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Genetic structure of *Fundulus heteroclitus* from PAH-contaminated and neighboring sites in the Elizabeth and York Rivers

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Abstract

Population genetic characteristics of mummichog, *Fundulus heteroclitus*, from the heavily industrialized Elizabeth River and nearby York River (Virginia USA) were assessed relative to sediment PAH concentrations. Allozyme genotype frequencies for all loci were consistent with random mating expectations at each locality and age class. Fish from all sites had comparable levels of enzyme polymorphism and heterozygosity regardless of the associated sediment PAH concentrations. Allozyme frequencies for 12 of 15 loci were homogeneous for mummichog from all localities except that allozyme frequencies were significantly different for the *Idh-2* locus of (adult and juvenile) mummichog at the heavily-contaminated Atlantic Wood site relative to all other sites. Additionally, allele frequency differences were noted for *Ldh-C* and *Gpi-1* among juvenile mummichog. Values for F_{st} were 0.0254 and 0.0141 in the juvenile and adult samples, respectively, indicating greater among-locality genetic differentiation for juvenile mummichog than for adults. Juvenile mummichog are more likely to remain in their natal area while adult samples reflect movement of fish during two or more winter seasons. Correlation analysis suggested that genetic differentiation was not correlated with geographic distance at the spatial scale studied here; however, there was a significant correlation between genetic distance and differences among sites in organic carbon-normalized PAH concentrations. Mummichog collected at the heavily PAH-contaminated AW locality were genetically distinct from those at neighboring sites.

Keywords: *Fundulus heteroclitus*; Fish; Population Genetics; PAH; Allozymes

1. Introduction

The Elizabeth River Estuary in Virginia, USA has a long history of physical and chemical perturbation. Dredging, industrialization, and urbanization led to significant fragmentation of habitat.

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High and spatially heterogeneous concentrations of polycyclic aromatic hydrocarbons (PAH), tributyltin (TBT), metals, and other toxicants have been reported from water, sediments, and organisms (Bieri et al., 1986; USEPA, 1999). For example, PAH concentrations of sediments from a superfund site adjacent to the former Atlantic Wood Industries (AW) wood treatment plant are as much as 2700 times higher than those of other sites along the Elizabeth River.

Mummichogs (*Fundulus heteroclitus*) are abundant in shallow marshes throughout the Elizabeth River. Discrete patches of suitable habitat are separated by unsuitable areas of shipyards and concrete debris along highly-modified reaches of the Elizabeth River such as the Southern Branch. Some contaminants reported in Elizabeth River sediments are directly toxic to mummichog at high concentrations (Williams, 1994) and chronically stressful at lower concentrations (Vogelbein et al., 1990; Huggett et al., 1992). Yet, mummichogs are consistently present despite the very high concentrations of PAHs in AW sediments.

Mummichogs are reported to exhibit strong site fidelity (Lotrich, 1975; Meredith and Lotrich, 1979). Summer home ranges in Delaware salt marshes were approximately 36 m along a creek side and few fish were observed to cross to the opposite bank (Lotrich, 1975). Although long-distance movement occurs occasionally (Lotrich reported recapture of fish 375 m from release), local mummichog can behave as semi-isolated subpopulations. Mummichog inhabiting heavily contaminated areas likely spend all or most of their lives exposed to potential stressors.

Isolated subpopulations, especially if small, can experience genetic drift and inbreeding. Population genetic theory predicts that, on average, such populations would exhibit a loss of genetic variation and an increase in homozygosity relative to larger non-fragmented populations. This prediction would be especially important in polluted environments if contaminant exposure increased mortality and reduced population size. Relative isolation of organisms in discrete habitat patches might also enhance acquisition of tolerance to local contaminant conditions. Thus, genetic variation might be further reduced for organisms in heavily contaminated habitat patches and differentiation of fish among habitat patches would be enhanced.

Exposure of mummichog to high PAH concentrations has been associated with an increased frequency of liver lesions and cancers (Vogelbein et al., 1990). Williams (1994) reported that mummichog from the AW location tolerated levels of PAHs that resulted in 100% mortality of fish from a reference locality on the nearby York River. Additionally, mummichog embryos from the AW site have a lower incidence of cardiac abnormalities than mummichog from other Elizabeth River and York River localities if exposed to AW sediments (Ownby et al., 2002). Taken together, these observations indicate that AW mummichog possess genetically-based enhanced tolerance to PAHs relative to fish from reference localities.

Several hypotheses may be forwarded to account for the persistence of mummichog in heavily-contaminated habitat. First, PAH exposure might not adversely affect mummichog. This seems unlikely. Second, the high levels of PAHs are harmful to fish and mortality of AW mummichog is high; however, fish are replenished through immigration from elsewhere in the estuary. The AW site acts as a sink in a metapopulation. A third possibility is that AW fish might be a locally stable subpopulation that is tolerant of high sediment PAH concentrations and immigration from neighboring subpopulations might be uncommon.

To discriminate among these hypotheses, we initially studied the microgeographic population genetic structure in mummichog along the highly-modified Southern Branch of the Elizabeth River. Mummichog from nine sites ranging from heavily contaminated to less contaminated were subjected to protein electrophoresis to assess the impact of stochastic processes, such as gene flow and drift, as well as the possibility of toxicant-associated selection. Allozymes are highly polymorphic and informative for mummichog in this region (Cashon et al., 1981). Allozymes should act as markers of population genetic structure and processes underlying it. None of the isozymes studied is explicitly related to contaminant tolerance although frequencies of allozyme genotypes (*Gpi-A*,

Ldh-B, *Idh*) have been associated with environmental variables and performance differences among allozyme genotypes have been described.

Two years after the survey of the Southern Branch of the Elizabeth River, the scale of this study was expanded to include a second branch of the Elizabeth River and sites in the adjacent York River estuary. The intent of this expansion was to determine whether the findings could be extended to include more geographically distant populations and populations with more extreme contaminant differences. Also, defining the genetic qualities of York River mummichog relative to Elizabeth River mummichog populations was important because past and ongoing studies compare with AW mummichog qualities to those of York River watershed reference populations.

Specifically, the following questions were addressed using the allozyme data. First, does the level of contamination influence the genetic structure of mummichog populations within a landscape mosaic of sites varying in sediment PAH concentrations? Genetic distance among mummichog would be determined only by geographical distance if differentiation among mummichog were unrelated to contamination. If high contaminant concentrations lead to high mortality in polluted habitat and mummichog were migrating into these areas from neighboring areas, we may expect high estimates of gene flow and little genetic differentiation among mummichog from different localities. Alternately, if mummichog exhibit strong site fidelity and minimal migration among patches, migration estimates would be low and mummichog from localities might be genetically differentiated. Second, does genetic variability decrease with increasing levels of PAH contamination? Third, is there evidence suggesting that potential or existing reference sites near the York River are genetically distinct from Elizabeth River sites? An affirmative answer to this last question would suggest that reference sites for studying AW mummichogs should be selected from within the Elizabeth River, not from the York River watershed.

2. Methods

2.1. Fish collection

Mummichogs were collected initially in October

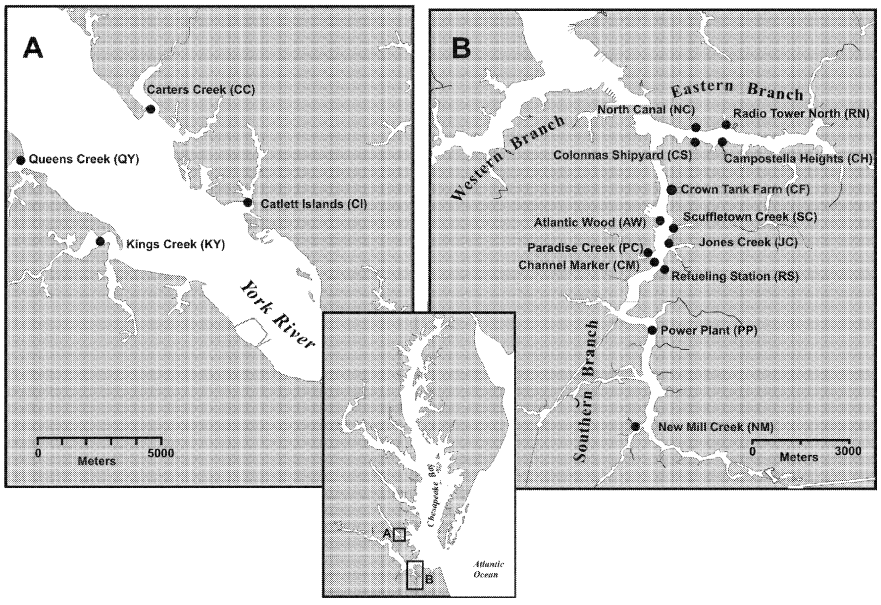

Fig. 1. Collection sites for mummichog on the Elizabeth and York Rivers. Panel A, York River and Panel B, Elizabeth River.

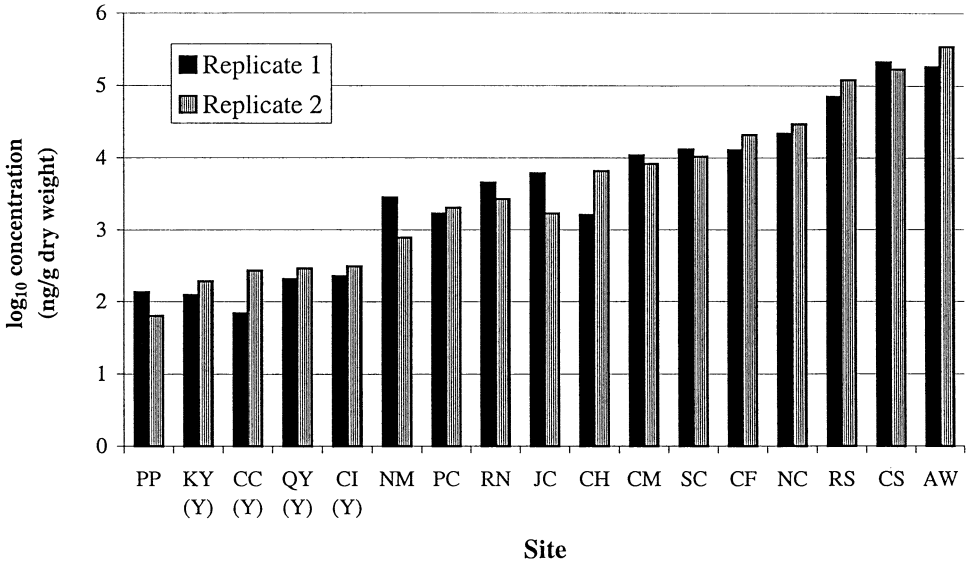

Fig. 2. PAH concentrations (ng/g dry weight) measured in duplicate sediment samples at each site. York River sites are identified with a (Y) below their site abbreviation.

1998 from the Southern Branch of the Elizabeth River (Fig. 1). Sites were chosen along a 12 km reach of the river. Each site was a discrete habitat patch separated from the others by industrial areas unsuitable for mummichog. Sample localities differed markedly in sediment PAH contamination (Fig. 2). Two sites (Atlantic Wood and Refueling Station, RS) were chosen specifically because of their proximity to historical sources of PAH. Several others were selected from nearby locations having notionally lower concentrations of PAHs. Approximately 30 juveniles (mean = 1.5 g wet weight, standard deviation (S.D.) = 0.6 g, $n = 286$) and 60 adults (mean = 7.9 g wet weight, S.D. = 2.5 g, $n = 537$) were collected with baited minnow traps for analysis. Equal numbers of male and female fish were sampled.

Additional mummichog populations were sampled in October 2000 from the Eastern Branch of the Elizabeth River and from the adjacent and relatively clean York River (Fig. 1). One Eastern Branch site (Colonnas Shipyard, CS) was selected because of the high sediment PAH concentrations reported earlier for that site. The York River watershed populations represented reference populations used in past studies of PAH exposure consequences to AW mummichog, e.g. Vogelbein et al. (1990), Williams (1994), Armknecht et al. (1998). Sixty adult fish (mean = 6.6 g wet weight, S.D. = 3.3 g, $n = 480$), but no juveniles, were collected from these eight additional sites. The primary intent of this expanded collection was to increase the number and range of populations relative to geographical distance and PAH concentrations. A secondary intent was to generate background genetic information with which to assess the appropriateness of using York River reference populations in studies of Elizabeth River mummichog populations.

2.2. Sediment collection

Sediments were collected from all 17 stations in the Elizabeth and York Rivers during the periods of fish sampling. At each station, three surface grabs were taken from *F. heteroclitus* habitat with a stainless steel Ponar grab and were then homogenized in a stainless steel bucket to produce a composite sample. Duplicate composite samples

were collected at each location and analyzed for contaminants. Sediment samples for organic compound analyses were placed in cleaned glass jars with teflon-lined lids, placed on ice in the field, and stored frozen until analyzed.

2.3. Analytical methods

Sediment samples were analyzed for organic contaminants using the protocol described by Greaves et al. (1991). Briefly, sediments were freeze-dried, spiked with surrogate standards, and extracted with dichloromethane by accelerated solvent extraction. The resulting extracts were fractionated by sequential gel permeation and silica gel chromatographies, and analyzed for aromatic or heterocyclic compounds by capillary gas chromatography with flame ionization detection and gas chromatography-mass spectrometry in the full scan electron ionization mode. Blanks, duplicates, and standard reference materials were analyzed in tandem with the samples. Polycyclic aromatic hydrocarbons were summed as per the appendix of Horness et al. (1998).

2.4. Protein electrophoresis

Eyes and caudal muscle tissue were taken for isozyme analysis and stored at -70 °C. Tissues were prepared for electrophoresis by grinding in approximately 250 μl of deionized water and centrifuging at 14 000 rpm for 45 s. Supernatant fluid was absorbed onto filter paper wicks and inserted into 12% (w/v) horizontal starch gels. Fourteen enzyme loci and one general protein were assayed. Tris-citrate pH 8.0 gels and buffer (Selander et al., 1971) were used to resolve lactate dehydrogenase (*Ldh-A*, *-B*, and *-C*), glucosephosphate isomerase (*Gpi-1*, *-2*), isocitrate dehydrogeanse (*Idh-1*, *-2*) using eye tissue, and malate dehydrogenase (Mdh-1, -2), NADP-dependent malate dehydrogeanse (*Me-1*), mannose phosphate isomerase (*Mpi*), and a non-specific protein (*Gp*) using muscle tissue. The lithium hydroxide gel-buffer of Selander et al. (1971) was used for phosphoglucomutase (*Pgm*) and aspartate aminotransferase (*Aat*). The Tris citrate pH 6.9 buffer (diluted 1:10 for buffer trays and 1:20 for gels) was used to resolve 6-phosphogluconate dehydrogenase (*Pgd*). After overnight (16 h) electrophoresis, gels were sliced and stained following standard methods (Hillis et al., 1996; Selander et al., 1971).

2.5. Data analysis

Isozyme data were analyzed using BIOSYS-2 (Swofford and Selander, 1981) to test for fit of the observed data to random mating expectations, determine homogeneity of allele frequencies using contingency χ^2 statistics, and evaluate divergence among populations using genetic distance (Nei, 1972) and *F*-statistics (Wright, 1978). The χ^2 tests were done only for cases where expected numbers of observations were greater than five and Bonferroni adjustments were made to maintain an experimentwise α of 0.05 if several statistical tests were done. Fish migration among Southern Branch sites was estimated from F_{st} values for juvenile and adult samples. Because of small sample sizes for individuals with many heterozygous loci, individual heterozygosity was grouped into the following categories for analysis: 0, 1, 2, 3, and >3. A three-way Mantel test (Smouse et al., 1986) was done with ARLEQUIN software (Schneider et al. 2000) using genetic distance, geographic distance, and contaminant distance between pairs of sites. Geographic distance was estimated 'over water' using nautical chart software (Capn 45). Contaminant concentration differences were square roots of the absolute differences in total sediment PAH concentration between sites. Subsequent correlation analysis of genetic distance and contaminant concentration difference were done using the PROC CORR procedure of the SAS statistical package (SAS Institute Inc., 1989) and PAH concentrations normalized to organic carbon in sediments.

3. Results

3.1. PAH in Elizabeth River sediments

Analysis of the non-polar aromatic fraction showed a predominance of unsubstituted and substituted PAHs. Total PAH concentrations ranged

from a low of 99 ng/g dry weight at the PP site to a high of 264 114 ng/g dry weight at the AW site. The PAH concentrations were arranged in Fig. 2 from the lowest to the highest to illustrate the wide gradient in PAH contaminations represented by the sites. Also shown in this figure, duplicate samples from each site showed low variability relative to that across sites.

3.2. Population genetics

Allozyme frequencies for the 15 loci for juvenile and adult mummichog from the Elizabeth River Southern Branch sampling are provided in Table 1. The χ^2 tests for all 17 sites indicate that genotypic distributions were consistent with random mating expectations for fish from any site and, in the case of the sites on the Southern Branch of the Elizabeth River, any age. There were no significant differences in allozyme frequencies between juvenile and adult mummichog at any Elizabeth River Southern Branch sites. Significant heterogeneity in allozyme frequencies for the Southern Branch of the Elizabeth River sites were observed for three of the 15 loci examined. The χ^2 tests for homogeneity of allozyme frequencies among juvenile mummichog from the Elizabeth River Southern Branch indicated significant differences at the *Ldh-B*, *Gpi-1*, and *Idh-2* loci (all $P < 0.01$). For Southern Branch adults, there were significant differences in the frequencies of *Idh-2* alleles among the sites ($P < 0.00003$) but there was no simple relationship between allozyme frequency and sediment PAH contamination of sites. The frequency of the *Idh-2* allozymes was consistently different for AW mummichog (Fig. 3). Both juvenile and adult fish from AW had a significantly lower frequency of the common *Idh-2* allozyme than fish from other sites along the Elizabeth River. Consistent with these results for the Southern Branch adult mummichog, χ^2 tests for homogeneity of allozyme frequencies indicated significantly lower frequency of the common *Idh-2* allele at AW relative to all 16 sites ($P < 0.001$).

There were no significant differences among localities in any measures of overall genetic variability (Table 2). The mean number of alleles per locus was comparable for juveniles and adults for the Southern Branch of the Elizabeth River sites. Mean heterozygosity was comparable among adult fish for all 17 sites. Genetic variability was not different between highly contaminated (e.g. AW, CS and RS) and less contaminated sites (Table 2).

The three-way Mantel tests indicated a positive (0.407), but statistically nonsignificant ($\alpha = 0.05$), correlation between the difference in sediment PAH contamination and genetic distance between populations of the nine Southern Branch sites. Very low and statistically nonsignificant correlation coefficients indicated no relationship existed between geographic distance and genetic distance for fish along the Southern Branch, nor for fish sampled at the wider geographical scale. Because geographical distance was not correlated with genetic distance for these sites, more focused correlation analyses were done for genetic distance versus differences in PAH concentrations with PAH concentrations being normalized to sediment organic carbon content (Fig. 4). These analyses were done with and without the AW site to assess if a correlation was present even in the absence of this extreme site. There was a highly significant correlation (Kendall τ correlation coefficient $= 0.302$, $P < 0.001$) in analyses of all 17 sites. After omitting the AW site, the correlation coefficient was lower (0.177) for the remaining 16 sites but still statistically significant ($P = 0.009$).

Wright's F_{st} value (Wright, 1978), a measure of genetic differentiation, was 0.025 for juvenile mummichog and 0.014 for adult fish from the Elizabeth River Southern Branch samples. These values were significantly different from zero (Workman and Niswander, 1970), indicating genetic structure among the Southern Branch sampling sites. The larger F_{st} value for the juvenile samples suggested that migration among sites is more likely for fish of the older age classes. Wright's F_{is} values ($F_{is} = 0.017$ and 0.011 for juveniles and adults, respectively) indicated greater relatedness for the juveniles within sites relative to that observed for the adults.

Variance component analysis of the genetic data for all 17 sites indicated that 99.3% of the total genetic variation was associated with variation within a sample site, 0.5% was associated with variation among sites within the river, and 0.2% was variation between the two rivers.

Table 1
Allozyme frequencies of mummichog from York and Elizabeth River sites

River	Site	Aat						Gp			Gpi-1						Gpi-2		
		1	2	3	4	5	7	2	3	4	1	2	3	4	5	9	2	3	4
York	Kings Creek (KY)		0.42	0.58					0.99	0.08	0.01	0.23	0.75	0.01			0.01	0.99	
	Carters Creek (CC)		0.44	0.56					1.00			0.21	0.78	0.01			0.01	0.99	
	Queens Creek (QY)	0.01	0.37	0.59	0.03				1.00			0.25	0.75				0.01	0.98	0.01
	Catlett Islands (CI)		0.42	0.57	0.01				0.99	0.01	0.01	0.16	0.83					1.00	
Eastern Elizabeth	Radio Tower North (RN)		0.48	0.50	0.02				0.98	0.02		0.23	0.78					1.00	
	Campostella Heights (CH)		0.49	0.51					0.98	0.03		0.18	0.81	0.01		0.01		1.00	
	North Canal (NC)		0.48	0.52			0.01		1.00			0.23	0.78					1.00	
	Colonnas Shipyard (CS)		0.46	0.53	0.02				1.00		0.01	0.13	0.86					1.00	
South Elizabeth	Power Plant (PP)		0.42	0.59					1.00			0.28	0.72					1.00	
			(0.45)	(0.55)				(0.01)	(0.99)			(0.20)	(0.80)				(0.02)	(0.98)	
	New Mill Creek (NM)		0.49	0.51					1.00			0.37	0.63					0.99	0.01
			(0.37)	(0.63)					(1.00)			(0.30)	(0.70)					(1.00)	
	Paradise Creek (PC)		0.33	0.67					1.00			0.30	0.70					1.00	
			(0.36)	(0.64)				(0.02)	(0.98)			(0.17)	(0.83)					(1.00)	
	Jones Creek (JC)		0.42	0.58	0.01				1.00			0.30	0.68	0.01	0.01			0.99	0.01
			(0.42)	(0.58)					(1.00)			(0.20)	(0.80)					(0.97)	(0.03)
	Channel Marker 2 (CM)		0.38	0.62					1.00			0.30	0.70					1.00	
			(0.41)	(0.59)					(1.00)			(0.23)	(0.77)					(1.00)	
	Scuffletown Creek (SC)		0.51	0.48		0.01			1.00			0.27	0.73					1.00	
			(0.35)	(0.65)		(0.00)			(1.00)			(0.29)	(0.71)					(1.00)	
	Crown Tank Farm (CF)		0.38	0.63					1.00			0.24	0.76					1.00	
			(0.48)	(0.52)					(1.00)			(0.38)	(0.62)					(1.00)	
	Refueling Station (RS)		0.37	0.63					1.00			0.19	0.81					1.00	
			(0.50)	(0.50)					(1.00)			(0.18)	(0.82)					(1.00)	
	Atlantic Wood (AW)		0.31	0.69					1.00			0.20	0.80					1.00	
			(0.31)	(0.69)					(1.00)			(0.10)	(0.90)					(1.00)	

		Idh-1				Idh-2						Ldh-A			Ldh-B		Ldh-C		
		2	3	4	8	2	3	4	5	8	9	2	3	4	2	3	2	3	4
York	KY		0.99			0.30	0.73	0.22	0.02	0.02			1.00		0.78	0.23		1.00	
	CC		1.00				0.71	0.28		0.01			0.98	0.02	0.77	0.23	0.01	0.98	0.01
	QY		0.99	0.01		0.01	0.70	0.27		0.03		0.01	0.99		0.80	0.20		0.98	0.02
	CI		1.00				0.70	0.28		0.02			1.00		0.80	0.20		1.00	
East Elizabeth	RN	0.01	0.99				0.78	0.21		0.01			1.00		0.86	0.14		0.99	0.01
	CH	0.01	0.99			0.01	0.83	0.17					1.00			0.80	0.20	1.00	
	NC		1.00				0.85	0.15					1.00			0.77	0.23	1.00	
	CS		1.00				0.78	0.23					1.00			0.84	0.16	1.00	
South Elizabeth	PP	0.02	0.98			0.01	0.79	0.19			0.01		0.99	0.01		0.83	0.17	1.00	
			(1.00)			(0.01)	(0.76)	(0.23)				(0.01)	(0.97)	(0.02)		(0.83)	(0.18)	(1.00)	
	NM		1.00					0.77	0.23				1.00			0.78	0.22	1.00	

Table 1 *(continued)*

		Idh-1				Idh-2						Ldh-A			Ldh-B		Ldh-C		
		2	3	4	8	2	3	4	5	8	9	2	3	4	2	3	2	3	4
			(1.00)				(0.83)	(0.17)					(1.00)		(0.84)	(0.16)		(1.00)	
	PC	0.01	0.99				0.85	0.15					0.98	0.02	0.84	0.16		1.00	
			(1.00)				(0.87)	(0.13)					(1.00)		(0.81)	(0.19)		(1.00)	
	JC	0.01	0.99			0.02	0.67	0.31					1.00		0.85	0.15	0.01	0.98	0.01
		(0.01)	(0.95)	(0.02)	(0.01)	(0.01)	(0.77)	(0.22)					(0.98)	(0.02)	(0.88)	(0.12)		(0.97)	(0.03)
	CM		1.00				0.77	0.23					0.99	0.01	0.81	0.19		1.00	
			(1.00)				(0.73)	(0.27)					(0.98)	(0.02)	(0.73)	(0.27)		(0.97)	(0.03)
	SC		1.00				0.86	0.14					0.99	0.01	0.88	0.12		1.00	
			(1.00)				(0.80)	(0.20)					(1.00)		(0.86)	(0.14)		(0.98)	(0.02)
	CF	0.01	0.98	0.02			0.78	0.22					1.00		0.85	0.15		1.00	
			(0.98)	(0.02)			(0.78)	(0.22)					(1.00)		(0.65)	(0.35)		(1.00)	
	RS		1.00				0.83	0.17				1.00		0.88	0.12		1.00		
			(1.00)				(0.72)	(0.28)				(0.02)	(0.98)		(0.69)	(0.31)		(1.00)	
	AW		0.99	0.01			0.58	0.42					1.00		0.83	0.17	0.01	0.97	0.02
			(0.98)	(0.02)			(0.58)	(0.42)					(1.00)		(0.87)	(0.13)		(0.97)	(0.03)

		Mdh-1			Mdh-2				Me-1					Mpi						
		2	3	4	1	2	3	4	2	3	4	5	6	1	2	3	4	5	6	7
York	KY		1.00			0.02	0.98		0.13	0.66	0.19	0.02			0.01	0.98	0.02			
	CC		1.00				1.00		0.10	0.75	0.13	0.02			0.01	0.95	0.02	0.02		
	QY	0.01	0.99				1.00		0.10	0.72	0.16	0.03			0.01	0.98	0.02			
	CI		1.00			0.03	0.96	0.01	0.09	0.74	0.17				0.01	0.95	0.03	0.02		
East Elizabeth	RN		1.00				1.00		0.08	0.66	0.22	0.04				0.88	0.07	0.05		
	CH		0.99	0.01			1.00		0.13	0.70	0.15	0.02			0.02	0.88	0.04	0.05		0.01
	NC		1.00				1.00		0.08	0.73	0.15	0.03				0.94	0.05	0.01		
	CS		1.00				1.00		0.09	0.64	0.23	0.03			0.01	0.92	0.06	0.02		
South Elizabeth	PP	0.01	0.99				0.99	0.01	0.18	0.68	0.11	0.03				0.94	0.03	0.03		
			(1.00)				(1.00)		(0.17)	(0.56)	(0.24)	(0.03)			(0.03)	(0.96)	(0.01)			
	NM		1.00		0.01	0.04	0.95		0.14	0.68	0.14	0.04		0.01	0.01	0.93	0.05	0.01		
			(1.00)				(1.00)		(0.08)	(0.67)	(0.23)	(0.02)				(0.97)		(0.03)		
	PC		0.99	0.01		0.02	0.98		0.15	0.66	0.16	0.03			0.01	0.93	0.05	0.01		
			(0.98)	(0.02)			(1.00)		(0.16)	(0.65)	(0.14)	(0.05)				(0.95)		(0.05)		
	JC		1.00				1.00		0.17	0.58	0.18	0.05	0.01		0.02	0.95		0.03	0.01	
			(1.00)				(1.00)		(0.12)	(0.73)	(0.13)	(0.02)				(0.93)	(0.02)	(0.05)		
	CM		1.00				1.00		0.19	0.57	0.17	0.07				0.90		0.03		
			(1.00)				(1.00)		(0.09)	(0.63)	(0.19)	(0.09)				(0.92)	0.07	(0.08)	0.01	
	SC		0.99	0.01			1.00		0.13	0.68	0.11	0.08			0.02	0.92	0.05	0.02		
			(1.00)				(1.00)		(0.14)	(0.54)	(0.29)	(0.03)				(0.97)	(0.01)	(0.02)		
	CF		1.00			0.01	0.99		0.12	0.65	0.16	0.07				0.92	0.06	0.02		
			(1.00)				(1.00)		(0.14)	(0.59)	(0.22)	(0.05)			(0.02)	(0.90)	(0.05)	(0.03)		
	RS		1.00			0.01	0.99		0.11	0.63	0.17	0.09				0.96	0.03	0.01		
			(1.00)			(0.02)	(0.98)		(1.03)	(0.74)	(0.10)	(0.03)				(0.98)		(0.02)		
	AW		1.00				1.00		0.12	0.64	0.17	0.07				0.92	0.07	0.01		
			(1.00)				(1.00)		(0.25)	(0.49)	(0.19)	(0.07)				(0.94)	(0.02)	(0.04)		

		Pgd				Pgm							
		2	3	4	5	1	2	3	4	5	6	7	8
York	KY		0.99	0.01			0.01	0.83	0.07	0.10			
	CC	0.01	0.87	0.13				0.83	0.08	0.10			

Table 1 *(continued)*

		Pgd				Pgm							
		2	3	4	5	1	2	3	4	5	6	7	8
	QY		0.98	0.03			0.02	0.84	0.04	0.10			
	CI		0.97	0.03			0.02	0.78	0.07	0.12	0.01		0.01
East Elizabeth	RN		0.97	0.03			0.01	0.79	0.08	0.10	0.03		
	CH	0.01	0.91	0.08		0.01	0.03	0.78	0.05	0.13			
	NC		0.98	0.03			0.01	0.83	0.04	0.11	0.01		
	CS		0.99	0.01				0.79	0.13	0.08			
South Elizabeth	PP		0.92	0.07				0.75	0.10	0.14			
		0.01	(0.86)	(0.14)				(0.82)	(0.11)	(0.07)		0.01	
	NM		0.91	0.09			0.01	0.82	0.09	0.08			
			(0.80)	(0.20)			(0.03)	(0.81)	(0.03)	(0.13)			
	PC		0.89	0.11		0.03		0.78	0.08	0.11			
			(0.95)	(0.05)		(0.01)	(0.02)	(0.72)	(0.06)	(0.19)			
	JC		0.94	0.06		0.01		0.80	0.04	0.15			
			(0.83)	(0.17)			(0.01)	(0.82)		(0.17)			
	CM	0.01	0.88	0.12			0.01	0.82	0.06	0.11			
			(0.86)	(0.12)	(0.02)			(0.72)	(0.03)	(0.25)			
	SC	0.01	0.91	0.08		0.01	0.02	0.77	0.09	0.11			
		(0.01)	(0.82)	(0.17)		(0.01)	(0.02)	(0.83)	(0.08)	(0.06)			
	CF		0.95	0.05		0.01	0.78	0.18	0.03				
			(0.95)	(0.05)			(0.76)	(0.10)	(0.12)	(0.02)			
	RS		0.87	0.13		0.01	0.80	0.12	0.06		0.01		
		(0.01)	(0.82)	(0.17)			(0.84)	(0.03)	(0.13)				
	AW		0.93	0.07			0.80	0.04	0.14		0.03		
			(0.92)	(0.08)		(0.01)	(0.81)	(0.06)	(0.12)				

Data for juveniles are placed in parentheses.

Table 2
Genetic variability in adult mummichog from the York and Elizabeth Rivers

River	Locality	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity
Elizabeth River–Southern Branch Juveniles	Power Plant (PP)	32.9 (0.1)	2.1 (0.2)	73.3	0.185 (0.052)
	New Mill Creek (NM)	31.9 (0.1)	1.8 (0.3)	53.3	0.163 (0.051)
	Paradise Creek (PC)	32.0 (0.0)	2.0 (0.3)	66.7	0.162 (0.048)
	Jones Creek (JC)	30.0 (0.0)	2.3 (0.2)	80.0	0.176 (0.043)
	Channel Marker 2 (CM)	32.0 (0.0)	2.0 (0.3)	60.0	0.189 (0.054)
	Scuffletown Creek (SC)	32.8 (0.2)	2.1 (0.3)	60.0	0.199 (0.059)
	Crown Tank Farm (CF)	29.0 (0.0)	2.0 (0.3)	60.0	0.189 (0.054)
	Refueling Station (RS)	30.9 (0.1)	2.0 (0.2)	73.3	0.161 (0.043)
	Atlantic Wood (AW)	33.7 (0.2)	2.0 (0.3)	66.7	0.192 (0.058)
Elizabeth River–Southern Branch Adults	Power Plant (PP)	59.5 (0.5)	2.3 (0.3)	80.0	0.185 (0.052)
	New Mill Creek (NM)	59.9 (0.1)	2.2 (0.3)	66.7	0.189 (0.049)
	Paradise Creek (PC)	59.7 (0.2)	2.2 (0.3)	80.0	0.161 (0.042)
	Jones Creek (JC)	59.9 (0.1)	2.5 (0.3)	73.3	0.189 (0.059)
	Channel Marker 2 (CM)	60.0 (0.0)	2.1 (0.3)	60.0	0.197 (0.055)
	Scuffletown Creek (SC)	59.4 (0.6)	2.4 (0.3)	66.7	0.177 (0.051)
	Crown Tank Farm (CF)	59.9 (0.1)	2.1 (0.3)	66.7	0.170 (0.048)
	Refueling Station (RS)	56.7 (0.8)	2.0 (0.3)	60.0	0.159 (0.045)
	Atlantic Wood (AW)	57.0 (0.0)	2.1 (0.3)	66.7	0.191 (0.052)
York River Adults	Kings Creek (KY)	59.7 (0.2)	2.5 (0.3)	80.0	0.165 (0.050)
	Carters Creek (CC)	59.9 (0.1)	2.4 (0.3)	73.3	0.171 (0.045)
	Queens Creek (QY)	59.8 (0.2)	2.5 (0.3)	86.7	0.180 (0.054)
	Catlett Islands (CI)	59.7 (0.2)	2.4 (0.4)	66.7	0.171 (0.048)
Elizabeth River–Eastern Branch Adults	Radio Tower North (RN)	59.9 (0.1)	2.3 (0.3)	73.3	0.178 (0.052)
	Campostella Heights (CH)	59.8 (0.1)	2.5 (0.4)	73.3	0.175 (0.045)
	North Canal (NC)	59.7 (0.3)	2.0 (0.3)	53.3	0.157 (0.048)
	Colonnas Shipyard (CS)	60.0 (0.0)	2.0 (0.3)	53.3	0.144 (0.045)

Standard errors for estimates are provided in parentheses.

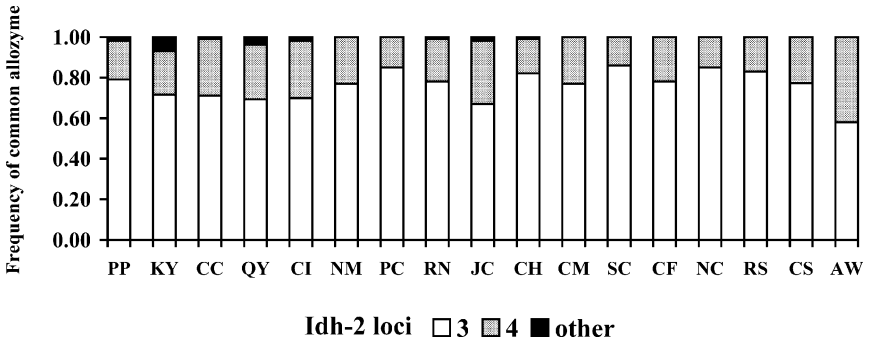

Fig. 3. *Idh-2* allele frequencies for loci with significant heterogeneity among adult mummichog at sites along the Elizabeth and York Rivers. Consistent with Fig. 2, sites are arranged in order of increasing sediment PAH contamination. The common allele (3) and allele 4 are separated from the remaining rare alleles ('other' = 2, 5, 8 and 9 alleles in Table 1).

4. Discussion

Insufficient data are currently available to determine the possible impact of environmental contaminants on the genetic qualities of natural populations. Mummichog occur in discrete habitat patches and are thought to exhibit strong site fidelity. These characteristics make them useful for testing the hypothesis that markedly different levels of environmental contamination have genetic consequences in exposed populations over relatively short distances.

The microgeographic genetic structure of mummichog on a spatial scale encompassing the Elizabeth and York Rivers presents a mixed picture relative to predictions that fish residing in contaminated localities would be genetically distinct from fish in neighboring, uncontaminated habitat. Mummichog from the most heavily contaminated locality, AW, were distinct from fish at other localities. Fish from the moderately contaminated RS site were less distinct from other fish. A statistically significant correlation was observed between genetic distance and sites differences in carbon-normalized contaminant concentrations (Fig. 4).

The minor genetic differentiation between the York and Elizabeth Rivers suggested that reference sites from York River are justifiable in studies of AW mummichog. No evidence was produced that suggested the need to select an Elizabeth River reference site in such studies.

Mummichogs from AW or other highly contaminated sites were not genetically depauperate. All measures of genetic diversity (percent of loci polymorphic, number of alleles per locus, and heterozygosity) were comparable at AW and other localities in the Elizabeth and York Rivers.

Genetic distinction was heavily influenced by differences in the frequencies of allozymes in the AW mummichog compared with fish from elsewhere in the Elizabeth and York River landscape. Allozyme frequencies for the *Idh-2* locus for the mummichog at the AW locality were consistently and significantly distinct from other localities. Es-

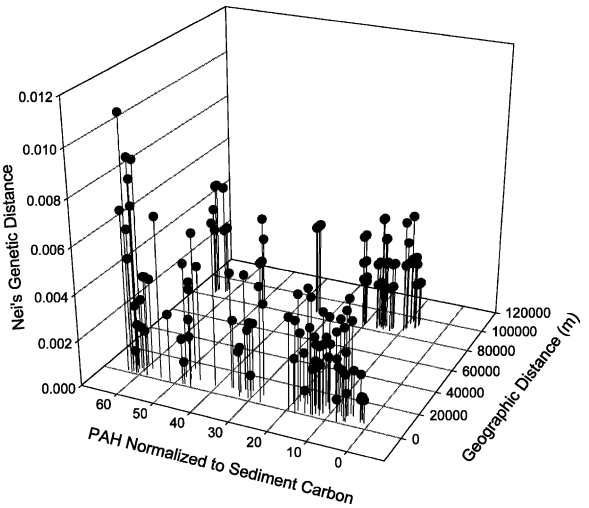

Fig. 4. Plot of mummichog genetic distance, geographic distance, and square root of absolute difference in organic carbon-normalized PAH concentrations.

timates for migration rates of mummichog in this study and previously published work (Brown and Chapman, 1991; Smith et al., 1998) indicate significant amounts of gene flow at this geographic scale. High rates of migration act to homogenize allozyme frequencies among fish from throughout the South Branch of the Elizabeth River. The distinct allele frequencies at the AW locality suggest that, although the potential for migration is high, migrants do not persist at the heavily contaminated location or do not participate successfully in reproduction. Although the mechanisms underlying genetic differentiation of mummichog at heavily contaminated localities remains unknown, population genetic processes have been impacted by contamination at AW.

Measures of performance in organisms experiencing environmental stress have been associated with metabolic efficiency, especially if resources are limited (Koehn and Bayne, 1989; Parsons, 1997). Lactate dehydrogenase-B has been linked to rates of cellular metabolism (Powers et al., 1991). Swimming endurance (DiMichele and Powers, 1982b) and hatch times (DiMichele and Powers, 1982a,) in mummichog differ among *Ldh-B* genotypes. Isozymes of glucosephosphate isomerase have been related to organism performance during abiotic stress. Several studies have suggested that the relationship occurs because the locus regulates metabolite flux through glycolysis (Watt, 1985; Riddoch, 1993). In the present study, there were no significant correlations between allozyme frequencies at specific loci and PAH concentration. However, genetic distance was correlated with site differences in organic carbon-normalized PAH concentration. The overall genetic divergence of the AW mummichog from other mummichog collected in the Elizabeth River was greater than divergences detected among other sites. Mummichogs from RS, the second most contaminated site, were also genetically divergent from other Elizabeth River localities but less divergent than mummichog at AW (Fig. 4).

Kirchoff et al. (1999) reported a discontinuity in the frequency of esterase allozymes downstream of a bleach kraft pulp mill in Miramichi Estuary, New Brunswick, Canada and argued that there was limited gene flow. Although there are numerous impediments to movement of mummichog along the Elizabeth River Southern Branch, no isolation-by-distance was observed and migration of fish among localities is probably not limited at this spatial scale. The model, $F_{st} = 1/(1 + 4N_e m)$, where N_e is the effective population size and m is the migration rate, was used to estimate the number of migrates per generation (Wright, 1978). For the Elizabeth River Southern Branch mummichog, the estimate of effective migration ($N_e m$) was 9.6 and 17.5 from the juvenile and adult data, respectively. Brown and Chapman (1991) estimated $N_e m$ to be 24 for mummichog along an 8.4 km shoreline. Kirchoff et al. (1999) reported estimates of $N_e m$ ranging from 3 to 125 for mummichog at five estuarine locations (between locality distances of 4 and 100 km) along the east coast of New Brunswick, Canada. Perhaps due to the numerous physical impediments to movement, migration estimates for the Elizabeth River mummichog were at the lower end of the other available estimates. Regardless, gene flow is sufficient to prevent differentiation due to genetic drift if $N_e m$ is greater than 1. The wide distribution of rare alleles also indicates gene flow because rare alleles are most likely to be dispersed by migration (Slatkin, 1985). Rare alleles were observed in mummichog throughout the Elizabeth River localities, including at the AW site.

Several characteristics contribute to the perception that mummichog is a low dispersal species. Adults exhibit strong site fidelity and summer movements have been reported to be 36 m in a Delaware Bay estuary (Lotrich, 1975). Mummichog eggs possess adhesive fibrils and so remain on vegetation (Able, 1984) or empty bivalve shells (DiMichele et al., 1986) onto which they were deposited. Taylor and DiMichele (1982) report that larval mummichog remain in shallow intertidal pools for as many as 6–8 weeks before joining adults in daily movements on and off the marshes. Winter movement of mummichog has been reported to be greater and might mitigate against isolation of populations (Able and Felley, 1986).

Our findings regarding mummichog population genetic structure are consistent with several pub-

lished studies of other mummichog populations. Brown and Chapman (1991) used mtDNA to investigate microgeographic population genetic structure in mummichog along 8.4 km of a Maryland salt marsh. They reported no significant genetic differentiation among samples and sufficient gene flow to prevent population differentiation associated with genetic drift. In a study of the northern form of mummichog in Connecticut, Leamon (1999) found a high degree of gene flow over a 41 km range. Additionally, he reported that, although population-level differentiation was low between sites, the population structure and allele frequency at a subpopulation (as little as 500 m) was a poor predictor for neighboring subpopulations. Leamon reported that the relative frequencies of alleles varied widely within the small spatial (i.e. 41 km) or temporal scales (i.e. 2 years) examined. Both of these studies argue that mummichog may exhibit greater movement than previously reported over scales ranging from 8 to 41 km. Relative to the use of York River reference populations in studies of AW fish, there was no rationale based on genetic divergence due to geographic distance for arguing that an Elizabeth River reference site would be preferable.

Factors contributing to significant differences in allele frequencies between sites include differences in female reproductive success and high rates of larval or juvenile mortality, possibly exceeding 99% (Meredith and Lotrich, 1979). In the Elizabeth River Southern Branch survey of this study, mummichogs were collected over approximately 12 km. Estimates of population genetic structure were similar to those reported in earlier studies. Genetic subdivision, as measured by F_{st}, was two times greater in adult mummichog than in juveniles. This observation is consistent with greater site fidelity among juveniles and movement of mummichog adults during the winter. Population genetic structure of mummichog appears to reflect diversifying forces (variance in reproductive success and low probabilities of reaching reproductive age) and homogenizing forces (adult seasonal movement).

Introduction of contaminants into the environment is often associated with reduction in population size or elimination of organisms due to toxicant-induced mortality. Populations might further be altered by differential elimination of particularly sensitive individuals. Organisms that persist in contaminated habitats can exhibit tolerance of conditions that would be deleterious to individuals from non-polluted areas. Contaminant-induced disturbance in populations may result in reduction in genetic diversity in impacted areas. Despite high levels of PAH contamination at AW, mummichog had genetic diversity comparable to that of fish at neighboring cleaner localities. However, genetic distance was significantly correlated with organic carbon-normalized PAH concentrations. Genetic distance was greater between mummichog of the AW locality and other sites than among other sites. Significant differences in allele frequencies for the *Idh-2* locus and greater overall divergence at the AW locality suggest that, although the potential for migration is high, allelic distributions were not homogenized. The highly contaminated habitat at AW apparently disrupts predicted population genetic processes. Additional studies have been completed (Ownby et al., 2002) that show that differences reflect genetically-based differential success of fish exposed to the sediments at the AW.

5. Conclusion

The level of contamination did appear to influence the genetic structure of mummichog populations within a landscape mosaic of sites varying in sediment PAH concentrations The mummichog at AW were genetically distinct from the other sampled populations despite high rates of migration among sites. However, genetic diversity was not correlated with the level of PAH contamination. There was no evidence that isolation by distance causes a problem relative to using reference populations from the York River watershed in studies of the AW population.

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