

Influence of stocking history on the population genetic structure of anadromous alewife (*Alosa pseudoharengus*) in Maine rivers

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Abstract Stocking programs have been used extensively to mitigate declines in anadromous fishes, but these programs can have long-term unintended genetic consequences. Stocking can homogenize population structure, impede local adaptation, and hinder the use of genetic stock identification as a fishery management tool. Using 12 microsatellite loci, we evaluate the spatiotemporal genetic structure of 16 anadromous alewife (*Alosa pseudoharengus*) populations in Maine, USA, to determine whether inter-basin stocking practices have influenced population structure and the genetic diversity of the species in this region. Although, no pre-supplementation samples exist, comparative analyses of stocked and non-stocked populations show that stock transfers have influenced alewife population genetic structure. Genetic isolation by distance (IBD) was non-significant among stocked populations, but significant among non-stocked populations. However, two populations, Dresden Mills and Sewell Pond, appear to have resisted genetic homogenization despite stocking. Non-significant genic and genetic differentiations were broadly distributed among alewife populations. Hierarchical AMOVA indicated highly significant differentiation

among temporal replicates within populations, and Bayesian clustering analysis revealed weak population structure. A significant correlation was observed between stocking (time and events) and pairwise F'_{ST} among alewife collections, and an analysis of IBD residuals showed a significant decline in the amount of genetic differentiation among populations as the extent of stocking activity increased. These findings call for an increased awareness of evolutionary processes and genetic consequences of restoration activities such as inter-basin stock transfers by fisheries management and conservation practitioners.

Keywords Stocking · Alewife · *Alosa* · Genetic structure · Fisheries management · Ecological restoration

Introduction

The long-term success of broad scale conservation programs requires the application of science-based solutions that are coordinated across jurisdictions, agencies, and ecosystems (López-Hoffman et al. 2010; Lauber et al. 2011). Conservation practitioners have called for the integration of evolutionary processes in conservation planning (Moritz and Potter 2013), and are increasingly aware that local-scale conservation actions can influence broader scale evolutionary processes (Beever et al. 2014). Indeed, conservation actions with evolutionary implications that are undertaken by an agency and which are not coordinated with other stakeholders may unintentionally compromise long-term conservation goals of the collective. These considerations apply to a wide range of taxa, but are particularly relevant to anadromous (i.e., migratory sea-run) fishes where multiple large entity stakeholders (e.g., local,

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state, and international governments) are often involved, and where stocking practices have played a substantial role in conservation efforts for several species (e.g., Ward 2006; Neff et al. 2011).

Anadromous fishes are of great ecological (Schindler et al. 2003; Hall et al. 2012) and economic (Hilborn et al. 2003; Schindler et al. 2010) value, and frequently occur across broad geographic ranges that span multiple jurisdictions (e.g., political boundaries) where various stakeholders have an interest in conservation and management. Conservation efforts for anadromous fishes often occur at the watershed/river scale because spawning and rearing in varying freshwater environments tends to foster philopatry and the formation of genetically distinguishable populations (e.g., Quinn et al. 2001). Nonetheless, many anadromous species undertake lengthy ocean migrations that typically involve mixed population assemblages before ascending natal rivers to spawn, and are therefore subject to anthropogenic stressors (e.g., overfishing, habitat loss and/or degradation) in marine and freshwater environments that increases their vulnerability and extinction risk (Wilcove and Wikelski 2008; Berger et al. 2014). Stocking programs (i.e., supportive breeding and stock transfers) have been employed by some agencies to mitigate these threats, and are often deemed successful when spawning stock biomass is shown to increase in response (Hasselman and Limburg 2012). However, these activities carry their own set of well documented risks (e.g., reduced genetic integrity and fitness of wild populations; Hindar et al. 1991; Lynch and O’Hely 2001; Araki et al. 2007; Lamaze et al. 2013; Valiquette et al. 2014) and can have long-term unintended negative consequences for population persistence and species’ evolutionary potential (Frankham 1995). These unintended side-effects often extend beyond the jurisdiction of the agency or entity conducting stocking activities.

Anadromous populations of alewife (*Alosa pseudoharengus*) are an important ecological component of coastal freshwater and marine food webs, and historically supported an important commercial fishery along the Atlantic coast of North America. However, precipitous declines in abundance since 1970 stemming from multiple anthropogenic stressors (i.e., overfishing, habitat loss and degradation; Hightower et al. 1996; Limburg and Waldman 2009) have resulted in historic lows, and the species is of increasing conservation concern (Atlantic States Marine Fisheries Commission (ASMFC) 2012). Despite a series of conservation efforts (e.g., fisheries moratoria, dam removal, stocking activities) the few consistent signs of recovery for many spawning populations (ASMFC 2012) suggest that marine mortality (e.g., bycatch in non-target commercial fisheries) may be substantial. Accordingly, the ASMFC has recently identified an increased understanding

of alewife marine ecology (i.e., spatial distribution of populations, extent of mixed population assemblages) as a high priority for conservation and recovery (ASMFC 2012). However, stock transfers used in restoration by some agencies may increase gene flow and homogenize otherwise genetically distinct populations. Artificially elevated levels of gene flow may impact local adaptation and compromise the ability of genetic stock identification methods to accurately assign individuals caught at sea to population of origin. These potential negative effects of stocking should be accounted for in conservation planning. Understanding the influence of stock transfers on spatial patterns of alewife genetic variation and population structure is important, because stocking activities in support of alewife restoration are increasing (MDMR 2009; MDEP 2009).

Stock transfers within and among drainages have been used extensively for alewife restoration in the state of Maine, USA, and were previously justified under early management regimes (Atkins 1887; Belding 1920) based on the assumption of extensive natural straying and gene flow (Rounsefell and Stringer 1945; Havey 1961). However, recent research has demonstrated that alewives exhibit stronger patterns of genetic differentiation on finer spatial scales than previously believed (Hasselman et al. 2010; 2013; Palkovacs et al. 2014; McBride et al. 2014), and stock transfers may have influenced the spatial patterns of alewife genetic structure. Extensive inter-basin stocking activities for alewife have been conducted by the Maine Department of Marine Resources (MDMR) during the last century, but stocking history documentation has been kept intermittently since the 1940s (MDMR 2010, Lewis Flagg, MDMR, personal communication). Pre-supplemented samples do not exist to assess the spatial patterns of genetic population structure prior to stocking. Using 12 microsatellite loci, we examine patterns of genetic variation among 16 populations of alewife in Maine that have experienced various intensities of inter-basin stock transfers over the previous century, and investigate whether stocking practices have altered spatial patterns of genetic structure.

Materials and methods

Sample collections

Anadromous alewives were collected from Maine as part of a larger study of river herring genetic variation in Northeastern North America (McBride 2013; McBride et al. 2014). Sample locations were chosen to provide broad geographic coverage within the study region, and included recently re-established spawning runs (e.g., Union River), and the primary source populations used in

statewide inter-basin stocking activities during the previous 30 years (i.e., Kennebec and Androscoggin Rivers). Sampling effort targeted 50 individuals per collection (i.e., sample location year⁻¹), replicated for a subset of locations over successive years, and resulted in the collection of 2216 fin clips from 19 sampling locations from nine drainages during 2005–2011 (Table 1; Fig. 1). Tissue samples were obtained in freshwater from adults, with the exception of those collected from Veazie Dam and Souadabscook Falls (Penobscot River), which comprised young-of-the-year specimens. Tissue was preserved in 95 % ethanol until DNA extraction. Species identifications based on field observations of peritoneal coloration (Scott and Crossman 1973; Messieh 1977) were confirmed using Bayesian clustering analyses of genetic data as described in Hasselman et al. (2014), with misidentified blueback herring (*A. aestivalis*) and hybrid individuals removed prior to analyses.

Laboratory protocols

DNA extraction and genotyping

We examined genetic variation at 14 polymorphic microsatellite loci developed for alewife (*Aps1*, *Aps2A*: Bentzen and Paterson 2005; *Ap010*, *Ap058*, *Ap071*: A'Hara et al. 2012) blueback herring (*Aa046*, *Aa070*, *Aa081*, *Aa082*, *Aa093*, *Aa039*: A'Hara et al. 2012), and American shad (*A. sapidissima*; *AsaD042*, *AsaC249*: Julian and Bartron 2007; *Asa8*: Waters et al. 2000). Details regarding DNA isolation and genotyping protocols are reported in McBride et al. (2014).

Data analyses

Data conformance to model assumptions

Genotyping artifacts were assessed using MICROCHECKER v.2.2.3 (van Oosterhout et al. 2004). Tests for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed among collections with GENEPOP v. 4.0.6 (Rousset 2008) using default parameters for all tests. Sequential Bonferroni adjustments were used to judge significance levels for all simultaneous tests (Holm 1979; Rice 1989). Selective neutrality of the microsatellite markers was confirmed previously (McBride et al. 2014).

Genetic diversity

Population-specific sample size (N), number of alleles (N_a), probability of departures from HWE (p val), observed (H_o)

and expected heterozygosity (H_e) was assessed using Arlequin v.3.1 (Excoffier et al. 2005). Allelic richness (A_e) per locus and location was estimated with FSTAT v.2.9.3.2 (Goudet 1995) standardized to a minimum sample size of 34 individuals (Leberg 2008). Population-specific inbreeding coefficient (F_{IS}) was estimated by GENEPOP (Rousset 2008).

Genetic differentiation

Allelic heterogeneity among drainages was assessed via genic tests in GENEPOP using default parameters. Tests were combined across loci using Fisher's method and sequential Bonferroni correction (Rice 1989) was used to determine the significance among pairwise comparisons.

Pairwise F_{ST} values (θ ; Weir and Cockerham 1984) were estimated using FSTAT (Goudet 1995). The effect of variation in genetic diversity on genetic differentiation (Hedrick 2005) was controlled for by calculating standardized estimates of differentiation (F'_{ST}) using RECO-DEDATA v.0.1 (Meirmans 2006) together with FSTAT to estimate $F_{ST(max)}$. Standardized estimates of differentiation were then calculated as $F'_{ST} = F_{ST}/F_{ST(max)}$ following Hedrick (2005). To quantify the patterns of genetic variation among locations, among years (temporal replicates) within locations and within locations, a hierarchical analysis of molecular variance (AMOVA) was assessed with Arlequin (10,000 permutations; Excoffier et al. 2005).

Relationships among collections

Genetic affinities among collections were examined with an unrooted neighbor-joining (NJ) analysis of Nei's D_A distance (Nei et al. 1983) bootstrapped over all loci (10,000 replications) using POPTREE2 (Takezaki et al. 2010). Genetic relationships among locations were assessed with a Principal Coordinate Analysis (PCoA) of pairwise F_{ST} values generated by FSTAT, using GenAlEx (v.6.41; Peakall and Smouse 2006). Gene flow was estimated among all alewife collections and among both stocked and non-stocked populations using the private allele method [N_m estimate; Slatkin (1985)] generated in GENEPOP (Rousset 2008).

Relationships within collections

COLONY v.2 (Jones and Wang 2009) was used to infer the number of sibship and parentage relationships within collections. We employed the full-likelihood analysis method with high precision and with a mating system of female and male polygamy with interbreeding and without the clone option. Default settings were used for the remaining

Table 1 Summary indicating the number of samples collected in Maine from 2005 to 2011, sample location and year, river name, watershed, coordinates, observed and expected heterozygosity (H_o , H_e), inbreeding coefficient (F_{IS}), allelic richness (A_e) and whether the population was stocked (S) or not (U)

Watershed	River name	Location name	Code	Type	Lat.	Long.	Sample year	Sample no.	H_o	H_e	F_{IS}	A_e	
St. Croix River	St. Croix River	Milltown Dam	05MIL	U	45.18	67.29	2005	55	0.477	0.491	0.026	4.809	
		Dennis Stream	05DEN	U	45.19	67.26	2005	48	0.494	0.497	0.002	4.459	
		Woodland	05WOO	U	45.19	67.20	2005	7	–	–	–	–	
Union River	Union River	Leonard Lake Dam	09LEO	S	44.51	68.43	2009	58	0.517	0.520	–0.030	5.003	
			11LEO				2011	49	0.553	0.546	–0.030	4.510	
Bagaduce River	Bagaduce River	Wight Pond	09WIG	S	44.42	68.68	2009	52	0.526	0.522	–0.019	4.606	
			11WIG				2011	60	0.539	0.538	0.000	4.462	
Orland River	Orland River	Orland Dam	10ORL	U	44.57	68.74	2010	60	0.489	0.495	0.010	4.743	
			11ORL				2011	53	0.526	0.511	–0.025	4.368	
Penobscot River	Penobscot River	Veazie Dam	09VEA	U	44.83	68.70	2009	71	0.495	0.496	–0.010	4.482	
			10VEA				2010	52	0.504	0.495	–0.013	4.294	
			11VEA				2011	52	0.482	0.513	0.061	4.719	
	Sedgeunkedunk	Sedgeunkedunk	Sedgeunkedunk Dam	09EDD	U	44.83	68.70	2009	16	–	–	–	–
				09SED	U	44.77	68.78	2009	14	–	–	–	–
				09SOU	U	44.76	68.86	2009	60	0.488	0.484	–0.019	4.752
				10SOU				2010	60	0.470	0.465	–0.014	4.289
St. George River	St. George River	Sennebec Pond	11SOU				2011	61	0.527	0.520	–0.023	4.703	
			08SEN	S	44.07	69.28	2008	48	0.531	0.533	–0.001	4.543	
			10SEN				2010	63	0.481	0.485	0.016	4.377	
Damariscotta River	Damariscotta River	Damariscotta Mills Dam	11SEN				2011	50	0.523	0.531	0.020	4.684	
			09DAM	S	44.03	69.53	2009	60	0.507	0.498	–0.033	4.107	
			10DAM				2010	52	0.503	0.479	–0.055	4.038	
Kennebec River	Eastern River	Dresden Mills Dam	11DAM				2011	53	0.491	0.503	0.016	4.410	
			08DRE	S	44.06	69.72	2008	39	0.566	0.575	0.020	5.246	
			09DRE				2009	29	0.555	0.576	0.042	4.951	
			10DRE				2010	58	0.540	0.530	–0.023	NA	
			11DRE				2011	53	0.545	0.549	0.001	5.340	
	Kennebec River	Lockwood Dam	09LOC	S	44.55	69.63	2009	64	0.506	0.522	0.023	4.545	
			11LOC				2011	35	0.455	0.497	0.082	4.728	
			09NEQ	U	43.91	69.78	2009	59	0.489	0.515	0.033	4.350	
	Nequasset Brook	Nequasset Lake Dam	10NEQ				2010	60	0.525	0.520	–0.022	4.603	
			11NEQ				2011	45	0.528	0.530	0.029	4.312	
			09BEN	S	44.54	69.55	2009	65	0.508	0.525	0.042	4.636	
	Sebasticook River	Benton Falls Dam	10BEN				2010	62	0.550	0.509	–0.087	4.362	
			11BEN				2011	61	0.525	0.538	0.029	4.711	
			09WEB	S	44.40	69.67	2009	37	0.482	0.504	0.055	4.537	
	Seven Mile Stream	Webber Pond Dam	10WEB				2010	59	0.471	0.464	–0.029	4.254	
11WEB						2011	52	0.486	0.523	0.068	4.532		
09SEW			S	43.88	69.78	2009	64	0.484	0.517	0.073	5.126		
Sewell Creek	Sewell Pond	11SEW				2011	51	0.535	0.537	–0.002	4.649		
		09AND	S	43.92	69.97	2009	59	0.511	0.511	0.001	4.463		
		10AND				2010	60	0.518	0.516	–0.012	4.389		
Androscoggin River	Androscoggin River	Brunswick Dam	11AND				2011	50	0.472	0.488	0.018	4.262	

NA indicates sample size ($N = 29$) was below the threshold ($N = 34$) to calculate allelic richness

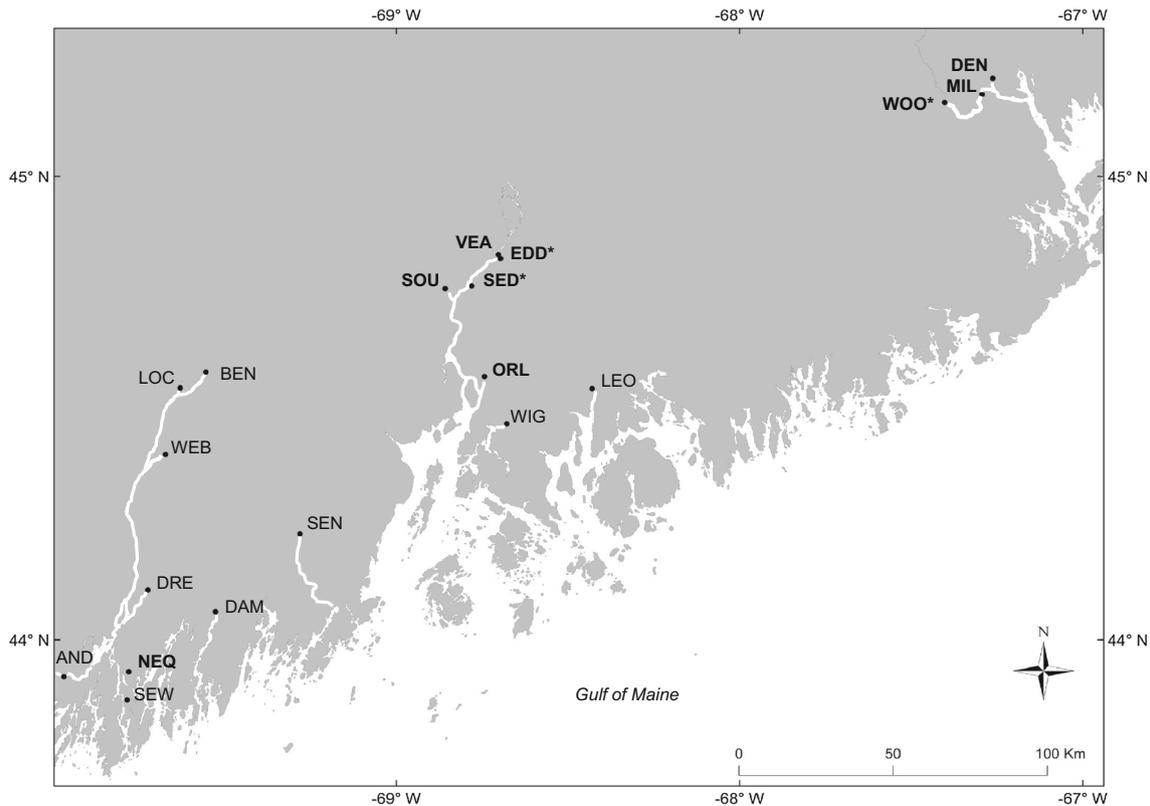


Fig. 1 Sampling locations for Maine alewife collections from 2005 to 2011. Locations in *bold* indicate no stocking history. Those populations with the asterisk (WOO, SED and EDD) were dropped

from analysis due to the small number of samples collected. Full names of sampling locations are provided in Table 1

parameters [i.e. species, run length, sibship relationships and run specification (Jones and Wang 2009)].

Population structure

STRUCTURE v. 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) was used to infer the number of genetically homogenous clusters among collections (Latch et al. 2006), and was conducted both with and without prior location information. For this analysis we specified a burn-in of 50,000 replicates followed by 500,000 replicates of the Markov chain Monte Carlo (MCMC) simulation, and employed the admixture model and correlated allele frequencies among populations. Three iterations of this parameter set were performed for number of clusters (*K*) from 1 to 10, 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. Following Vähä et al. (2007) hierarchical STRUCTURE analysis was conducted until all recovered genetic clusters resulted in *K* = 1. Results from all STRUCTURE iterations were combined using CLUMPP

(Jakobsson and Rosenberg 2007) and visualized with DISTRUCT (Rosenberg 2004).

Isolation by distance

Analysis of isolation by distance (IBD) was conducted among drainages to test for correlations between geographic distance and genetic differentiation using 10,000 permutations of the Mantel Test implemented in the R package ‘vegan’ (Oksanen et al. 2013; R Development Core Team 2013). Pairwise F'_{ST} values were linearized [$F'_{ST}/(1 - F'_{ST})$] following Rousset (1997). Geographic distance between sampling locations were measured following the shortest path distance within 5 km of the Maine coastline using ArcGIS 10.2. Briefly, a base map with a Projected Coordinate System of NAD83 (North American Datum 1983) Maine 2000 East Zone (Meters) served as a template, with georeferenced locations for sampling locations overlaid using a standard Geographic Coordinate Systems. A polygon shape file was constructed within 5 km of the Maine coastline, with distances among sampling locations estimated following along the outer edge of this buffer.

Effects of stocking

Available inter-basin stocking data were extracted from agency reports and databases provided by MDMR and from published accounts (Rounsefell and Stringer 1945; Havey 1961). Stocking data were summarized into four categories describing: (i) whether documented stocking had occurred between sample locations, (ii) the extent of stocking (i.e., the total number of individuals stocked between sample locations), (iii) the number of stocking events (i.e., the total number of stock transfers that have been documented between sample locations), and (iv) years of stocking (i.e., the duration of stock transfers). Stocking information is summarized in Supplemental Table S1. These four categories provided complementary information about the scope of stocking activities in Maine, and were used to assess the influence of inter-basin stock transfers on the genetic variation among populations using linear regression models implemented in R Development Core Team (2013).

Results

Data conformance to model assumptions

Evidence for null alleles resulted in the exclusion of two loci (*Aa082*, *Ap071*) prior to analyses (Microchecker). Remaining loci were retained as evidence for null alleles was sporadic among loci and collections. Exact tests indicated that genotypic frequencies were largely in accordance with HWE ($p > 0.05$; sequential Bonferroni correction for 40 comparisons). However, HWE departures remained for eight locus-collection comparisons, seven of which were associated with Kennebec River collections (Supplemental Table S2). Exact tests of LD revealed that loci were physically unlinked and statistically independent. Collections with small sample sizes (i.e., WOO, EDD, SED; see Table 1) were removed prior to further analyses.

AMOVA revealed a highly significant ($p < 0.001$; 0.28 %) component of genetic variation partitioned among temporal replicates (Supplemental Table S3), and suggested that binning replicated collections was not an appropriate strategy for analyses. Therefore, we treated each collection as a separate group.

Genetic diversity

The amount of genetic polymorphism observed varied among loci and locations (Supplemental Table S2). The number of alleles per locus ranged from two (*Aps1*, *Aa046*) to 10 (*Ap010*) with eight loci exhibiting \leq five alleles.

Allelic richness per locus and location ranged from 1.64 (*Aps 1*; 11DRE) to 9.74 (*Ap010*; 11DRE) and observed heterozygosity varied between 0.019 (*Aps 1*; 11DRE) and 0.865 (*Aa081*; 10VEA). Across all loci allelic richness ranged from 4.31 (10DAM) to 5.34 (11DRE; Table 1), with mean allelic richness of 4.57 (range 4.04–5.34) among stocked populations and 4.58 (range 4.29–4.75) among non-stocked populations. Observed heterozygosity varied between 0.455 (11LOC) and 0.566 (08DRE; Table 1), with the mean observed heterozygosity of 0.509 (range 0.455–0.566) among stocked populations and 0.513 (range 0.470–0.528) among non-stocked populations.

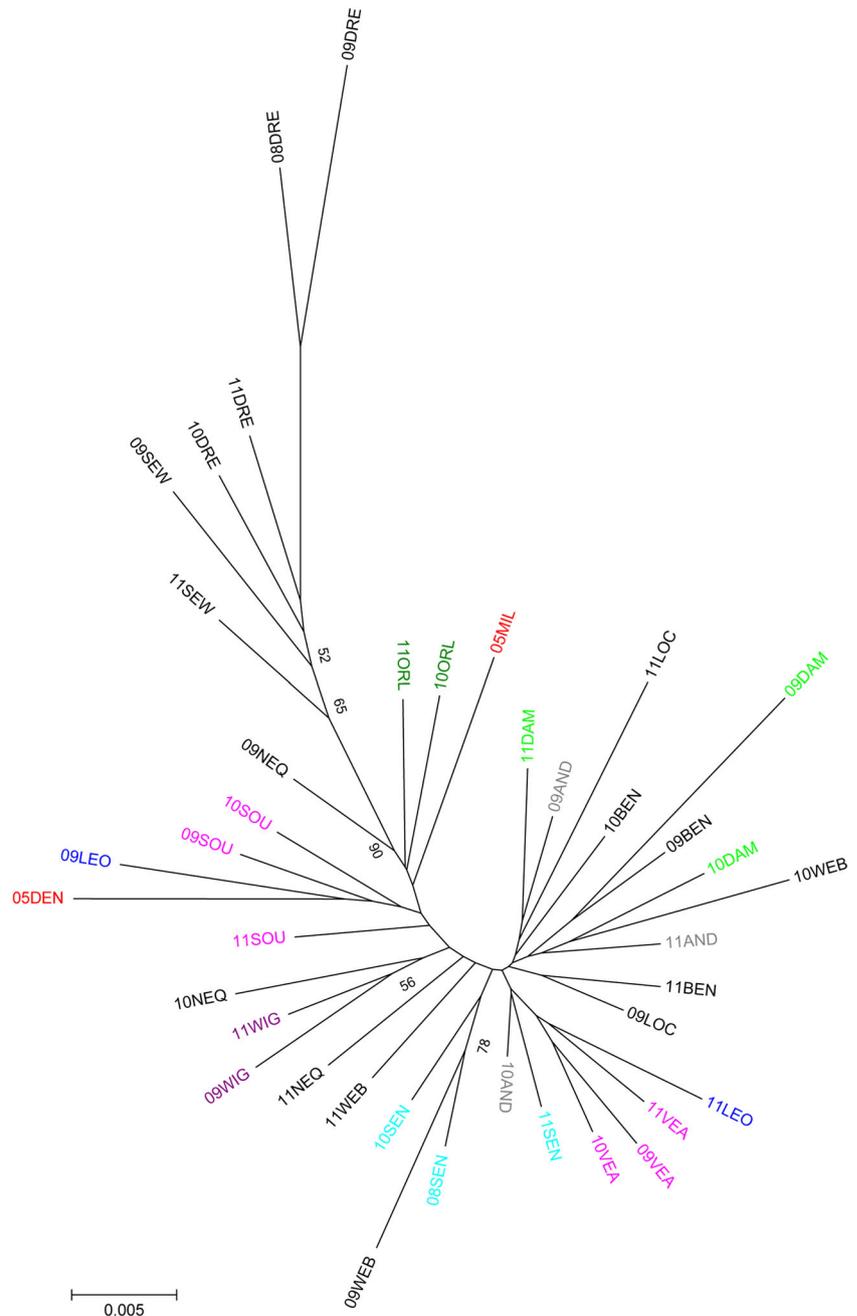
Genetic differentiation

Significant ($p < 0.05$) genic differentiation was observed for 334/748 pairwise comparisons after Bonferroni correction (Supplemental Table S4). Instances of non-significant genic differentiation were broadly distributed among collections (Supplemental Table S4). Standardized pairwise estimates of genetic differentiation (F'_{ST}) ranged from -0.01 to 0.137 [$F_{ST} = -0.005$ – 0.066 ; Table S5]; multilocus global $F'_{ST} = 0.024$ ($F_{ST} = 0.012$). Mean F'_{ST} among non-stocked populations was 0.015 (range -0.019 – 0.094), whereas mean F'_{ST} among stocked populations was 0.013 (range -0.009 – 0.043). Non-significant ($p > 0.05$) instances of genetic differentiation (497/780) were broadly distributed among collections (Supplemental Table S5). The majority of significant pairwise comparisons (191/227) were associated with the temporally replicated collections of Dresden Mills Dam (DRE) and Sewell Pond (SEW).

Relationships among collections

Neighbor-joining analysis using Nei's D_A revealed a complex relationship among alewife collections. There was little tendency for temporal replicates to cluster by location, or for locations to cluster by watershed. Most of these relationships were only weakly supported (<50 % bootstrap support; Fig. 2). However, associations among temporally replicated collections from DRE and SEW were supported more strongly (>50 % bootstrap support), and suggested a different relationship among these collections relative to the others. Similar patterns of genetic differentiation among populations, including relatively strong genetic differentiation between DRE and SEW populations and all remaining sample sites (mean $F_{ST} = 0.028$), were observed in a PCoA (Supplemental Fig. S1). Although high gene flow was detected among all alewife collections (N_m estimate = 16.084) and non-stocked populations (N_m estimate = 15.758), higher rates of gene flow were revealed among stocked populations (N_m estimate = 21.347). Gene

Fig. 2 Unrooted neighbor-joining tree analysis, using Nei's D_A , displaying bootstrap support >50 %. Sampling locations are colored by watershed for ease of interpretation of tree topology. Full names of sampling locations are provided in Table 1



flow rates increased (N_m estimate = 23.760) when DRE and SEW were omitted from analysis.

Relationships within collections

Sibship analyses in COLONY showed that all population samples consisted largely of individuals unrelated at the full- or half-sib level. In each case, the number of family groups closely approached the total number of individuals in the population sample (Supplemental Table S6), indicating that estimates of genetic differentiation and diversity were not biased by the presence of large family groups.

Population structure

STRUCTURE analyses revealed the maximum value of $\ln Pr(X|K)$ at $K = 2$, regardless of whether prior location information was used or not (Supplemental Fig. S2a). Estimates of ΔK also revealed the largest increase in the likelihood of the number of clusters at $K = 2$ (Supplemental Fig. S2b). Visualization of individual admixture proportions revealed that temporal replicates from DRE and SEW comprised one cluster, while all other collections comprised the other (Supplemental Fig. S3). Hierarchical STRUCTURE analysis revealed no further substructure.

These results are consistent with those of the Neighbor-joining analysis and PCoA. Despite historical stocking, SEW and DRE collections were significantly different from all other Maine populations including neighboring populations and thus were ‘outliers’ for all analyses. We consequently removed them from further consideration.

Isolation by distance

Mantel tests revealed a highly significant ($p < 0.001$; $r^2 = 0.251$) pattern of IBD among all collections (data not shown). When collection pairs were examined based on stocking history, linear regression revealed a highly significant ($p < 0.001$; $r^2 = 0.277$) relationship between genetic differentiation and geographic distance among non-stocked collections, but a non-significant ($p < 0.914$; $r^2 = 0.000$) relationship among stocked collections (Fig. 3a). To compare IBD patterns for stocked and non-stocked collection pairs on a similar spatial scale (~ 300 km) we removed pairwise comparisons with collections from the St. Croix River. Despite this omission a significant relationship was still revealed among non-stocked collections ($p > 0.001$, $r^2 = 0.155$; data not shown). Examination of the distribution of residuals from a linear regression of the IBD pattern among all collections revealed a general decline in the residual values for stocked collection pairs greater than 120 km apart (Fig. 3b), suggesting lower genetic differentiation than what might be expected based on geographic distance alone.

Effects of stocking

Linear regressions among collections revealed a highly significant ($p < 0.001$, $r^2 = 0.036$) decline in genetic differentiation among stocked versus non-stocked pairs (data not shown). We observed a highly significant ($p < 0.001$) decline in the amount of genetic differentiation as the extent of inter-basin stocking activity increased (i.e., number of fish stocked, number of years stocked, number of stocking events; Fig. 4 a, b, c).

Discussion

Effective conservation planning requires coordination to ensure that achieving short-term conservation results (e.g., population size) does not undermine long-term conservation goals (e.g., retention of genetic diversity, local adaptation, and population viability) (López-Hoffman et al. 2010; Lauber et al. 2011; Valiquette et al. 2014). Stocking activities have been used extensively for the conservation of anadromous fishes, but can have long-term unintended consequences that can extend beyond the jurisdiction of a

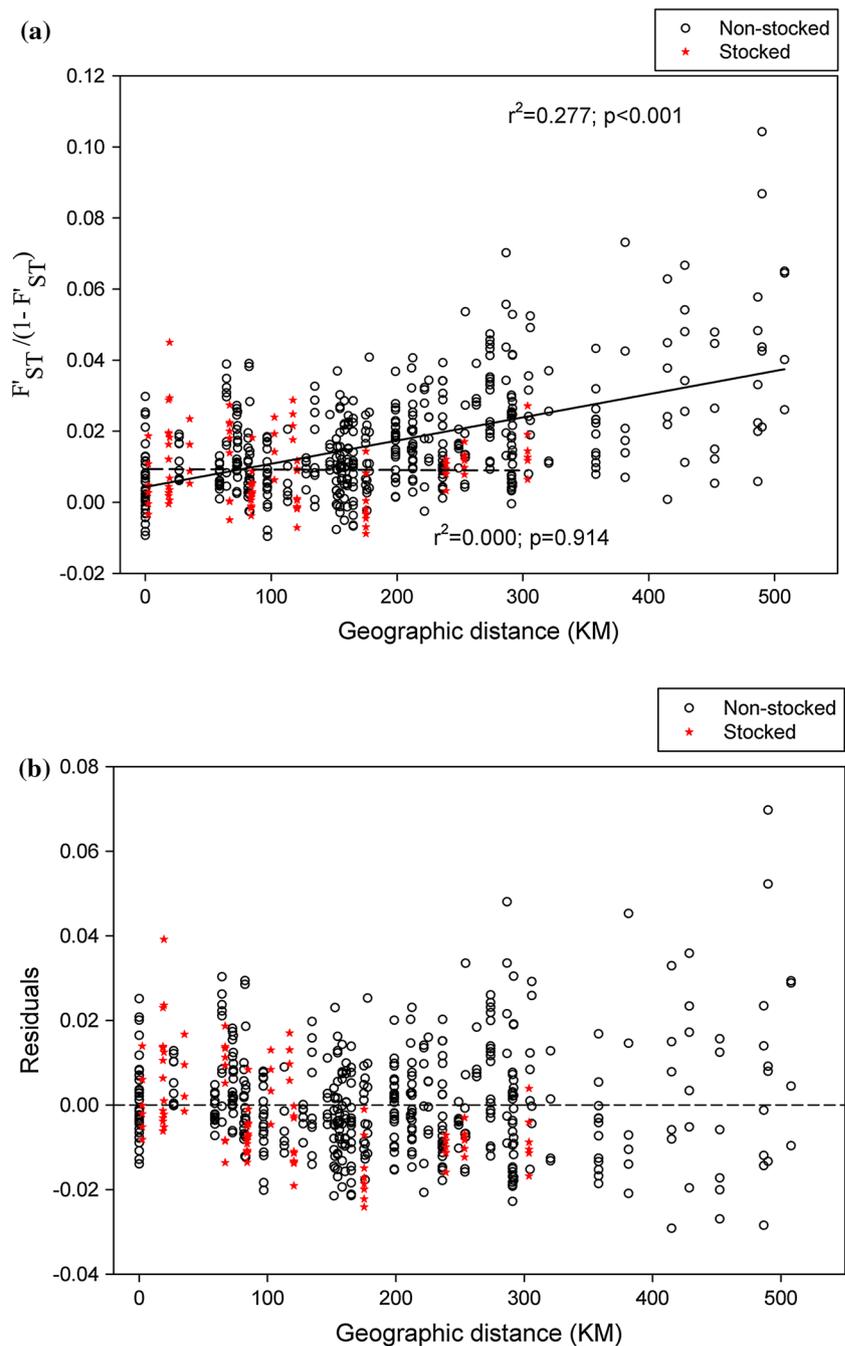
given agency and inadvertently hamper long-term conservation planning. We investigated the impacts of inter-basin stock transfers on the genetic structure of anadromous alewife populations in Maine, USA. The absence of pre-supplementation samples precluded direct estimates of the effects of inter-basin stocking on altered patterns of genetic variation among alewife populations; but nonetheless, our analyses of contemporary populations did provide evidence that inter-basin stocking activities have significantly influenced spatial patterns. Our results have potential implications for local adaptation, the utility of genetic stock identification as a tool for understanding marine ecology, and for developing conservation and management plans.

Stocking effects on the spatial pattern of genetic variation

Compared to other studies of alosine fishes in various portions of their ranges (e.g., alewife: Palkovacs et al. 2008, 2014; Labbe 2012; McBride et al. 2014; blueback herring: Palkovacs et al. 2014; American shad: Hasselman et al. 2013; Aunins et al. 2014), we generally observed weak population structure for alewife in Maine. Under a stepping stone model of population structure (typical of philopatric anadromous fishes; Hasselman et al. 2010; Carlson et al. 2011), we expect natural gene flow to occur primarily among neighboring and geographically proximal populations, and anticipate spatially structured patterns of genetic variation and differentiation. However, we observed no spatially consistent pattern in the arrangement of non-significant genic tests among populations (Supplemental Table S4), levels of genetic differentiation among populations (Supplemental Table S5), topology of the NJ Tree (Fig. 2) or PCoA (Supplemental Fig. S1). This result contrasts with what was recently reported from the Canadian portion of the species range, where stocking has not occurred. McBride et al. (2014) observed strong population structure, and evidence of natal homing with some degree of natural straying and gene flow. Our results suggest that the contemporary spatial pattern of genetic variation for alewife in Maine deviates from a stepping stone model of population structure that is more typical of philopatric anadromous fishes, and may be influenced by historical stock transfers that moved fish across watersheds.

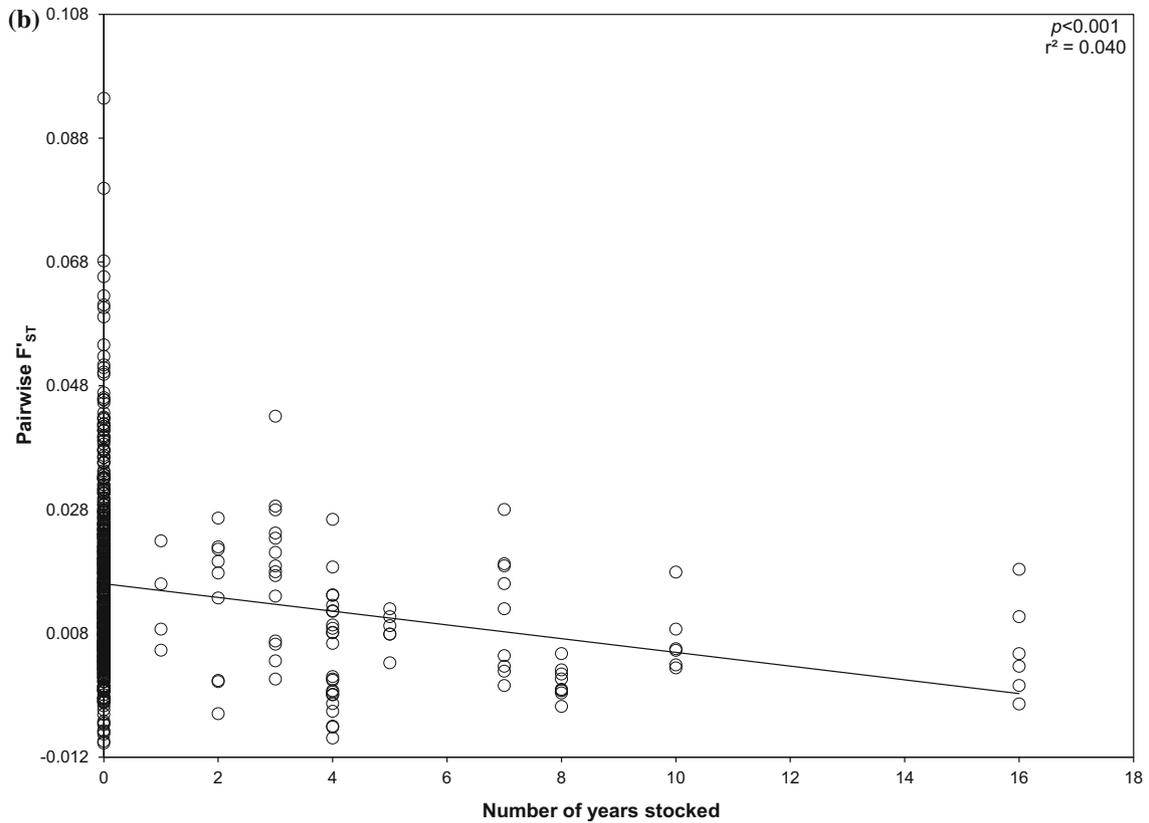
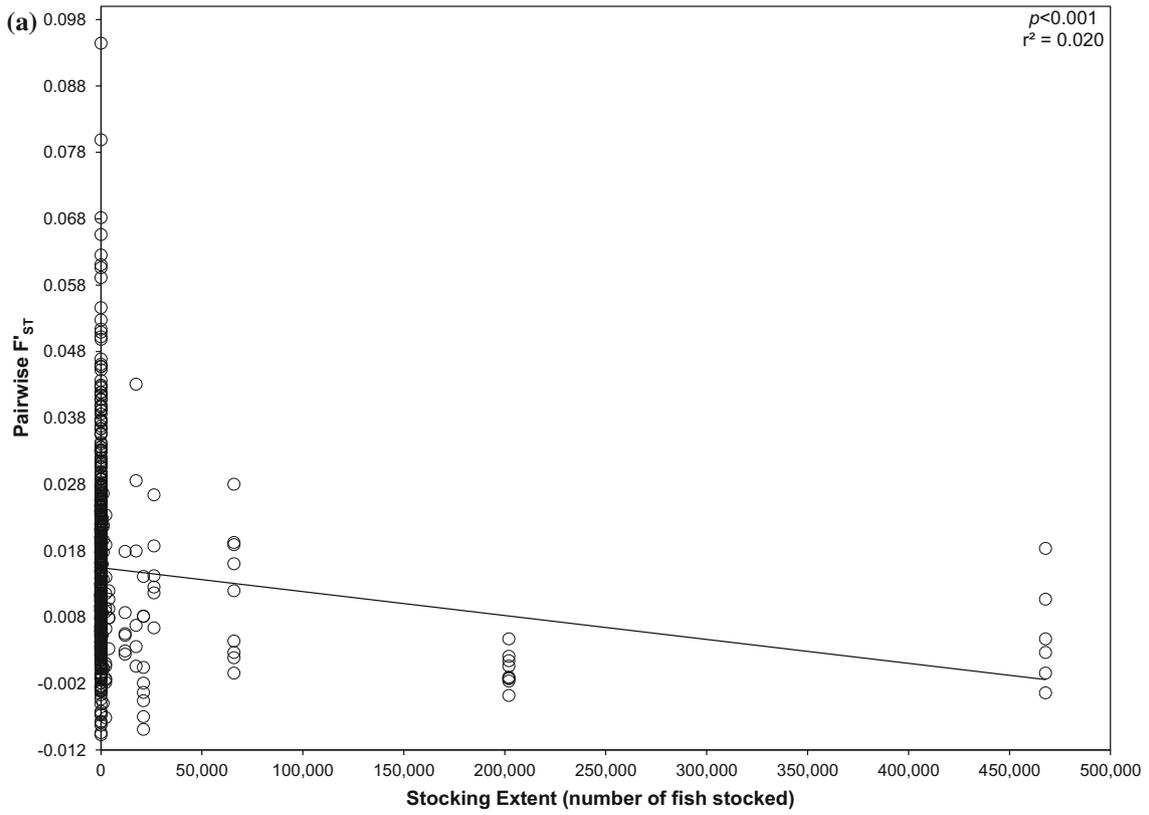
Bayesian clustering analysis revealed the presence of two groups: one comprised of admixed individuals from two stocked locations on the Kennebec River (i.e., Dresden Mills and Sewell Pond), and the other comprised of all remaining populations. This result was unexpected, especially since Dresden Mills and Sewell Pond have been stocked (Supplemental Table S1). Studies investigating stocking effects on genetic variation among fish populations have indicated a correlation between stocking intensity and duration with a

Fig. 3 a Isolation by distance for stocked and non-stocked locations, where IBD is highly significant for stocked locations ($p < 0.001$, $r^2 = 0.277$), excluding Dresden Mills Dam and Sewell Pond, and for non-stocked locations ($p < 0.914$, $r^2 = 0.000$). **b** Residual plot of stocked and non-stocked locations from IBD relationship



decrease in genetic differentiation (Eldridge and Naish 2007; Marie et al. 2010; Aunins et al. 2014; Valiquette et al. 2014). Yet, genic tests (Table S4), pairwise genetic differentiation (Supplemental Table S5), NJ tree topology (Fig. 2) and PCoA (Supplemental Fig. S1) each suggest that these two locations are unusually divergent from the remaining populations. Further, these two populations appear to spawn earlier than other alewife populations in the region (T. Willis, unpublished data.), suggesting that perhaps

differences in spawning time driven by local adaptation and/or different timing of environmental cues at these sites may sustain reproductive isolation and prevent introgression. Similar observations have been seen for other anadromous fish species (e.g., *Oncorhynchus kisutch*, *Salmo salar* L., and *S. trutta*) where native populations have been shown to resist genetic swamping by large releases of fish from outside their drainage (Nielson et al. 2001; Hansen 2002; Eldridge et al. 2009).



◀ **Fig. 4** Linear regression analyses identified a highly significant ($p < 0.001$) correlation between genetic differentiation and (a) stocking extent; (b) years stocked; (c) stocking events. A general decline in genetic variation among collection pairs was identified when stocking activities occurred and when intensity or duration increased

significant relationship between the extent and duration of inter-basin stocking activities and the decline of genetic differentiation among populations. These results suggest that inter-basin stocking has influenced the genetic structure of alewife populations in Maine. Similar observations

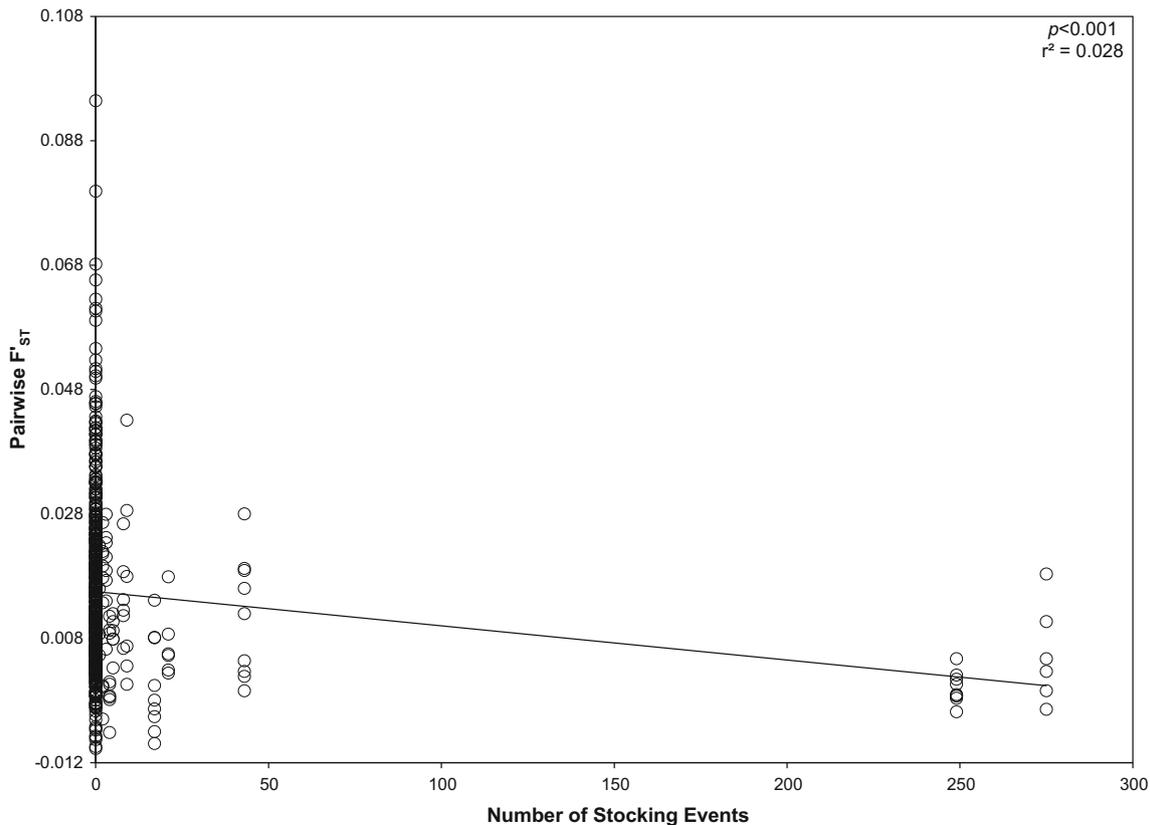


Fig. 4 continued

Hierarchical AMOVA detected a highly significant, albeit small, amount of genetic variation among years within locations (Table S3); we observed a number of significant genic tests among collections within populations, suggesting temporal instability of allele frequencies within populations. This result could be attributed to drift associated with small population sizes, sampling effects, and/or influences from stocking. These results contrast with Hasselman et al. (2010) where temporally stable allele frequency distributions were found for American shad populations from the Canadian portion of the species' range where inter-basin stocking has not occurred.

Linear regressions revealed that inter-basin stocking activities (i.e., stocked or not, number of years stocked, number of fish stocked, number of stocking events) were correlated with reductions in genetic variation among anadromous alewife populations in Maine. There was a

of long-term stocking effects and intensity have been seen in other species (e.g., brook charr (*Salvelinus fontinalis*), Marie et al. 2010; Arctic charr (*Salvelinus alpinus*), Brunner et al. 1998; Coho salmon (*O. kisutch*), Eldridge and Naish 2007; American shad, Hasselman and Limburg 2012; Aunins et al. 2014; lake trout (*Salvelinus namaycush*), Valiquette et al. 2014).

IBD analysis revealed a significant relationship between geographic distance and genetic differentiation, consistent with the pattern observed among alewife populations within the Gulf of St. Lawrence (McBride et al. 2014). These results suggest that inter-basin stocking has not entirely eliminated the signal of natural dispersal among populations. Palkovacs et al. (2014) found significant IBD for alewife populations across their US range, but non-significant differentiation within some areas such as Long Island Sound (see also Palkovacs et al. 2008) and Albemarle Sound.

We found a highly significant relationship between geographic distance and genetic differentiation among non-stocked populations, consistent with studies of alosines in regions not subject to stocking activities (Hasselman et al. 2010; McBride et al. 2014). This result suggests that non-stocked alewife populations in Maine are exchanging migrants at a rate inversely proportional to their geographic distance (Wright 1943). IBD analysis of stocked populations revealed a non-significant relationship and analysis of the IBD residuals revealed a general decline in residual values, especially for those population pairs greater than 120 km apart. This finding suggests that human-mediated migration has influenced the genetic relationships among alewife populations relative to what would be expected based on geographic distance alone (Laike et al. 2010; Pearse et al. 2011; Hasselman and Limburg 2012).

Conservation and management implications

Multiple anthropogenic stressors such as overfishing, habitat loss and habitat degradation have resulted in historic low abundances for anadromous alewife populations (Hightower et al. 1996; Limburg and Waldman 2009). In response to these declines, alewife has been identified as a high priority species for conservation and recovery (ASMFC 2012). Restoration efforts have included harvest restrictions, dam removal and fish passage projects, stream habitat restoration, and stock transfers. Unfortunately, pre-supplementation samples were unavailable, thereby precluding direct estimates of the genetic effects of inter-basin stocking on spatial patterns of genetic variation within Maine. Nonetheless, our study of contemporary populations, which takes into account differing histories of stocking and stocking intensity, does provide strong evidence that inter-basin stocking has influenced patterns of genetic variation among alewife populations in Maine. These effects have implications for planning alewife conservation measures. Specifically, genetic homogenization may negatively impact local adaptation and hinder the use of genetic stock identification to assign alewife bycatch to river of origin. Inter-basin stocking may thereby negatively impact broad-scale conservation goals (ASMFC 2012).

Several recent studies underscore the potential effects of stocking on anadromous salmonids. Pearse et al. (2011) showed that the historically strong relationship between genetic differentiation and geographic distance in California populations of steelhead trout was significantly reduced as a direct effect of stocking. Lamaze et al. (2013) demonstrated that stocking brook charr has impacted the expression of genes that have important biological functions which may be related to fitness, thereby compromising the longevity and adaptability of populations. Valiquette et al. (2014) identified a strong decrease in the

extent of genetic differentiation among stocked populations when compared to non-stocked populations, and found the declining genetic differentiation to be significantly correlated with stocking intensity. In response to this mounting evidence, inter-basin stocking for anadromous salmonids has been largely abandoned as a conservation strategy, although hatcheries currently stock extensively from natal broodstock (Hansen et al. 2000; Eldridge et al. 2009). Despite the appropriate abandonment of inter-basin stocking by anadromous salmon managers, inter-basin stock transfers are increasing being implemented as a restoration tool for anadromous alosines. The persistence of this restoration strategy is perhaps due to longstanding perceptions of naturally high straying rates and short-term increases to spawning adult run sizes (Belding 1920; Rounsefell and Stringer 1945; Havey 1961). We call for a critical re-appraisal of the use of inter-basin stock transfers as a population supplementation strategy for anadromous alosines, including alewife, when an existing spawning run persists. Our results point to important impacts that may hinder long-term population recovery.

Conclusions

It was traditionally believed that river herring strayed extensively and that stock transfers would not affect gene frequencies. However, recent work has demonstrated that alosines exhibit genetic structure on a finer spatial scale than previously thought (Hasselman et al. 2010; Palkovacs et al. 2014; Hasselman et al. 2014; McBride et al. 2014). Thus, stock transfers carry risks to alosine local adaptation, long-term persistence, and evolutionary potential (Frankham 1995; Hasselman and Limburg 2012). This study adds to a growing body of literature that emphasizes the importance of conserving genetic integrity of anadromous populations in the face of stocking activities and restoration strategies designed to optimize spawning run revitalization. Alternative management strategies, such as habitat restoration and fish ladder modifications have proven successful at revitalizing alewife spawning runs in Maine (Crane 2009; Willis 2009; Hall et al. 2011), and pose considerably lower risk to genetic integrity. Given the need for a holistic approach to alewife management that accounts for population viability at all life stages, promoting conservation actions that maintain genetic diversity, (i.e., natural recolonization) may be the most prudent course for future alewife restoration efforts.

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References

- A'Hara SW, Amouroux P, Argo EE, Avand-Faghih A, Barat A, Barbieri L, Bert TM (2012) Permanent genetic resources added to molecular ecology resources Database 1 Aug–30 Sep 2011. *Mol Ecol Resour* 12:185–189
- Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 318:100–103
- ASMFC (Atlantic States Marine Fisheries Commission) (2012) River herring benchmark stock assessment. Vol 1. Stock Assessment Report No. 12-02 of the Atlantic States Marine Fisheries Commission, Washington, DC
- Atkins CG (1887) The river fisheries of Maine. In: C.G. Goode (ed) *The Fisheries and Fishery Industries of the United States*, vol. 1, Sect. V. U.S. Govt, pp 673–728
- Aunins AW, Epifanio JM, Brown BL (2014) Genetic evaluation of supplementation-assisted American shad restoration in the James River, Virginia. *Mar Coast Fish* 6:127–141
- Beever EA, Mattson B, Germino M, Post Van der Burg M, Bradford J, Brunson M (2014) Successes and challenges of 11 broad-extent conservation programs, from formation to implementation. *Conserv Biol* 2:302–314
- Belding DL (1920) The preservation of the alewife. *Trans Am Fish Soc* 49:92–104
- Bentzen P, Paterson I (2005) Genetic analyses of freshwater and anadromous alewife (*Alosa pseudoharengus*) populations from the St. Croix River, Maine/New Brunswick. Final Report to Maine Rivers 3
- Berger J, Cain SL, Cheng E, Dratch P, Ellison K, Francis J, Frost HC, Gende S, Groves C, Karesh WA, Leslie E, Machlis G, Medellin RA, Noss RF, Redford KH, Soukup M, Wilcove D, Zack S (2014) Optimism and challenge for science-based conservation of migratory species in and out of U.S. National Parks. *Conserv Pract Policy* 28:1–9
- Brunner PC, Douglas MR, Bernatchez L (1998) Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr (*Salvelinus alpinus*) (Teleostei: Salmonidae) from central Alpine lakes. *Mol Ecol* 7:209–223
- Carlson SM, Quinn TP, Hendry AP (2011) Eco-evolutionary dynamics in Pacific salmon. *Heredity* 3:438–447
- Crane J (2009) Setting the river free: the removal of the Edwards dam and restoration of the Kennebec river. *Water Hist* 1:131–148
- Eldridge WH, Naish KA (2007) Long-term effects of translocation and release numbers on fine-scale populations structure among coho salmon (*Oncorhynchus mykiss*). *Mol Ecol* 16:2407–2421
- Eldridge WH, Myers JM, Naish KA (2009) Long-term changes in the fine-scale population structure of coho salmon populations (*Oncorhynchus kisutch*) subject to extensive supportive breeding. *Heredity* 103:299–309
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587
- Frankham R (1995) Conservation genetics. *Annu Rev Genet* 29:305–327
- Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *J Hered* 86:485–486
- Hall CJ, Jordaan A, Frisk MG (2011) The historic influence of dams on diadromous fish habitat with a focus on river herring and hydrologic longitudinal connectivity. *Landsc Ecol* 26:95–107
- Hall CJ, Jordaan A, Frisk MG (2012) Centuries of anadromous forage fish loss: consequences for ecosystem connectivity and productivity. *Bioscience* 62:723–731
- Hansen MM (2002) Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. *Mol Ecol* 11:1003–1015
- Hansen MM, Nielson EE, Ruzzante DE, Bouza C, Mensbery KLD (2000) Genetic monitoring of supportive breeding in brown trout (*Salmo trutta* L.), using microsatellite DNA markers. *Can J Fish Aquat Sci* 57:2130–2139
- Hasselman DJ, Limburg KE (2012) Alosine restoration in the twenty first century: challenging the status quo. *Mar Coast Fish* 4:174–187
- Hasselman DJ, Bradford RG, Bentzen P (2010) Taking stock: defining populations of American shad (*Alosa sapidissima*) in Canada using neutral genetic markers. *Can J Fish Aquat Sci* 67:1021–1039
- Hasselman DJ, Ricard D, Bentzen P (2013) Genetic diversity and differentiation in a wide ranging anadromous fish, American shad (*Alosa sapidissima*), is correlated with latitude. *Mol Ecol* 22(6):1558–1573
- Hasselman DJ, Argo EE, McBride MC, Bentzen P, Schultz TF, Perez-Umphrey AA, Palkovacs EP (2014) Human disturbances causes the formation of hybrid swarm between two naturally sympatric fish species. *Mol Ecol* 23(5):1137–1152
- Havey KA (1961) Restoration of anadromous alewives at Long Pond, Maine. *Trans Am Fish Soc* 90:281–286
- Hightower JE, Wicker AM, Endres KM (1996) Historical trends in abundance of American shad and river herring in Albermarle Sound, North Carolina. *N Am J Fish Manag* 16:257–271
- Hilborn R, Quinn TP, Schindler DE, Rogers DE (2003) Biocomplexity and fisheries sustainability. *Proc Natl Acad Sci* 100:6564–6568
- Hindar K, Jonsson B, Ryman N, Staahl G (1991) Genetic relationships among landlocked, resident, and anadromous brown trout, *Salmo trutta* L. *Heredity* 66:83–91
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70
- Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801–1806
- Jones O, Wang J (2009) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol Ecol Resour* 10:551–555
- Julian SE, Bartron ML (2007) Microsatellite DNA markers for American shad (*Alosa sapidissima*) and cross-species amplification within the family Clupeidae. *Mol Ecol Notes* 7:805–807
- Labbe EM (2012) Influence of stocking history and geography on the population genetics of alewife (*Alosa pseudoharengus*) in Maine rivers. MSc thesis. University of Southern Maine
- Laike L, Schwartz MK, Waples RS, Ryman N, The GeM Working Group (2010) Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. *Trends Ecol Evol* 25:520–529

- Lamaze FC, Garant D, Bernatchez L (2013) Stocking impacts the expression of candidate genes and physiological condition in introgressed brook charr (*Salvelinus fontinalis*) populations. *Evol Appl* 6:393–407
- Latch EK, Dharmarajan G, Glaubitz JC, Rhodes OE Jr. (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv Genet* 7:295–302
- Lauber TB, Stedman RC, Decker DJ, Knuth BA (2011) Linking knowledge to action in collaborative conservation. *Conserv Biol* 6:1186–1194
- Leberg P (2008) Estimating allelic richness: effects of sample size and bottlenecks. *Mol Ecol* 11:2445–2449
- Limburg KE, Waldman JR (2009) Dramatic declines in North Atlantic diadromous fishes. *Bioscience* 59:955–965
- López-Hoffman L, Varady RG, Flessa kw, Balvanera P (2010) Ecosystem services across borders: a framework for transboundary conservation policy. *Front Ecol Environ* 8:84–91
- Lynch M, O’Hely M (2001) Captive breeding and the genetic fitness of natural populations. *Conserv Genet* 2:363–378
- Marie AD, Bernatchez L, Garant D (2010) Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*). *Mol Ecol* 19:2025–2037
- McBride MC (2013) Population structure of river herring (Alewife, *Alosa pseudoharengus*, and Blueback herring, *Alosa aestivalis*) examined using neutral genetic markers. MSc thesis. Dalhousie University
- McBride MC, Willis TV, Bradford RG, Bentzen P (2014) Genetic diversity and structure of two hybridizing anadromous fishes (*Alosa pseudoharengus*, *Alosa aestivalis*) across the northern portion of their ranges. *Conserv Genet* 15:1281–1298
- MDEP (Maine Department of Environmental Protection) (2009) January 1, 2009 status reports: hydropower projects in maine, DEPLW0363-2009, and Dam removals in Maine and dams subject to regulated minimum flow releases
- MDMR (2009) Operational plan for the restoration of diadromous fishes to the Penobscot River, Maine Department of Marine Resources
- MDMR (2010) Species information: Maine River herring. Retrieved July 2010. <http://www.maine.gov/dmr/searunfish/alewife/index.htm>
- Messieh SN (1977) Population structure and biology of alewives (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) in the Saint John river, New Brunswick. *Environ Biol Fishes* 2:195–210
- Moritz CC, Potter S (2013) The importance of an evolutionary perspective in conservation policy planning. *Mol Ecol* 24:5969–5971
- Neff BD, Garner SR, Pitcher TE (2011) Conservation and enhancement of wild fish populations: preserving genetic quality versus genetic diversity. *Can J Fish Aquat Sci* 68:1139–1154
- Nei M, Tajima F, Tatenos Y (1983) Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J Mol Evol* 19:153–170
- Nielson EE, Hansen MM, Batch LA (2001) Looking for a needle in a haystack: discovery of indigenous Atlantic salmon (*Salmo salar* L.) in stocked populations. *Conserv Genet* 2:219–232
- Oksanen JF, Blinchet FG, Kindt R, Legendre P, Minchin PR, O’Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2013) Package ‘vegan’. <http://cran.r-project.org>, <http://vegan-forge.r-project.org/>. Accessed June 2014
- Palkovacs EP, Dion KB, Post DM, Caccione A (2008) Independent evolutionary origins of landlocked Alewife populations and rapid parallel evolution of phenotypic traits. *Mol Ecol* 17:582–597
- Palkovacs EP, Hasselman DJ, Argo EE, Gephard SR, Limburg KE, Post DM, Schultz TF, Willis TV (2014) Combining genetic and demographic information to prioritize conservation efforts for anadromous alewife and blueback herring. *Evol Appl* 7:212–226
- Pearse DE, Martinez E, Garza JC (2011) Disruption of historical patterns of isolation by distance in coastal steelhead. *Conserv Genet* 12:691–700
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Quinn TP, Kinnison MT, Unwin MJ (2001) Evolution of Chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: patterns, rate, and process. *Genetica* 112–113:493–513
- R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>. Accessed June 2014
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Mol Ecol Notes* 4:137–138
- Rounsefell G, Stringer L (1945) Restoration and management of the New England alewife fisheries with special reference to Maine. *Trans Am Fish Soc* 73:394–424
- Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* 145:1219–1228
- Rousset F (2008) GENEPOP’007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Mol Ecol Resour* 8:103–106
- Schindler DE, Schneuerell MD, Moore JW, Gende SM, Francis TB, Palen WJ (2003) Pacific salmon and the ecology of coastal ecosystems. *Front Ecol Environ* 1:31–37
- Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. *Nature* 465:609–612
- Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. *Bull Fish Res Board Can* 184:1–966
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution* 39:53–65
- Takezaki N, Nei M, Tamura K (2010) POPTREE2: software for constructing population trees from allele frequency data and computing other populations statistics with windows Interface. *Mol Biol Evol* 27:747–752
- Vähä JP, Erkinaro J, Niemela E, Primmer CR (2007) Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Mol Ecol* 16:2638–2654
- Valiquette E, Perrier C, Thibault I, Bernatchez L (2014) Loss of genetic integrity in wild lake trout populations following stocking: insights from an exhaustive study of 72 lakes from Quebec, Canada. *Evol Appl* 7:625–644
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Ward RD (2006) The importance of identifying spatial population structure in restocking and stock enhancement programmes. *Fish Res* 80:9–18
- Waters J, Epifanio J, Gunter T, Brown B (2000) Homing behaviour facilitates subtle genetic differentiation among river populations of *Alosa sapidissima*: microsatellites and mtDNA. *J Fish Biol* 5:622–636
- Weir B, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370

- Wilcove DS, Wikelski M (2008) Going, going, gone: is animal migration disappearing? PLoS Biol 6:e188
- Willis TV (2009) How policy, politics, and science shaped a 25-year conflict over alewife in the St. Croix river, New Brunswick Maine. In: Haro A, Smith KL, Rulifson RA, Moffitt CM, Klauda RJ, Dadswell MJ, Cunjak RA, Cooper JE, Beal KL, Avery TS (eds) Challenges for Diadromous fishes in a dynamic global environment. American Fisheries Society, Bethesda, pp 739–811
- Wright S (1943) Isolation by distance. Genetics 28:114–138