

pair-wise comparison	t	p
SAM vs. APM	2.43	0.0138*
SAM vs. BBM	1.57	0.0990
SAM vs. HIM	2.36	0.0006*
SAM vs. TBM	2.27	0.0068*
SAM vs. CHM	2.19	0.0130*
APM vs. BBM	1.21	0.2544
APM vs. HIM	2.12	0.0016*
APM vs. TBM	1.83	0.0402*
APM vs. CHM	4.30	0.0002*
BBM vs. HIM	0.44	0.9246
BBM vs. TBM	0.94	0.4096
BBM vs. CHM	2.32	0.0132*
HIM vs. TBM	1.40	0.0976
HIM vs. CHM	3.11	0.0004*
TBM vs. CHM	3.24	0.0006*

Table 3. Confusion matrix displaying the results of the cross-validation procedure used to assess reclassification-to-estuary success rates. The classifier was based on a random forest (RF) derived from mean otolith elemental concentrations from age-0/2009 juvenile gag. Results are expressed as percentages; rows sum to 100% for each estuary; correct classifications of "unknowns" are shown along the diagonal, with an overall success rate of 80%. Abbreviations of sites are defined in Table 2, with locations depicted in Figure 1.

	SAM	APM	BBM	HIM	TBM	CHM
SAM	33.3	44.4	-	11.1	-	11.1
APM	-	80.0	-	-	13.3	6.7
BBM	-	-	-	71.4	14.3	14.3
HIM	-	-	6.3	81.3	6.3	6.3
TBM	-	5.3	-	-	94.7	-
CHM	-	-	-	-	2.9	97.1

Table 4. Reclassification-to-estuary success rates calculated for six age-0/2009 juvenile gag collection sites using the proportional chance criterion. Location of sites are depicted in Figure 1.

Estuary	Correct Classification (%)
St. Andrews Bay	0.81
Apalachicola Bay	2.25
Big Bend Region	0.49
St. Joseph Sound	2.56
Tampa Bay	3.61
Charlotte Harbor	11.56
Total Correct	21.28

Table 5. Estimated mixing proportions (Θ) of the subadult population of offshore gag derived from the normalized votes generated by a random forest (RF) applied to otolith elemental concentration data from the juvenile core of age 1–3 fishes from the 2009 year class. Location of sites are depicted in Figure 1.

Estuary	Mixture Proportion
St. Andrews Bay	0.1924
Apalachicola Bay	0.0942
Big Bend Region	0.1390
St. Joseph Sound	0.2393
Tampa Bay	0.0662
Charlotte Harbor	0.2689

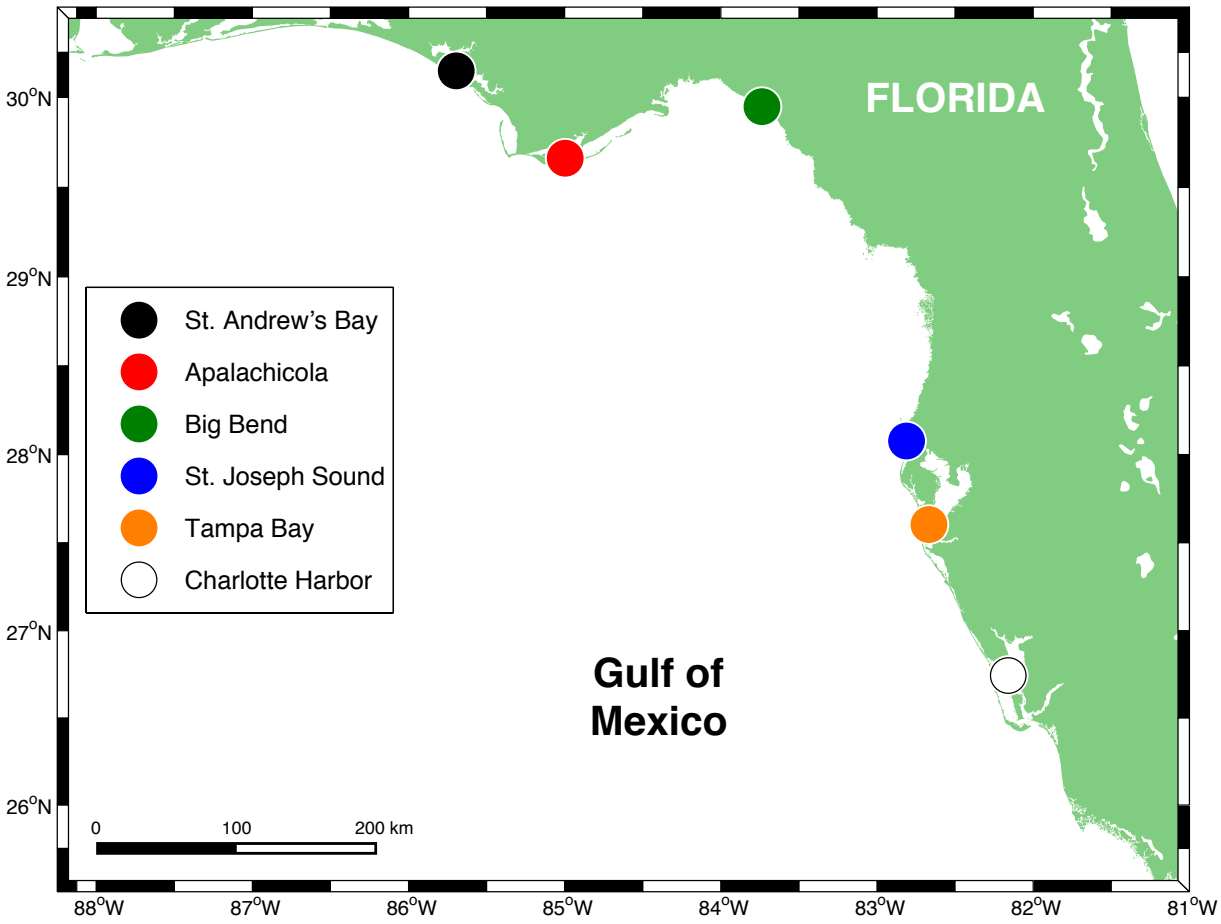

Figure 1. Map of the study area depicting the locations of age-0/2009 juvenile gag collection sites. Fish were taken by FWRI fishery-independent monitoring (FIM) surveys within six estuaries along the west coast of Florida as indicated.

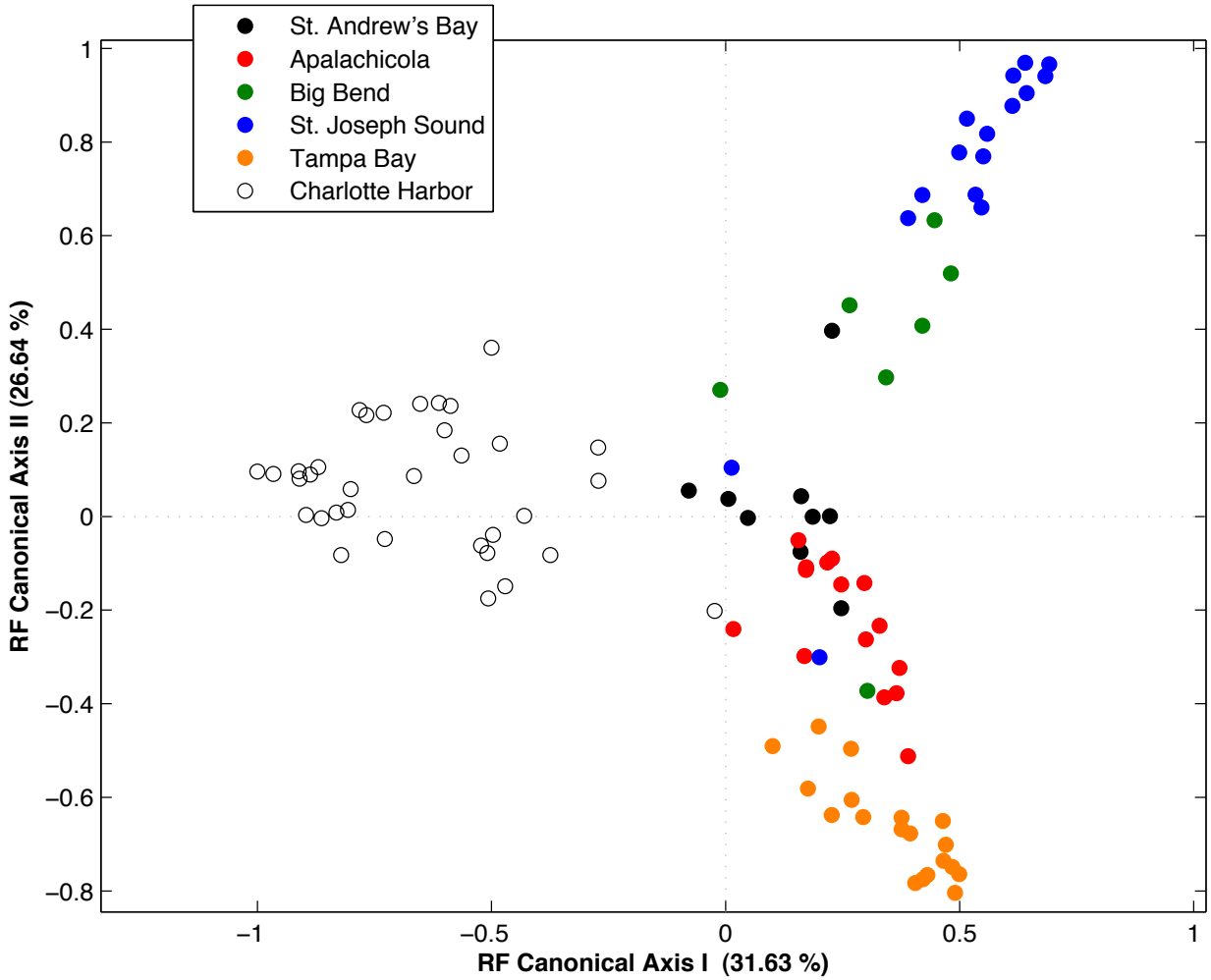

Figure 2. Multivariate visualization of the spatial variation in otolith elemental nursery signatures of age-0/2009 juvenile gag from six estuaries located along the western coast of Florida. Each symbol represents an individual otolith ($n = 100$) with differences in multivariate space proportional to differences in elemental concentration. The ordination diagram was constructed from a principal coordinate analysis (PCoA) of the proximities obtained from a random forest (RF) classifier. A plot of the corresponding correlation vectors is provided in Figure 3 and the locations of estuaries are depicted in Figure 1.

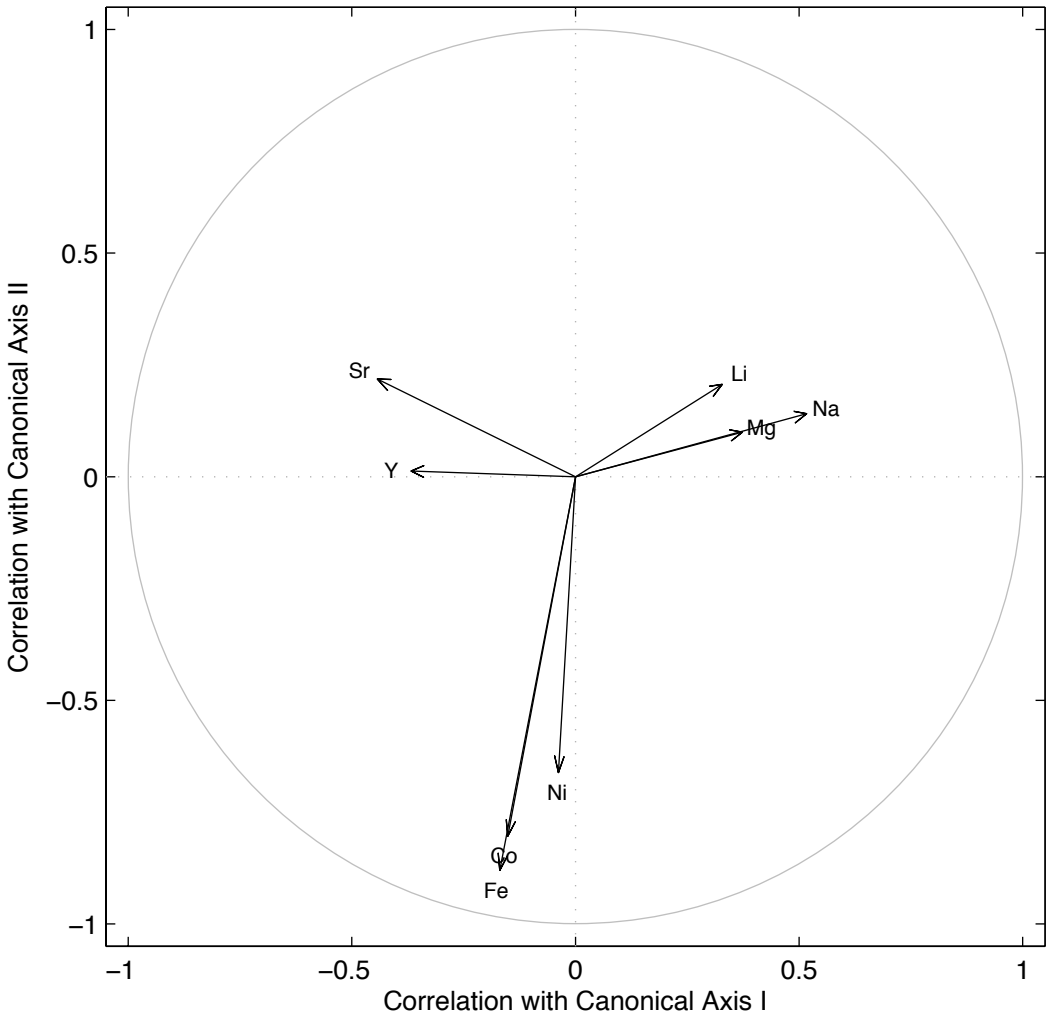

Figure 3. Correlation vectors for the 8 elements found to be most influential in separating estuaries based on otolith nursery signatures. Vectors represent correlations of otolith elemental concentration with the sample scores along the first two canonical axes depicted in Figure 2. The magnitude (length) of each element's vector is proportional to its discrimination power, while the heading of each vector indicates the direction of the underlying gradient in concentration.

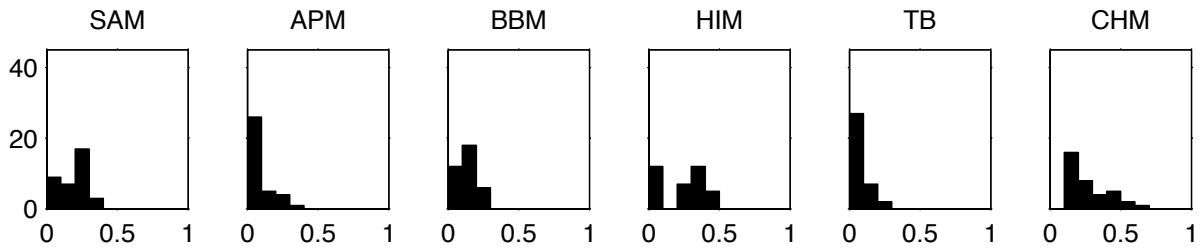

Figure 4. Histograms depicting the marginal posterior probabilities that each of $n = 36$ subadult gag previously exploited six putative estuarine nursery habitats during its juvenile stage. Posterior probabilities were derived from the normalized votes generated by a random forest (RF) classifier comprised of $n = 1000$ trees constructed from the otolith microchemistry of $n = 100$ age-0 juveniles from the 2009 year class. Abbreviations of sites are defined in Table 2, with locations depicted in Figure 1.

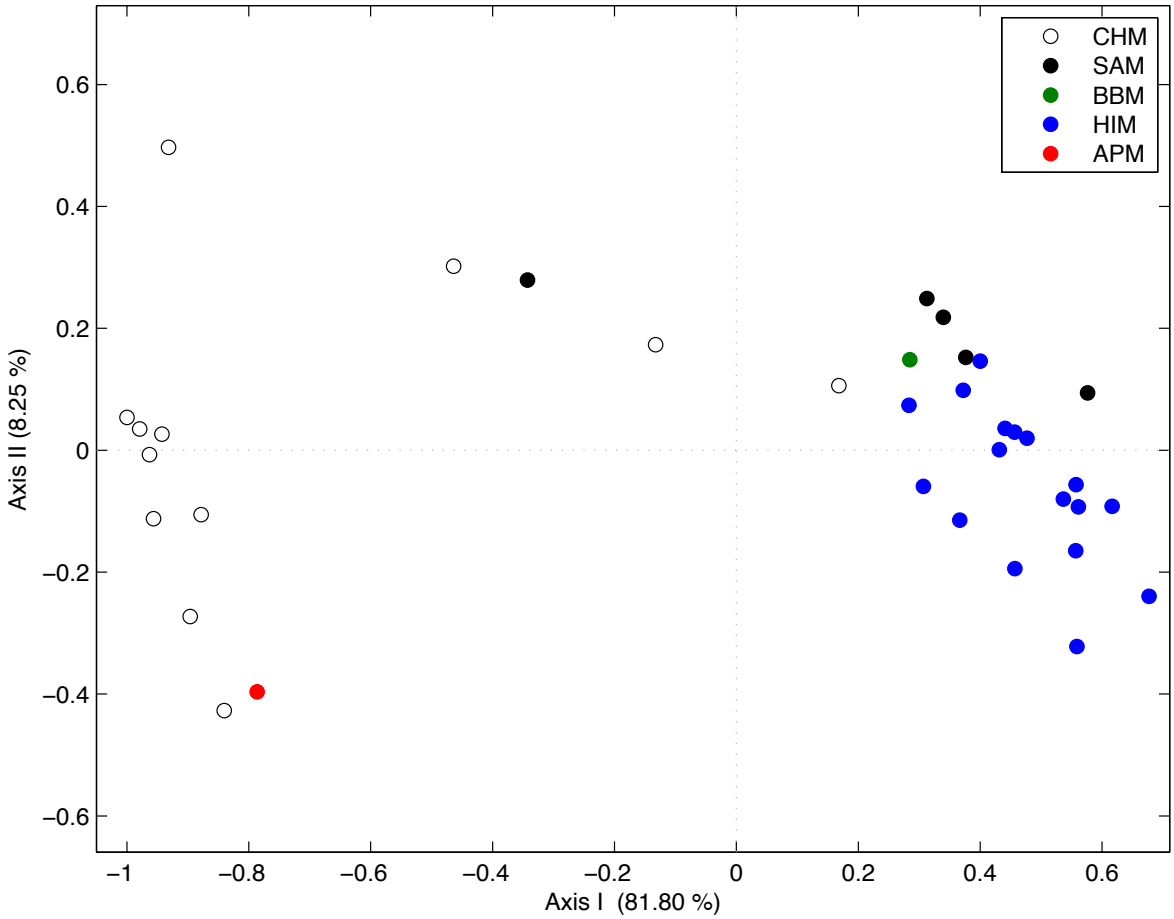

Figure 5. Principal coordinate analysis (PCoA) of the marginal posterior probabilities obtained during estimation of mixing proportions (Θ) of subadult gag. Each symbol represents an otolith ($n = 36$) and indicates the putative nursery habitat of each fish, with differences in multivariate space proportional to the relative affinity individuals displayed for each of six estuaries. A plot of the corresponding correlation vectors is provided in Figure 6. Abbreviations of sites are defined in Table 2, with locations depicted in Figure 1.

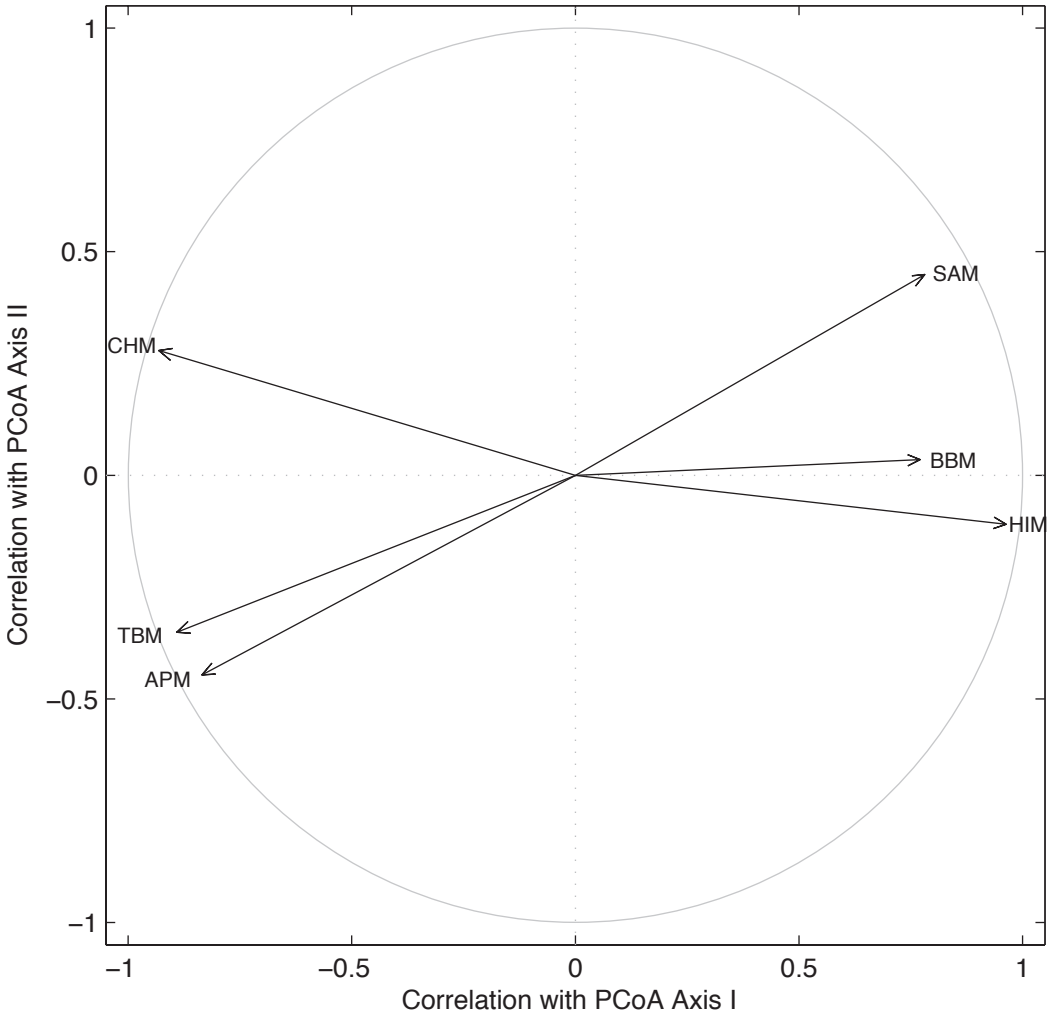

Figure 6. Plot of the correlations of the sample scores along the first two PCoA axes depicted in Figure 5 with the posterior probabilities associated with each of six estuarine nursery habitats. Abbreviations of sites are defined in Table 2, with locations depicted in Figure 1.

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