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The Evolution of Pollution Resistance in the Atlantic Killifish

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Rapid Evolution of Pollution Resistance in Atlantic Killifish

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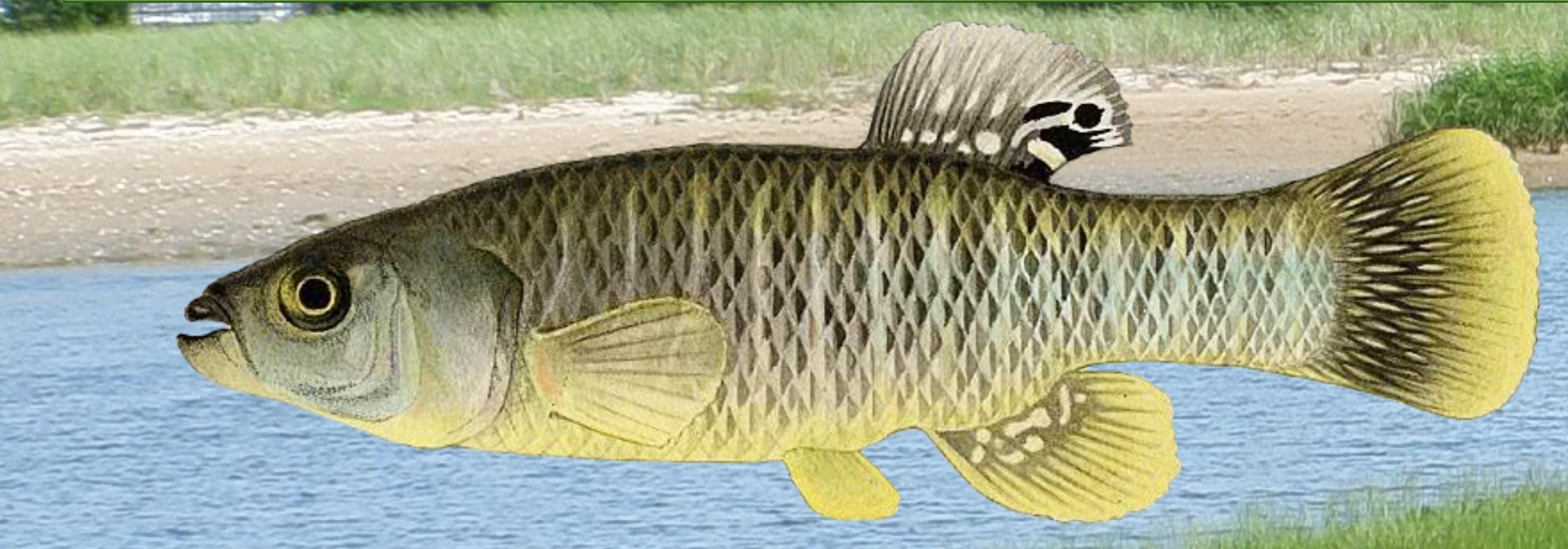
Summary

We examined genetic variation in *Fundulus heteroclitus* populations to better understand their ability to persist in Superfund sites like New Bedford Harbor (NBH) (MA, USA) which is contaminated with a mix of organic toxicants and heavy metals. We isolated DNA from killifish along the Rhode Island and Massachusetts coast at 11 different locations. We identified allele frequency differences at loci consistent with this adaptation. Overall, we observed the expected pattern of genetic isolation by distance when all loci were examined as a group. However, genotypes at some loci are consistent with selection at polluted locations. As predicted by other researchers, loci associated with the Aryl Hydrocarbon Receptor (AHR) system show large differences in allele frequencies between polluted and non-polluted sites.

Background

New Bedford Harbor (NBH) is contaminated with a mix of organic toxicants including Polychlorinated Biphenyls and heavy metals released in the early 20th century. PCBs harm many tissues, including developing hearts, causing 'tube heart'. **However, a large population of Atlantic Killifish thrives in New Bedford Harbor. Lab experiments show that this tolerance is heritable (Nacci et al, 2010).**

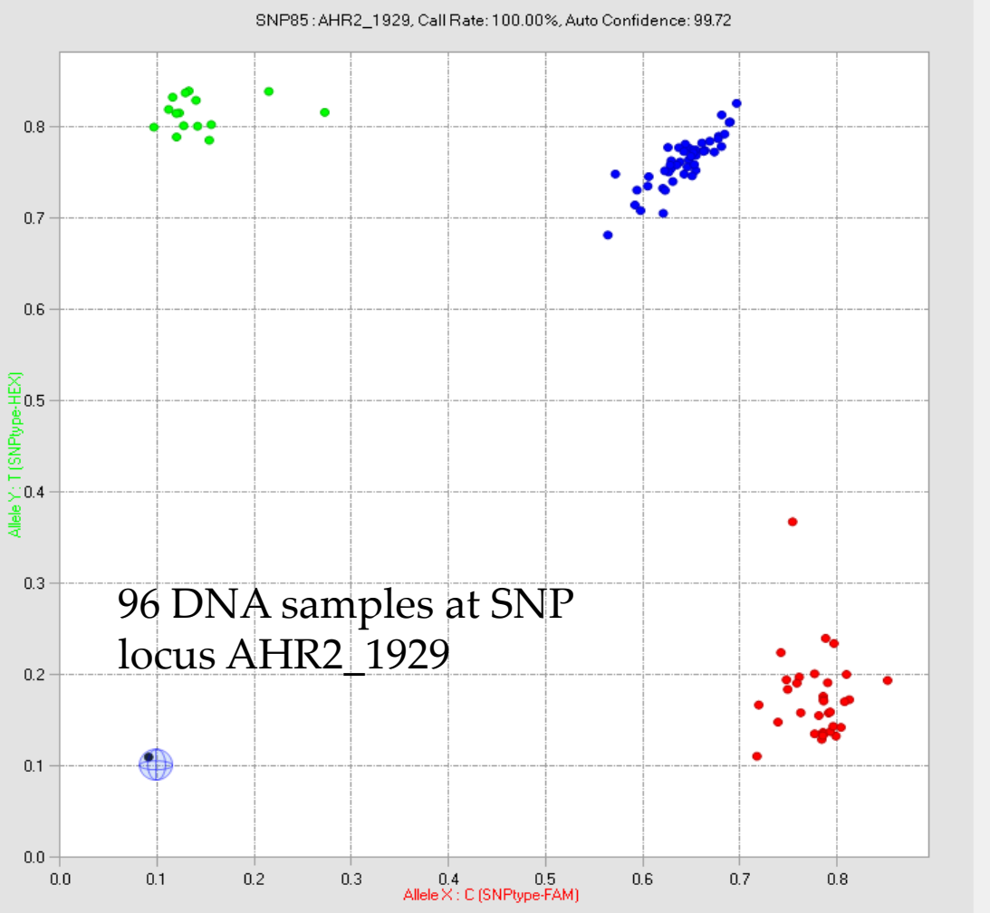
Fundulus heteroclitus embryonic development: 0 normal embryo, 1 & 2 PCB exposed embryos (Di Giulio and Clark, 2015)



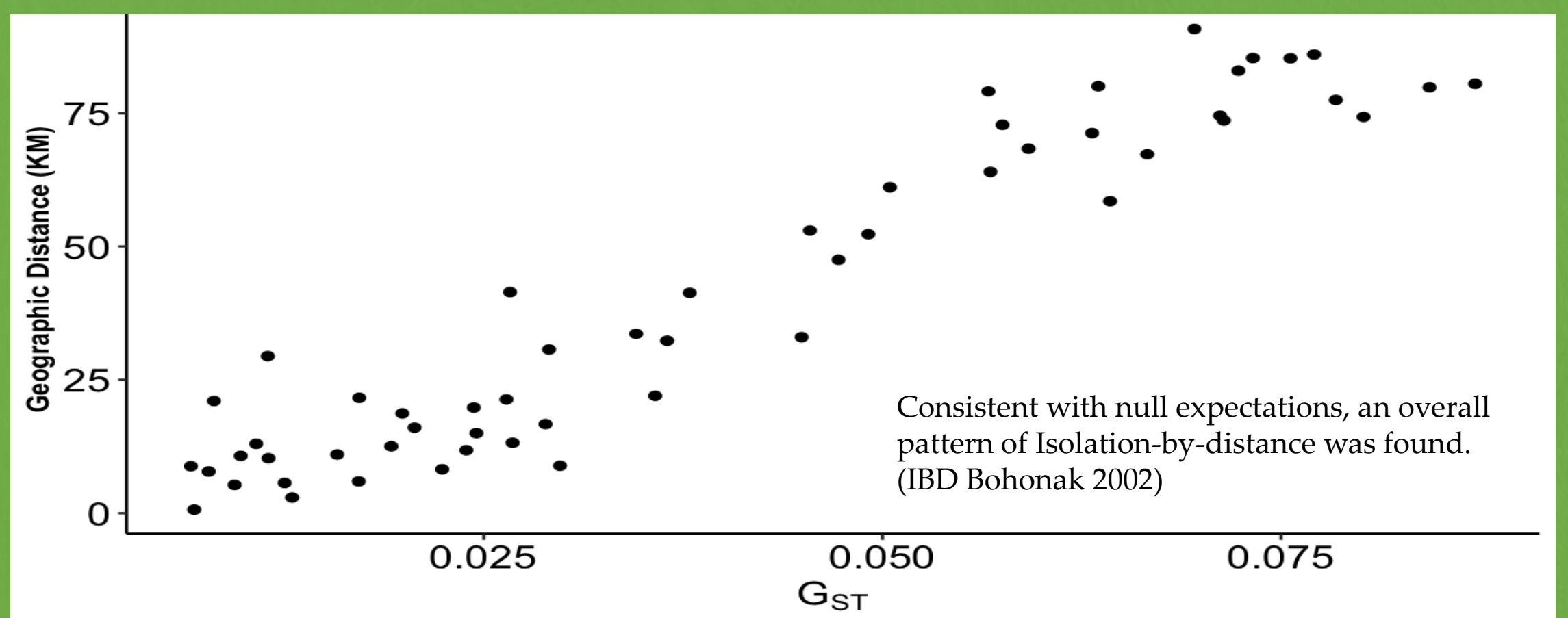
Sampling and Broad Patterns

~500 DNA samples from 11 sites were genotyped at 192 putative SNP loci using a Fluidigm EP-1 system. Of these, 55 loci were variable and repeatable.

96 DNA samples genotyped with 96 sets of SNP primers (Fluidigm)

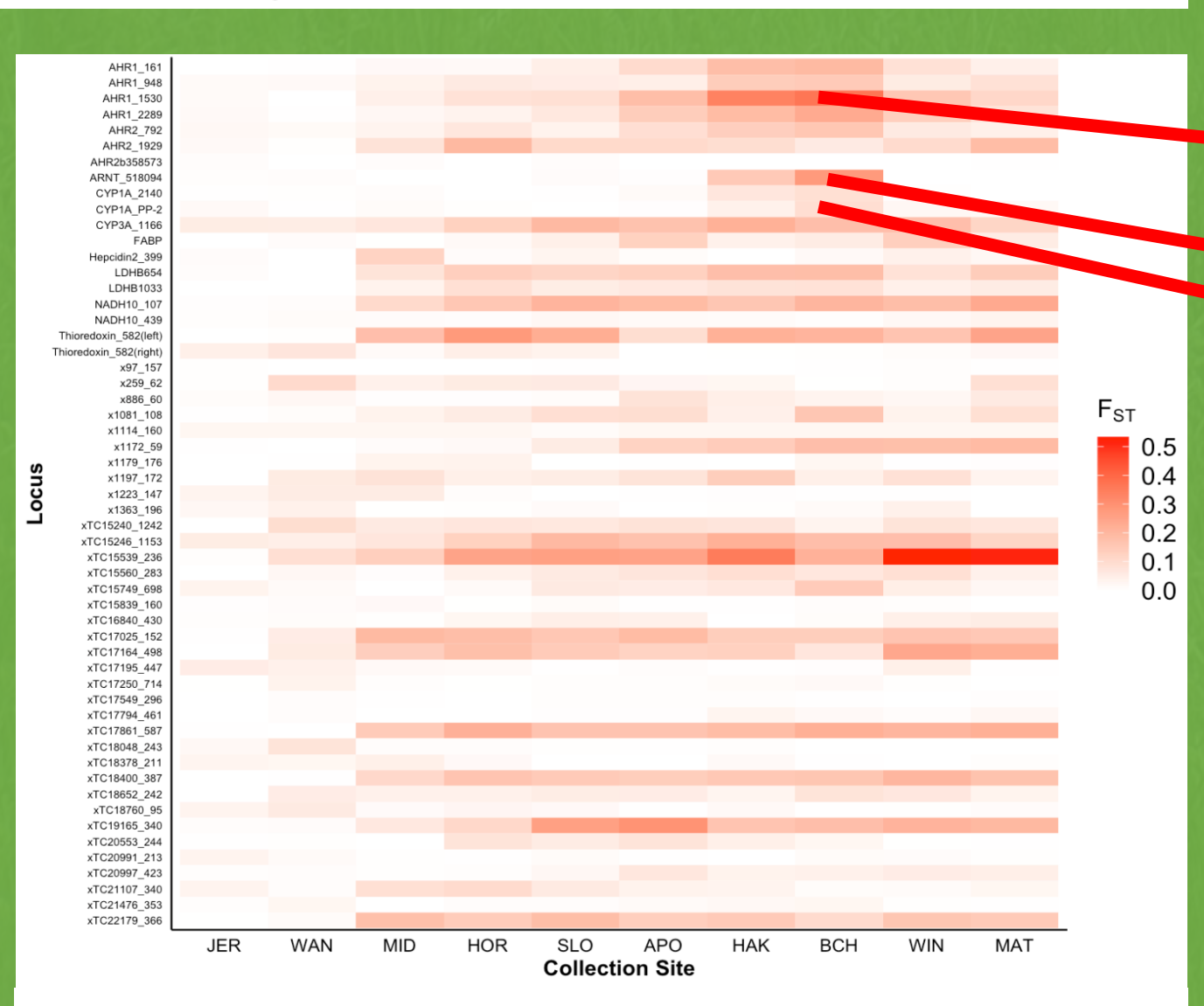


96 DNA samples at SNP locus AHR2_1929



Consistent with null expectations, an overall pattern of Isolation-by-distance was found. (IBD Bohonak 2002)

Despite the overall IBD pattern, between site comparisons at several loci show higher than expected levels of divergence between polluted and clean sites.



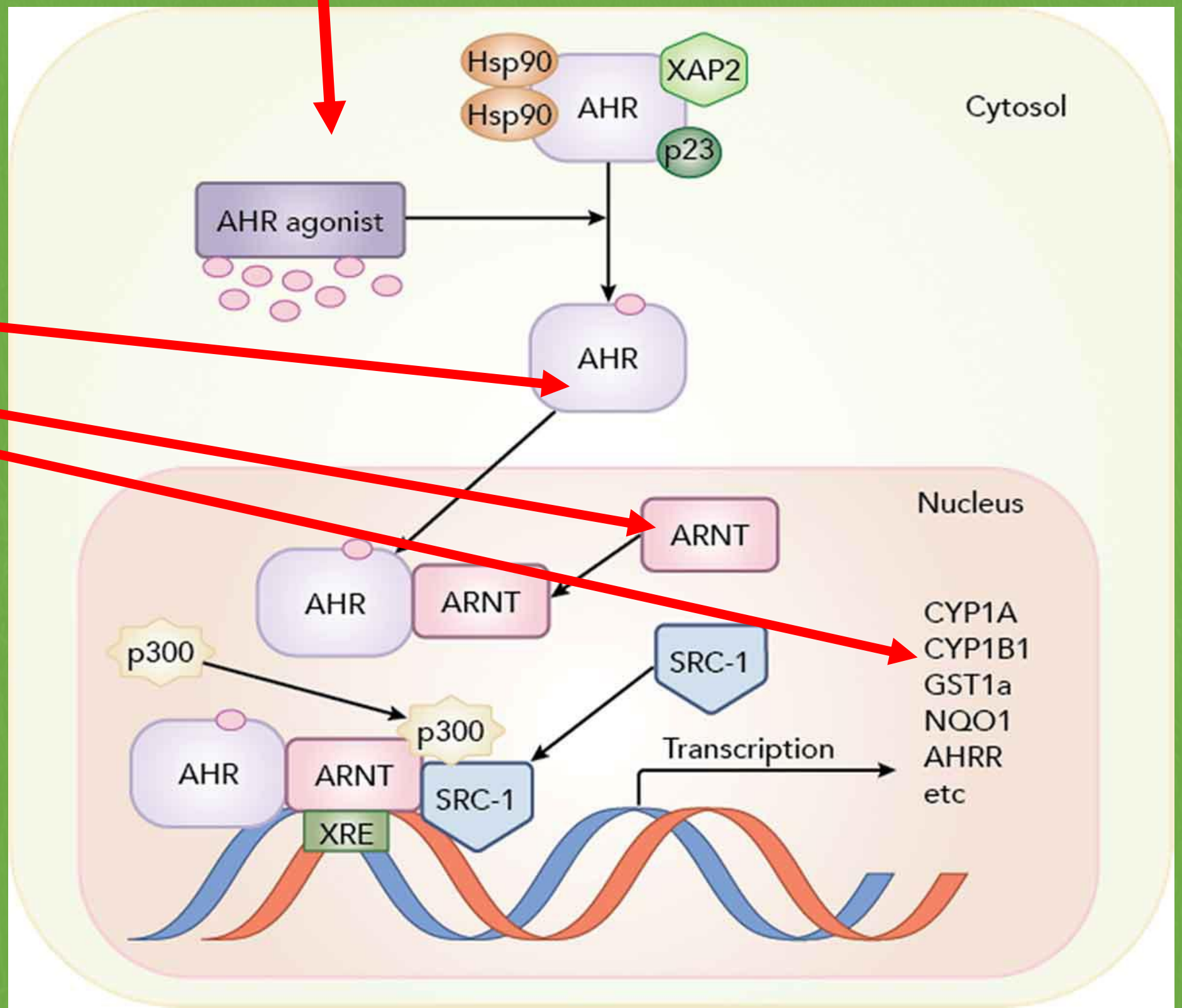
Heatmap of G_{st} values across three sites compared to a "clean" site in Jerusalem, RI at the west end of our survey area. Mattapoisett and Slocum's River are relatively clean sites to the east end near the very polluted NBH

Amino Acid Changes

Minor alleles at 27 of the 55 SNPs are consistent with missense or nonsense mutations, not all loci code for known proteins. Many of these are part of the **AHR pathway.**

SNP_NAME	Common Amino Acid	Common Condon	Alternate Amino Acid	Alternate Codon	Mutation Type
AHR1_161	Alanine	GCC	Glycine	GGC	Missense
AHR1_1530	Glycine	GGG	Arginine	CGG	Missense
AHR1_2289	Methionine	ATG	Isolucine	ATC	Nonesense
AHR2b358573	Glutamic Acid	GAG	Lysine	AAG	Missense
ARNT_518094	Arginine	CGC	Serine	AGC	Missense
CYP1A_2140	Lysine	AAA	Asparagine	AAT	Missense
CYP1A_PP-2	Proline	CCG	Arginine	CGG	Missense
CYP3A_1166	Valine	GTC	Isolucine	ATC	Missense
FABP	Arginine	CGC	Leucine	CTC	Missense
LDHB1033	Aspartic Acid	GAC	Alanaine	GCC	Missense
LDHB654	Alanine	GCC	Serine	TCC	Missense
Thioredoxin_582(left)	Methionine	ATG	Arginine	AGA	Nonesense
Thioredoxin_582(right)	Arginine	CGA	STOP	TGA	Nonesense
x97_157	Serine	TCG	Leucine	TTG	Missense
x259_62	Glutamic Acid	GAG	STOP	TAG	Nonesense
x1081_108	Glutamic Acid	GAG	Lysine	AAG	Missense
x1223_147	Threonine	ACA	Isolucine	ATA	Missense
x1363_196	Histidine	CAT	Arginine	CGT	Missense
xTC15240_1242	Arginine	CGA	STOP	TGA	Nonesense
xTC15246_1153	Valine	GTC	Isolucine	ATC	Missense
xTC17025_152	Methionine	ATG	Threonine	ACG	Missense
xTC17497_137	Proline	CCT	Alanine	GCT	Missense
xTC17794_461	Serine	AGC	Arginine	AGG	Missense
xTC17861_587	Threonine	ACA	Arginine	AGA	Missense
xTC19165_340	Threonine	ACT	Serine	TCT	Missense
xTC20997_423	Glutamic Acid	GAG	Aspatric Acid	GAC	Missense

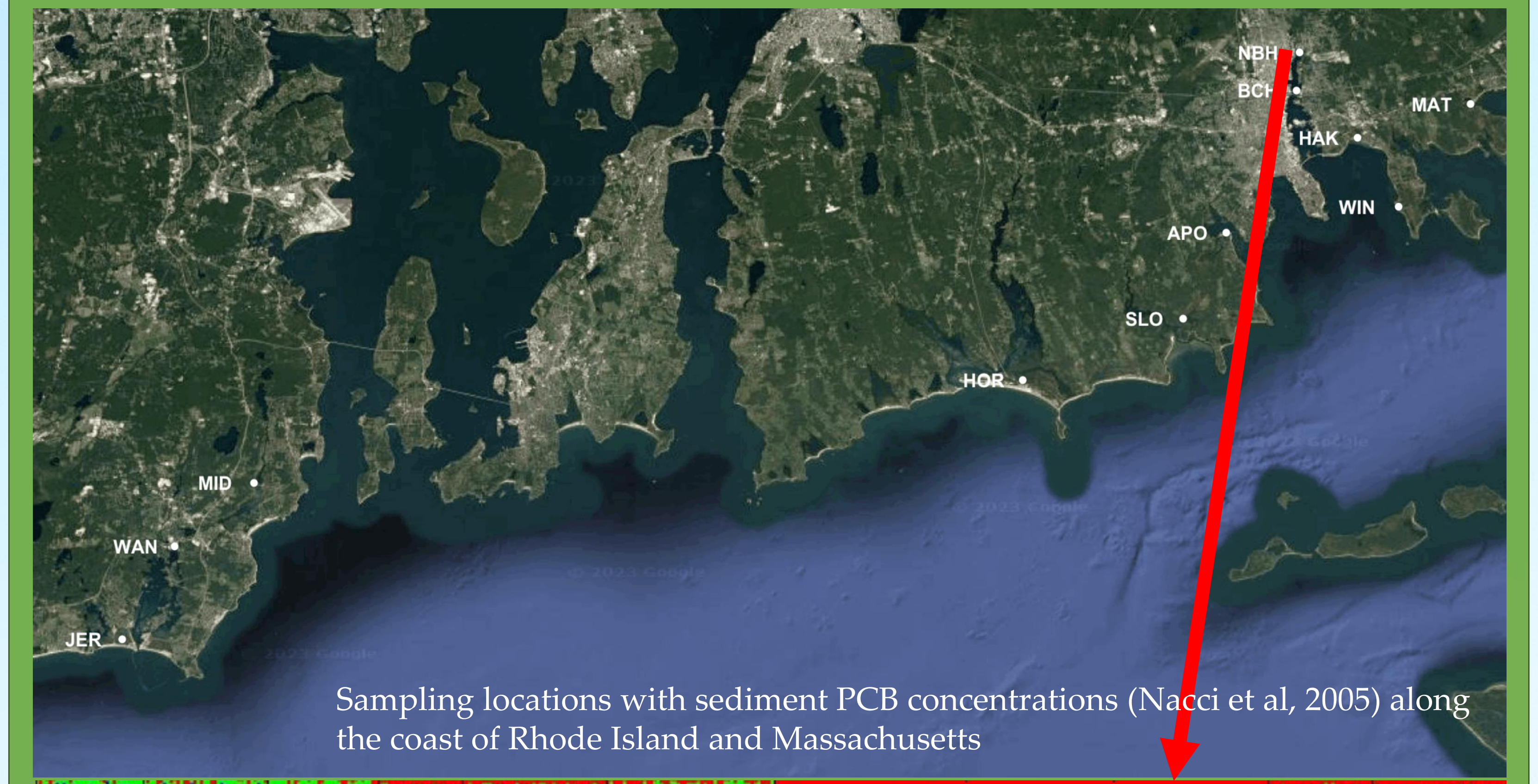
Changes in the amino acid sequence of the protein coding regions leading to missense and nonsense mutations.



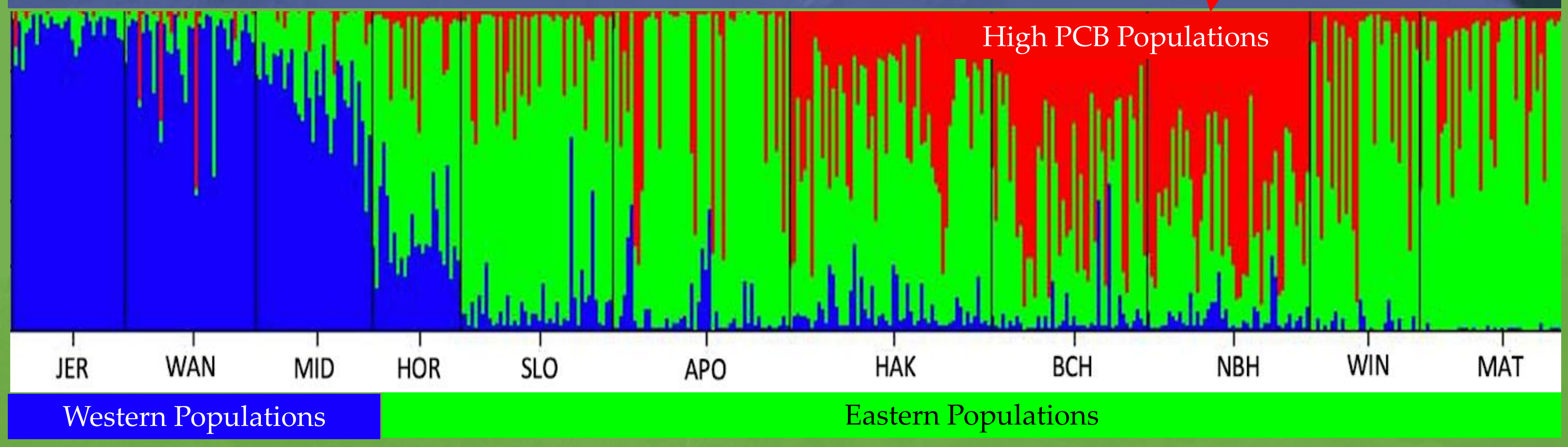
The aryl hydrocarbon receptor pathway. (Di Giulio and Clark, 2015)

The Aryl hydrocarbon receptor pathway also participates in the detoxification of many foreign toxicants and activates phase I and phase II detoxification pathways. The AHR pathway also effects immune development, metabolite signaling, and internal organ communication. The AHR pathway appears to lose function in *F. heteroclitus* living in New Bedford Harbor.

Variation and Clustering



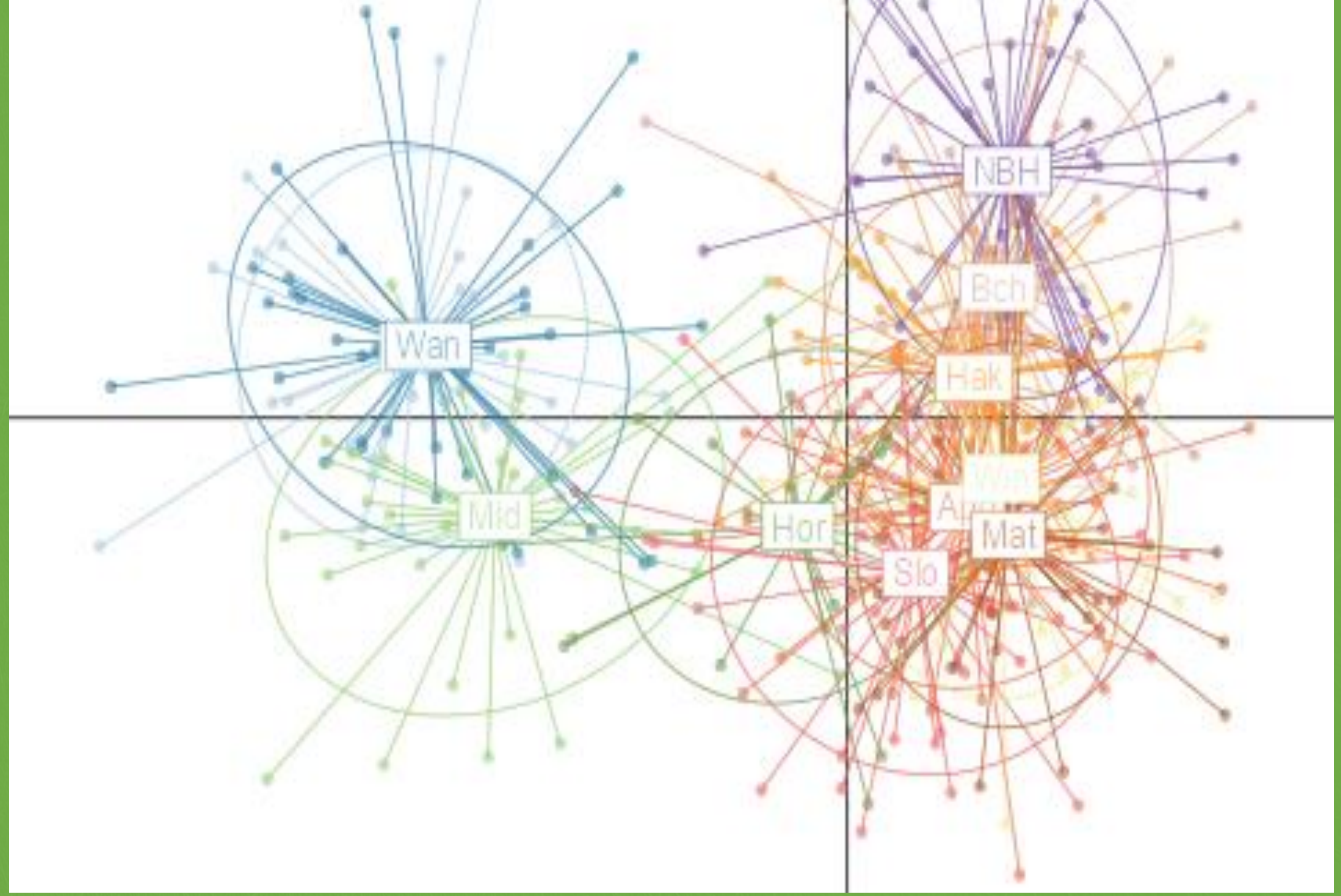
Sampling locations with sediment PCB concentrations (Nacci et al, 2005) along the coast of Rhode Island and Massachusetts



STRUCTURE analysis (above) suggests 3 genetically distinct units spread across the 11 collection sites. The red cluster is associated with higher levels of sediment PCBs at NBH, and perhaps evolved from the eastern cluster in the mid 20th century

Principal Components Analysis with Adegenet/DAPC

The first Principal Component (X axis) explains 43% of the overall variation and is consistent with geographic variation. The second Principal Com-ponent explains 21% of the variation. Allele loadings on this second axis cause us to hypothesize that this variation reflects population genetic changes in response to PCBs in the sediment.



References

Bohonak, A. J. "IBD (isolation by distance): a program for analyses of isolation by distance." *Journal of Heredity* 93.2 (2002): 153-154.

Jombart T (2008). "Adegenet: a R package for the multivariate analysis of genetic markers." *Bioinformatics*, 24, 1403-1405. doi:10.1093/bioinformatics/btn129.

Kumar, Sudhir, Koichiro Tamura, and Masatoshi Nei. "MEGA: molecular evolutionary genetics analysis software for microcomputers." *Bioinformatics* 10.2 (1994): 189-191.

Nacci, Diane E., Denise Champlin, and Saro Jayaraman. "Adaptation of the estuarine fish Fundulus heteroclitus (Atlantic killifish) to polychlorinated biphenyls (PCBs)." *Estuaries and Coasts* 33.4 (2010): 853-864.

Pritchard, Jonathan K., Matthew Stephens, and Peter Donnelly. "Inference of population structure using multilocus genotype data." *Genetics* 155.2 (2000): 945-959.

Reitzel, Adam M., et al. "Genetic variation at aryl hydrocarbon receptor (AHR) loci in populations of Atlantic killifish (Fundulus heteroclitus) inhabiting polluted and reference habitats." *BMC evolutionary biology* 14.1 (2014): 1-18.

Richard T. Di Giulio & Bryan W. Clark (2015) The Elizabeth River Story: A Case Study in Evolutionary Toxicology, Journal of Toxicology and Environmental Health, Part B, 18:6, 259-298, DOI: 10.1080/15320383.2015.1074841

Whitehead, Andrew, et al. "When evolution is the solution to pollution: Key principles, and lessons from rapid repeated adaptation of killifish (Fundulus heteroclitus) populations." *Evolutionary applications* 10.8 (2017): 762-783.

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