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Genetics and juvenile abundance dynamics show congruent patterns of population structure for depleted river herring populations in the upper Chesapeake Bay

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<'A'>Abstract

River herring (Alewife and Blueback Herring) populations have declined dramatically along the US Atlantic coast. Conservation efforts are currently inhibited by an incomplete understanding of stock structure for the upper Chesapeake Bay which once supported some of the largest spawning runs across the species' ranges. We collected genetic samples from 512 adult river herring from five rivers and used microsatellites to explore genetic differentiation and population structure. Juvenile abundance indices were also evaluated for spatiotemporal patterns using time series analyses. Statistically significant allelic heterogeneity was observed among most collections, and we identified genetically distinguishable groups for each species. Regression analysis indicated stable or declining juvenile abundance and empirical orthogonal function analysis supported groupings of tributaries based on temporal patterns in abundance. Results suggest a divide between Eastern Shore and Western Shore tributaries with the Susquehanna River and Head of the Bay showing similarities to both groups and possible temporal shifts in genetic structure due to straying. The Patuxent River likely represents a third genetic group for Blueback Herring. Cumulatively, our results suggest at least two genetically distinguishable groups of spawning populations for Alewife and at least three for Blueback Herring that should be considered separately for conservation and management.

<'A'>Introduction

River herring, collectively Alewife (*Alosa pseudoharengus)* and Blueback Herring (*A. aestivalis),* are anadromous fish that spawn in freshwater streams along the Atlantic coast of North America. They are an important source of nutrients and forage in freshwater and estuarine ecosystems (MacAvoy et al. 2000, Yako et al. 2000, Hall et al. 2012, McDermott et al. 2015, Twining et al. 2016), and once supported extensive commercial and subsistence fisheries. River herring populations have declined by an order of magnitude since the mid-20th century due to a series of anthropogenic factors, including overfishing, reduced access to spawning habitat via dam construction and inadequate fish passage, pollution and climate change. Management and conservation efforts have been hampered by a lack of information on stock structure (ASMFC 2012), which can be complicated by hybridization (Hasselman et al. 2014, McBride et al. 2014) and genetic homogenization due to stocking (McBride et al. 2015). A recent evaluation of stock structure among US populations revealed three regional genetic stocks of Alewife and four regional genetic stocks of Blueback Herring, with Chesapeake Bay river herring included in the mid-Atlantic stock of each species (Palkovacs et al. 2014). This information has provided new opportunities for conservation and management, including evaluation of the genetic assignment of bycatch in commercial ocean fisheries to specific genetic stocks (Hasselman et al. 2016). However, Palkovacs et al. (2014) did not have samples from several important spawning streams of the upper Chesapeake Bay, including the Potomac and Susquehanna rivers that historically supported the largest recorded commercial harvests in the region (US Bureau of Fisheries 1916). Prior genetic and stable isotope data suggest some level of population structuring among Chesapeake Bay tributaries (Palkovacs et al. 2014, Turner et al. 2015). As river herring

conservation efforts have expanded throughout the Chesapeake Bay watershed, determining the scale of population structure has become a priority.

The potential for stock structure among Chesapeake Bay tributaries has important implications for the design of conservation, management and monitoring strategies. Fishery independent monitoring of Chesapeake Bay spawning streams has been limited primarily to juvenile abundance indices (ASMFC 2012), but there is increased interest in developing comprehensive monitoring of adult spawning populations (Ogburn et al. 2016). However, there is insufficient information for evaluating the number and distribution of spawning runs that should be monitored to adequately characterize population dynamics within the Chesapeake Bay. Mid-Atlantic stocks of Alewife and Blueback Herring are being caught at disproportionately high levels in marine bycatch (Palkovacs et al. 2014, Hasselman et al. 2016), but the impact on Chesapeake Bay spawning populations remains poorly understood. River herring bycatch primarily occurs in Atlantic Herring *Clupea harengus* and Atlantic Mackerel *Scomber scombrus* midwater trawl fisheries (Bethoney et al. 2014, Hasselman et al. 2016), and bycatch limits are currently in place for both fisheries for 2016-2018 (NMFS 2016a, 2016b). All fisheries targeting river herring in the upper Chesapeake Bay and its rivers have been under moratoria since 2012 (ASMFC 2012). Stocking of hatchery-reared fish has been used in several rivers in attempts to rebuild populations, with at least one case (i.e., Patapsco River) of stock transfer among rivers (Stence et al. 2015). In other parts of the species' ranges, stock transfers have resulted in losses of genetic diversity (McBride et al. 2015), which could have lasting effects on population resilience (Carlson and Sattherthwaite 2011, Hasselman and Limburg 2012 and references therein). The present study explores genetic variation among river herring stocks of the upper Chesapeake Bay, evaluates trends in juvenile abundance indices, and examines potential

relationships between genetic structure and variation in juvenile abundance indices. We then consider the implication of these results for conservation, management, and monitoring strategies.

<'A'>Materials and Methods

<'B'>Sample collections and species identification

Collections of adult river herring (Alewife: n=277; Blueback Herring: n=234; unidentified: n=1) were obtained from rivers tributary to the upper Chesapeake Bay (i.e., Choptank River, Nanticoke River, Susquehanna River, Patuxent River, and Potomac River) during the spring of 2014 and 2015 using a combination of fyke nets and boat electrofishing equipment (Table 1; Figure 1). Choptank (2014), Nanticoke (2014) and Potomac River (2015) collections were pooled samples from weekly sampling programs, whereas Susquehanna (2015) and Patuxent River (2015 Blueback Herring only) collections were obtained on a single day for each species. Specimens were initially identified to species in the field based on external morphology and most were assessed for peritoneal coloration in a laboratory (Scott and Crossman 1973). Species identification was confirmed as described below using multi-locus genotypes from known adult Alewife and Blueback Herring specimens (n=100 per species) and the Bayesian model-based clustering method implemented in STRUCTURE v.2.3.3 (settings: K=2; correlated allele frequencies; admixture model; burn-in=50,000 steps; 250,000 steps of the Markov chain Monte Carlo (MCMC) algorithm) (Pritchard et al. 2000, Falush et al. 2003). Hybrid specimens were identified using the above method and following the procedure outlined in Hasselman et al. (2014). Alewife and Blueback Herring that were misidentified using morphological criteria were re-classified to their correct species prior to analyses. Hybrid

individuals and specimens not genotyped across a minimum of six microsatellites were removed from analyses. These adjustments resulted in a dataset for Alewife (n=228) and Blueback Herring (n=203) that was used to examine population genetic structure in upper Chesapeake Bay. Note that data from Palkovacs et al. (2014) were not included in the present study due to instrument repairs/upgrades that prevented direct comparison of data from the two studies, and that Patuxent River Alewife samples were collected then accidentally discarded prior to genetic analysis.

<'B'>Laboratory protocols

Specimens were genotyped across a suite of 15 polymorphic microsatellite loci developed for Alewife (*Ap010*, *Ap033*, *Ap037*, *Ap038*, *Ap047*, *Ap058*, *Ap070*, *Ap071*) and Blueback Herring (*Aa046*, *Aa070*, *Aa074*, *Aa081*, *Aa082*, *Aa091*, *Aa093*) by A'Hara et al. (2012). These same loci were previously used to genotype adult specimens for assessment of population genetic structure across the species' US ranges (Palkovacs et al. 2014). Details regarding DNA isolation and genotyping protocols were consistent with methods previously reported in (Palkovacs et al. 2014). Briefly, genomic DNA was extracted from tissue using one of two methods: Promega Wizard® SV Genomic DNA Purification System or 10% Chelex 100 (Bio-Rad, Richmond, CA). Amplification, size-fragment analysis, and scoring were conducted following (A'Hara et al. 2012). To confirm consistency in scoring and reproducibility of genotypes, positive and negative controls were used.

<'B'>Genetic data analyses

Data conformance to model assumptions.–

Tests for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were performed with GENEPOP v. 4.0.6 (Rousset 2008) using default parameters for all tests, and utilizing sequential Bonferroni adjustments to judge significance levels for all simultaneous tests (Holm 1979, Rice 1989). Deviations from HWE resulted in the exclusion of four loci for Alewife (*Aa082*, *Ap047*, *Ap070*, *Ap071*) and one for Blueback Herring (*Aa082*) from further analyses. Selective neutrality of loci was previously confirmed (Palkovacs et al. 2014).

Population genetic structure–

Allelic heterogeneity among rivers was assessed via genic tests in GENEPOP v.4.0.6 (Rousset 2008) using default parameters for all tests. Tests were combined across loci or collections using Fisher's method. Overall and pairwise F_{ST} values (θ) (Weir and Cockerham 1984) were estimated using FSTAT V.2.9.3.2 (Goudet 2001). The effect of variation in genetic diversity on genetic differentiation (Hedrick 2005) was accounted for by calculating standardized estimates of differentiation (F'_{ST}) using RECODEDATA v. 0.1 (Meirmans 2006) together with FSTAT to estimate $F_{ST(max)}$ for each pairwise comparison. Standardized estimates of differentiation were then calculated as $F'_{ST} = F_{ST} / F_{ST(max)}$ (Hedrick 2005). Genetic affinities among rivers were examined using Factorial Correspondence Analysis (FCA) implemented in Genetix v.4.0.5 (Belkhir et al. 2004).

The Bayesian model-based clustering method implemented in STRUCTURE v. 2.3.3 was used to infer the number of genetically homogenous clusters among rivers (Latch et al. 2006). For this analysis, we used a burn-in of 50,000 replicates followed by 250,000 replicates of the Markov chain Monte Carlo (MCMC) simulation, employing the admixture model and correlated

allele frequencies among populations. Five iterations of this parameter set were performed for K (number of clusters) from 1-4 for Alewife and 1-5 for Blueback Herring, allowing an estimation of the most likely number of clusters. Both the plateau of likelihood values (Pritchard et al. 2000) and ΔK (*i.e.,* second order rate of change between successive K values) (Evanno et al. 2005) were estimated. Admixture proportions were visualized using DISTRUCT v. 1.1 (Rosenberg 2004).

<'B'>Juvenile abundance

The dynamics of juvenile indices of abundance were compared among species and study streams using time series analyses. Maryland Department of Natural Resources (MD DNR) has conducted standardized beach seine surveys for juvenile fish in most of the study tributaries annually since 1966 and in the Patuxent River since 1983, providing the only long-term fishery-independent data for comparison of population trends among study streams (Durell and Weedon 2015). The seine survey is conducted using a quarter-turn method with a 30.5-m x 1.24-m bagless beach seine. Alewife and Blueback Herring were identified to species in the field and counted, and the geometric mean catch per unit effort (CPUE) per seine haul and 95% confidence interval were calculated. Regression analysis was used to determine if each index exhibited a positive or negative trend across the entire time series. Abundance data (y) were transformed prior to regression analysis by calculating ln (y+1). Second, empirical orthogonal function (EOF) analysis, which reduced the data to a set of uncorrelated functions and their principal component time series (RCs) (Wilks 2011), was used to compare temporal patterns in CPUE among time series. Analyses were conducted using data for the Head of Bay (near the mouth of the Susquehanna River), Potomac River, Choptank River and Nanticoke River using

Matlab version R2016a, to identify principal component time series explaining a substantial portion of the total variability. These PCs were then correlated with each time series of CPUE, and the strength of each correlation was characterized using the Cohen scale (i.e., $0.1<|r|<0.3$ is considered a weak correlation, $0.3<|r|<0.5$ is moderate, and $|r|>0.5$ is strong).

<'A'>Results

<'B'>Species identification

Morphological criteria provided a reliable means to identify adult river herring in rivers tributary to the upper Chesapeake Bay. Field and lab based identifications were confirmed for 92% of Alewife and 96% of Blueback Herring using genetic data for specimens of known species (Table 2). In total, three Alewife were misidentified as Blueback Herring, five Blueback Herring were misidentified as Alewife, and 20 hybrids were identified. This hybridization rate (4.6%) is similar to that detected previously for Chesapeake Bay rivers (Hasselman et al. 2014).

<'B'>Population genetic structure

Genic tests revealed significant ($P<0.05$) allelic heterogeneity between all pairwise comparisons for both Alewife and Blueback Herring; suggesting important differences in multilocus allele frequency distributions among collections for both species (data not shown). Standardized pairwise estimates of genetic differentiation (F'_{ST}) among Alewife ranged from 0.018-0.146 (F_{ST}= 0.007-0.046) (Table 3); multilocus global F'_{ST}=0.074 (F_{ST}=0.025). Non-significant ($p>0.05$) genetic differentiation was observed between the Choptank and Nanticoke Alewife collections (Table 3). For Blueback Herring, F'_{ST} ranged from 0.046-0.648 (F_{ST}= 0.018-0.170) (Table 4); multilocus global F'_{ST}=0.198 (F_{ST}=0.069). Non-significant ($p>0.05$) genetic

differentiation was observed between the Choptank and Nanticoke Blueback Herring collections and among all pairwise comparisons with the Susquehanna collection (Table 4).

FCA revealed three factors that explained 100% of the genetic variation among Alewife collections (Figure 2a) and 90.11% of the genetic variation among Blueback Herring collections (Figure 2b) in the upper Chesapeake Bay. For both species, the Choptank and Nanticoke collections clustered together and exhibited nearly complete overlap in FCA scores across the first two axes of variation. For Blueback Herring, the Patuxent collection exhibited some overlap with the Choptank and Nanticoke cluster. However, the Potomac and Susquehanna collections exhibited only slight overlap with the Choptank and Nanticoke cluster in both species.

For Alewife, the maximum value of lnPr(X|*K*) using STRUCTURE was observed at *K*=3 (-5228.46). Estimates of Δ*K* supported this result, and revealed the largest increase in the likelihood of the number of clusters at *K*=3 (Figure S1a). However, inspection of the STRUCTURE admixture plot at K=3 revealed several specimens from the Potomac collection that exhibited admixture patterns consistent with those from the Nanticoke and Choptank collections (data not shown). Indeed, 37.5% of Alewife collected from the Potomac River during 4–18 April 2014 (N=16) appeared to belong to a different genetic group than nearly all individuals (96%) collected from 25 April to 9 May 2014 (N=24). The inclusion of those specimens in the Potomac collection may have created admixture and inflated the estimate of the correct number of clusters. Based on these results, it seems that K=2 more accurately reflects the true number of clusters for Alewife among the collections examined in this study (Fig 3a). For Blueback Herring the maximum value of lnPr(X|*K*) using STRUCTURE was observed at *K*=3 (-

5745.22) and was supported by estimates of ΔK (FigureS1b). STRUCTURE admixture plots were consistent with K=3 for Blueback Herring (Fig. 3b).

<'B'>Juvenile abundance

Juvenile abundance indices (Fig. 4) exhibited substantial spatial and temporal variation, with the first three PC time series of the EOF analysis explaining over 97% of the variability (Fig. 5). Statistically significant declines in juvenile abundance indices were observed during the period of record for Alewife in the Potomac River ($p = 0.028$; $r^2 = 0.094$) and Blueback Herring in the Nanticoke River ($p = 0.002$; $r^2 = 0.174$), but there was no significant trend for the other time series. There was no apparent increase in juvenile abundance after 2012 when inshore fisheries were placed under moratorium. The majority of variance was explained by the first PC of the EOF analysis (77%), with the second PC explaining 16% and the third PC explaining 4%. Correlations between juvenile abundance indices and the first PC were strong for Blueback Herring in the Head of Bay and Nanticoke River, moderate for Alewife in the Head of Bay and Choptank, Nanticoke and Patuxent rivers, and weakly correlated with Blueback Herring in the Choptank and Patuxent rivers. Correlations with the second PC were strong for Alewife in the Head of Bay and Potomac River, and weak for Blueback Herring in the Head of Bay, Nanticoke, Potomac and Patuxent rivers and Alewife in the Patuxent River. The third PC was strongly correlated with Blueback Herring in the Potomac and Patuxent rivers, moderately correlated with Alewife in the Potomac River and Blueback Herring in the Choptank River, and weakly correlated with Blueback Herring in the Nanticoke River.

<'A'>Discussion

Alewife and Blueback Herring populations in the upper Chesapeake Bay exhibited significant allelic heterogeneity and genetic differentiation among most spawning populations (Table 3,4). However, we detected non-significant genetic differentiation between the Choptank and Nanticoke rivers for both Alewife and Blueback Herring, and non-significant genetic differentiation among all pairwise comparisons with the Susquehanna River for Blueback Herring. Bayesian clustering results for Alewife suggests two genetic clusters among upper Chesapeake Bay rivers, with the Choptank and Nanticoke River populations on Maryland's Eastern Shore in one cluster, and the Potomac and Susquehanna rivers on the Western Shore and at the Head of Bay in another cluster. The STRUCTURE admixture plot for Alewife (Fig 3a) also revealed substantial mixing between these clusters and may reflect some natural level of straying among upper Chesapeake Bay tributaries on relatively small spatial scales. For instance, 37.5% of the Alewife specimens collected from the Potomac River in early-mid April grouped with the Nanticoke-Choptank cluster, whereas 96% of those collected later in the season grouped with the Potomac-Susquehanna cluster (Figure 3a). Although we detected admixture for the Susquehanna River collection, detection of a similar temporal pattern was not possible due to a single sample collection date.

Bayesian clustering results for Blueback Herring suggests that there are three genetic clusters in the upper Chesapeake Bay. The Choptank and Nanticoke rivers form a single cluster, the Patuxent River represents a second cluster, and the Potomac and Susquehanna rivers form a third cluster (Fig 3b). We observed substantial admixture between Susquehanna River with both the Patuxent and Potomac rivers that is perhaps not surprising given their proximity in the upper Chesapeake Bay (Fig 1). However, we detected little admixture between the Potomac and Patuxent rivers which was surprising in light of their close geographic proximity.

The extent of genetic differentiation we observed among Alewife and Blueback Herring populations within upper Chesapeake Bay is broadly consistent with that observed among rivers in other portions of the species' ranges. Genetic differentiation of the scale we detected has also been observed in regional and coast-wide studies for river herring, with greater isolation by distance observed for Alewife than Blueback Herring (McBride et al. 2014, Palkovacs et al. 2014), and in other anadromous fishes including other *Alosa* spp. (Jolly et al. 2012, Hasselman et al. 2013) and Striped Bass *Morone saxatalis* (Gauthier et al. 2013). A previous study of river herring genetic structure revealed that Alewife exhibited significant allelic heterogeneity and genetic differentiation (albeit weak; F_{ST}=0.009) between the Nanticoke and Rappahannock rivers (Palkovacs et al. 2014). Conversely, Blueback Herring from these same rivers exhibited allelic homogeneity and non-significant genetic differentiation (F_{ST}=0.000) (Palkovacs et al. 2014). However, allelic heterogeneity for Blueback Herring was detected between the Rappahannock and James rivers, but not between the Nanticoke and James rivers, and each of these comparisons revealed non-significant genetic differentiation (Palkovacs et al. 2014). Taken together, these studies suggest a complex scenario of varying levels of genic and genetic differentiation among Alewife and Blueback Herring populations in the Chesapeake Bay. There are many other known spawning runs of river herring in upper Chesapeake Bay (Klauda et al. 1991) that have not been sampled in this or other studies of stock structure, and which might reveal additional genetic structure.

Interestingly, Alewife from the Potomac River appeared to originate from two distinct genetic groups. For Alewife collected in the Potomac River during 4–18 April 2014, 37.5% were similar to the Eastern Shore group and 62.5% were characteristic of the upper Western Shore group. In contrast, for Alewife collected from 25 April to 9 May 2014, only one individual was

similar to the Eastern Shore group and the remaining 96% were characteristic of the upper Western Shore group. These differences in run timing are consistent with spawning run counts in which both Alewife and Blueback Herring tend to arrive 1-3 weeks earlier in Eastern Shore tributaries (Choptank and Nanticoke rivers) than upper Western Shore tributaries (Patapsco and Susquehanna rivers) (Ogburn, unpublished data). Stream temperature is one important driver of spawning run timing of river herring (Ellis and Vokoun 2009, Ogburn et al. 2016), but other mechanisms potentially driving observed differences in run timing are poorly understood. Temporal shifts in the genetic composition of river herring spawning runs in Chesapeake Bay tributaries warrant further attention and will require weekly sampling in more rivers and multiple years to determine whether it is a common phenomenon potentially explaining observed patterns of genic and genetic differentiation.

Juvenile abundance was stable or declining, with no evidence of a response to the moratorium on inshore fisheries in the few years of data available since it was put in place. All time-series of juvenile abundance fluctuated through periods of higher and lower abundance, but most did not exhibit any consistent trend over time. The exceptions were Potomac River Alewife and Nanticoke River Blueback Herring, both of which exhibited minor but statistically significant declines in abundance since 1966. This is in stark contrast to a decline of over 95% in adult river herring landings in the Chesapeake Bay during the period 1966–2011 (ASMFC 2012). Notably, there has been no apparent increase in juvenile abundance since 2012 that would indicate a positive population response to the inshore fishing moratorium. One reason for the lack of response may be bycatch in offshore fisheries, estimated for the Southern New England Atlantic Herring fishery during 2012–2013 at 727,400 Alewife and 1,069,000 Blueback Herring (Hasselman et al. 2015). This level of bycatch is substantial compared to the 2014 spawning run

count of 581,275 ± 31,970 Alewife and 726,450 ± 39,995 Blueback Herring in the largest documented Chesapeake Bay spawning run (Ogburn et al. 2017) and other estimated run sizes in the region (ASMFC 2012). There may also have been insufficient time for the population to recover, or juvenile abundance indices may be relatively insensitive to changes in adult populations.

Patterns in time series supported the results of genetic analysis, suggesting at least two groups of sampling sites with distinct temporal trends that largely matched genetic groups. The first group, correlated with PC1 and best represented by Head of Bay Blueback Herring, was comprised of all juvenile abundance indices except the Potomac River. The second group, correlated with PC2 and best represented by Head of Bay Alewife, included Potomac and Patuxent River Alewife, and Potomac River Blueback Herring (with Head of Bay and Patuxent River Blueback Herring also correlated with a negative rather than positive relationship). A third group exhibited a distinct temporal pattern despite the fact that PC3 explained only 4% of the overall variance. This third group was best represented by Potomac River Blueback Herring and also included Alewife in the Potomac River and Blueback Herring in the Patuxent, Choptank and Nanticoke rivers. These patterns are consistent with genetic data in suggesting a divide between Eastern Shore (Choptank and Nanticoke rivers) and Western Shore tributaries (both species in the Potomac River and Blueback Herring in the Patuxent River) and similarity of Head of Bay (Susquehanna River) data with both groups. The mechanisms behind patterns in juvenile abundance are unknown, but the similar spatial patterns in both genetic and juvenile abundance data, as well as distinct otolith isotopic signatures differentiating Nanticoke River and Head of Bay fish of both species (Turner et al. 2015), hint at a combination of external factors (geography, environmental variability, harvest) and biological responses to those factors

(behavior, adaptation, evolution) potentially leading to both genetic differentiation and demographic independence (Waples and Gaggiotti 2006, Palkovacs et al. 2014).

The results of this study have important implications for river herring conservation, management and monitoring in the Chesapeake Bay. There is strong evidence for genetic structure among some Chesapeake Bay spawning streams for both Alewife and Blueback Herring, including potential differences in regional groupings by species. For Alewife, these results suggest at least two potential management and monitoring units along the Eastern and Western Shores, each of which likely exhibit unique demography and population dynamics that are shared by fish in the Susquehanna River. For Blueback Herring, the Eastern Shore, Potomac River, and Patuxent River are each potential management units, with the Susquehanna River population again sharing characteristics of both groups. Temporal shifts in genetic composition observed in the Potomac River complicate this picture, suggesting that run counts or indices of relative abundance could be comprised of more than one genetic stock possibly due to straying. Additional bay-wide sampling at weekly intervals and genetic analysis is likely needed to fully understand the stock structures of Alewife and Blueback Herring in the Chesapeake Bay. With fisheries in each of these rivers currently under moratorium and historical spawning habitat largely accessible (with the Susquehanna River a notable exception), understanding and reducing the impact of ocean bycatch on these stocks will be critical to rebuilding populations which have yet to exhibit a positive response following implementation of the inshore fishing moratorium.

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Table 1: Alewife and Blueback Herring genetic sample collections by river. Choptank and Nanticoke River samples were collected in 2014, whereas all other samples were collected in 2015. Samples were pooled from weekly collections, although some samples were collected on a single day (as indicated by an asterisk).

River	Latitude	Longitude	Gear	Alewife	Blueback Herring	Unknown
Choptank	38.9918	-75.7888	Electrofisher	84	85	0
Nanticoke	38.7029	-75.7753	Electrofisher	104	58	1
Susquehanna*	39.6132	-76.1484	Electrofisher	31	34	0
Patuxent*	38.8182	-76.6970	Electrofisher	0	30	0
Potomac	38.6875	-77.1923	Fyke net	58	27	0

Table 2: Specimen identification in the field (Field ID column) for Alewife (ALE) and Blueback Herring (BBH), subsequent genetic identification (ALE, BBH or Hybrid), and percent of field identifications that matched genetic identifications (% correct).

Field ID	n	ALE	BBH	Hybrid	% correct
ALE	245	226	5	14	92.25
BBH	207	3	198	6	95.65

Table 3: Alewife genetic differentiation. Pairwise F_{ST} values (θ; Weir and Cockerham 1984) below diagonal (non-significant values in bold) and standardized F_{ST} values (F'_{ST}; Hedrick 2005) above diagonal.

	Choptank	Nanticoke	Potomac	Susquehanna
Choptank	.	0.018	0.121	0.111
Nanticoke	**0.007**	.	0.146	0.121
Potomac	0.039	0.046	.	0.098
Susquehanna	0.035	0.038	0.030	.

Table 4: Blueback Herring genetic differentiation. Pairwise F_{ST} values (θ; Weir and Cockerham 1984) below diagonal (non-significant values in bold) and standardized F_{ST} values (F'_{ST}; Hedrick 2005) above diagonal.

	Choptank	Nanticoke	Patuxent	Potomac	Susquehanna
Choptank	.	0.0456	0.1120	0.6481	0.1955
Nanticoke	**0.0179**	.	0.0596	0.6476	0.1636
Patuxent	0.0431	0.0237	.	0.5778	0.0686
Potomac	0.1700	0.1672	0.1653	.	0.5997
Susquehanna	**0.0655**	**0.0551**	**0.0264**	**0.1561**	.

Figure captions

Figure 1. Map of sample collection locations in Maryland (MD) waters of the upper Chesapeake Bay and location of study along US Atlantic coast (inset). Virginia (VA) rivers sampled by Palkovacs et al. (2014) are labelled, as are adjacent states Delaware (DE), New Jersey (NJ), and Pennsylvania (PA).

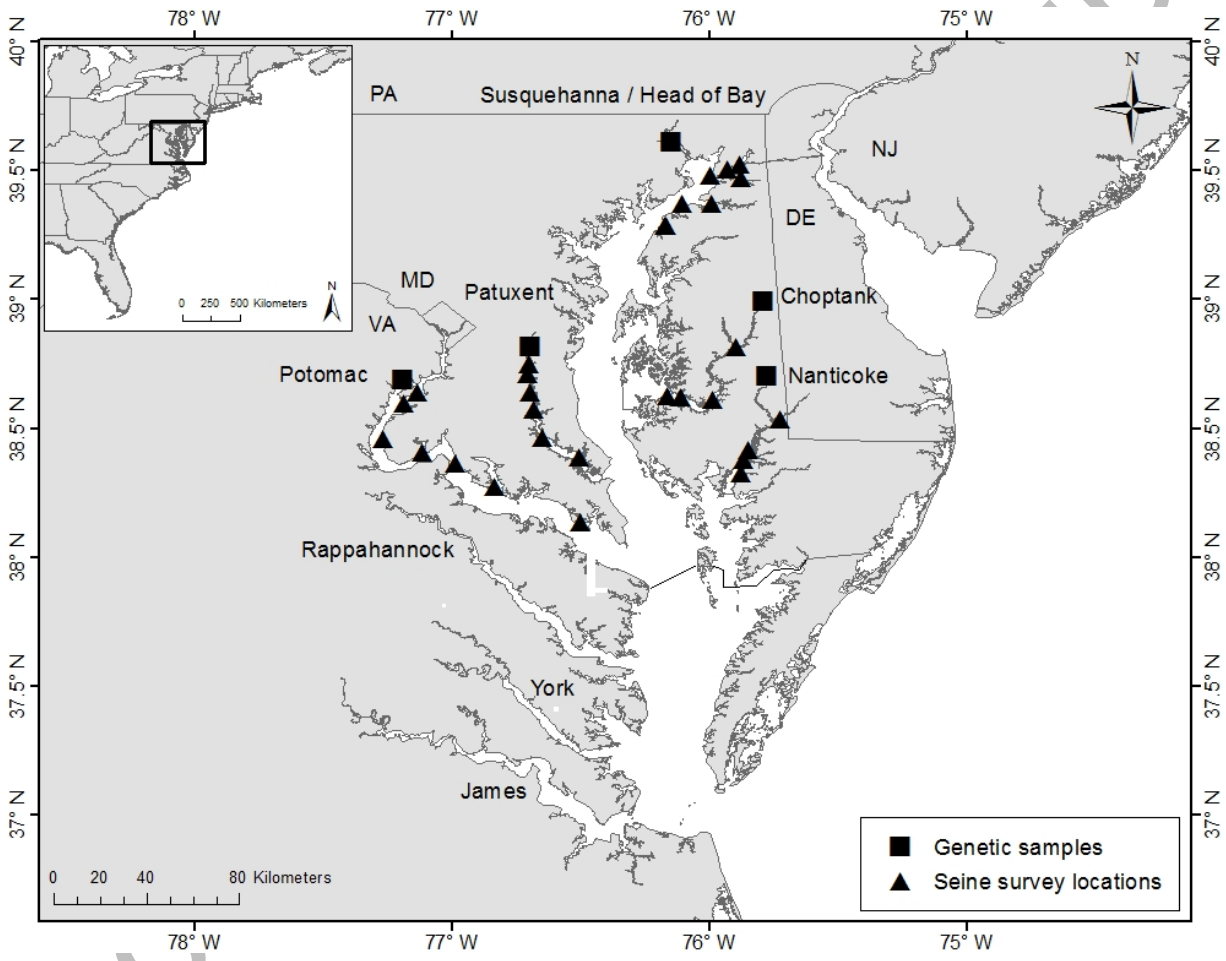


Figure 2. FCA plot for a) Alewife and b) Blueback Herring.

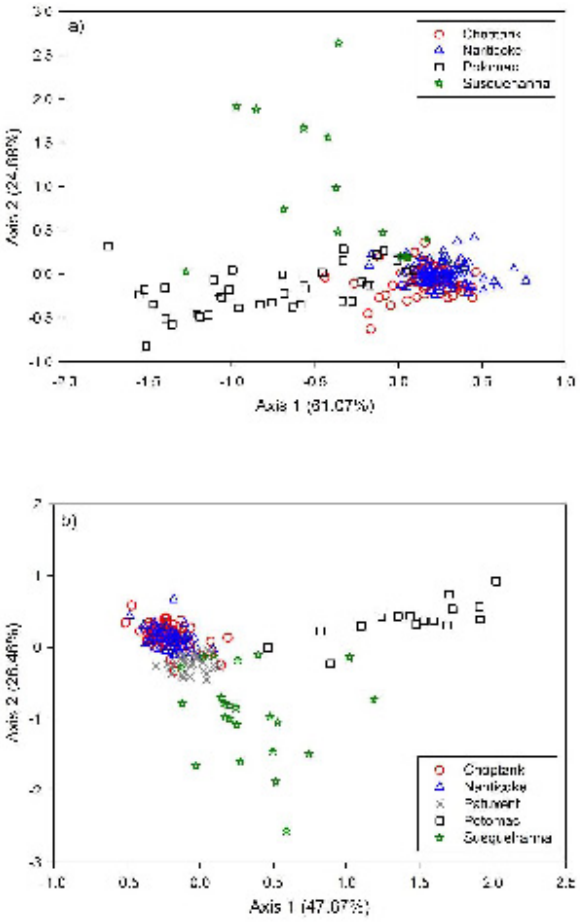


Figure 2. Ogburn et al.

Figure 3. Structure barplots showing admixture proportions for a) Alewife and b) Blueback Herring. The dashed line indicates the break between early season (4–18 April) and late season (25 April – 9 May) collections made in the Potomac River in 2014.

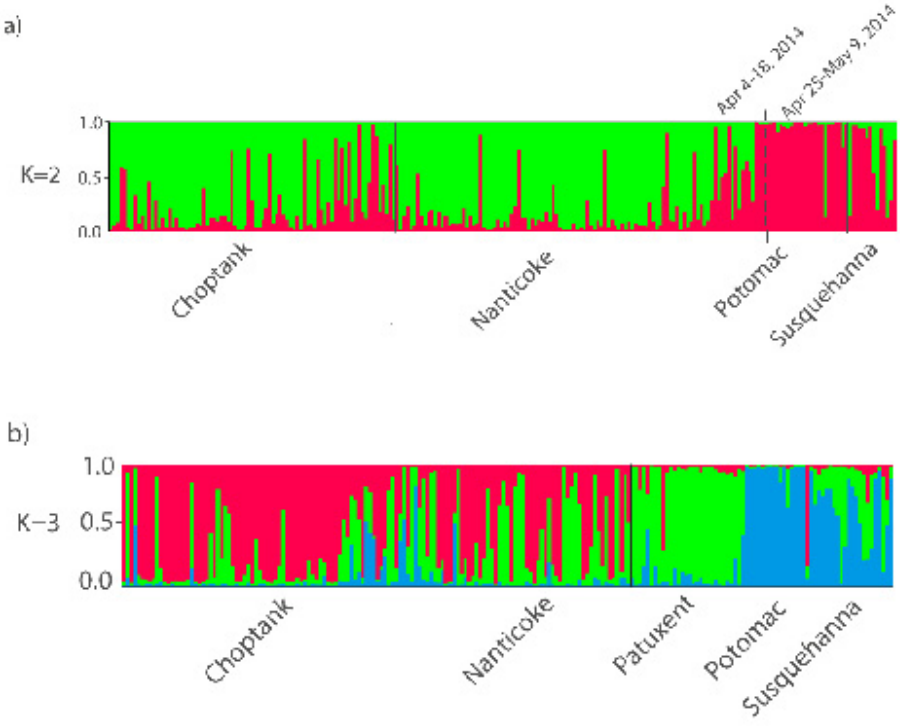


Figure 3. Ogburn et al.

Figure 4. Juvenile abundance indices (geometric mean catch per unit effort [CPUE]) for Head of Bay (A, F), Patuxent River (B, G), Potomac River (C, H), Choptank River (D, I), and Nanticoke River (E, J) for Alewife (A-E) and Blueback Herring (F-J). Note differences in scales of y-axes. Correlation coefficients (r) for which |r|>0.1 are reported for comparisons with the first three principal component time series of empirical orthogonal functions (PC1-3). The dashed lines indicate the onset of sampling in the Patuxent River.

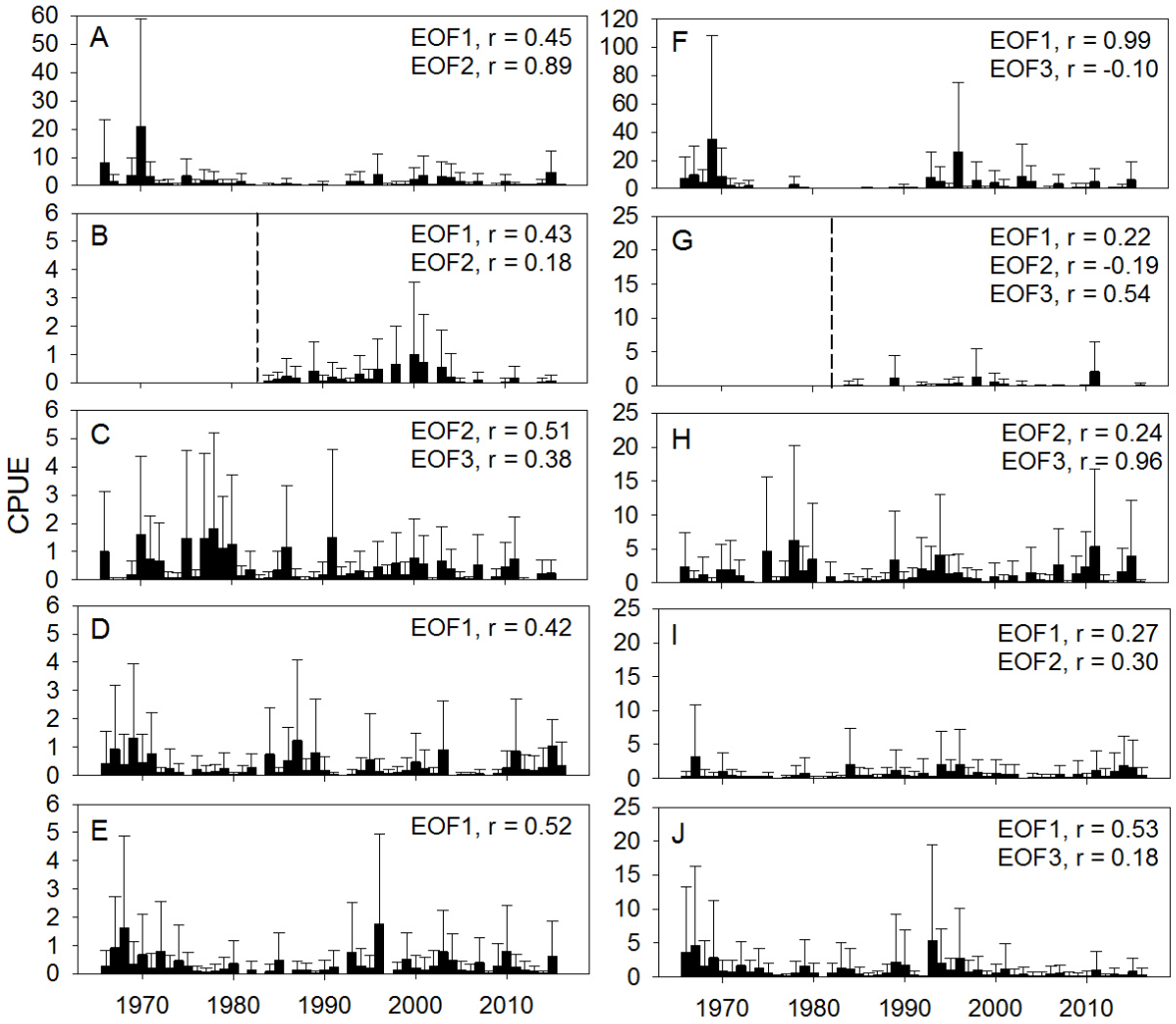
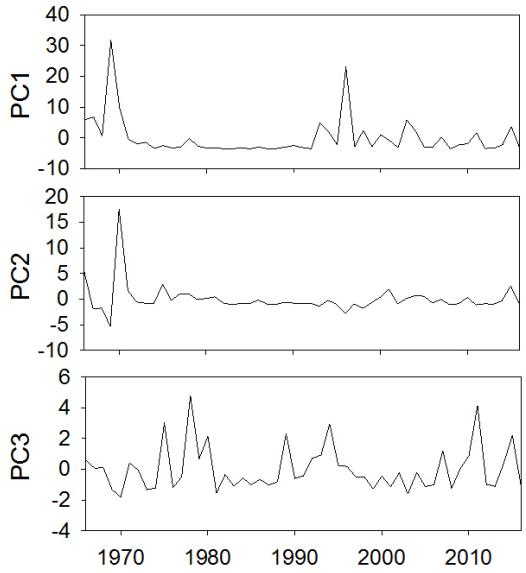


Figure 5. The first three principal component (PC) time series of the empirical orthogonal function analysis. Y-axis values are unit-less.



Ogburn et al. Figure 5