

Title: Epigenetics in a marine fish: Role of DNA methyltransferases in embryonic development

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Animals have the capacity to express a variety of morphological and behavioral phenotypes under different environmental conditions. Genetic differences determine much of this phenotypic variability. It is increasingly becoming clear that this variability cannot be completely explained by genetic mechanisms alone. Recent studies suggest that environmental factors cause epigenetic modifications to DNA. DNA methylation, the covalent modification of cytosine nucleotides by the addition of a methyl group, is the most common epigenetic DNA modification in eukaryotes, and it can result in heritable changes in gene expression patterns.

Some populations of killifish inhabiting highly contaminated estuaries along the North Atlantic coast of the United States have evolved resistance to anthropogenic stressors such as polynuclear aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) (Figure 1). This heritable adaptation allows them to thrive in otherwise inhospitable environments. Several studies have attempted to understand the genetic basis of this adaptation, but the answer is still elusive. The overall *hypothesis* of this work is that epigenetic mechanisms play an essential role in the development of the resistant phenotype. We tested this hypothesis by determining the differences in DNA methylation profiles and the expression of DNA methyltransferase (DNMT) genes between sensitive and resistant populations. In addition, we also cloned and sequenced the full length sequences of DNMT genes for future characterization.

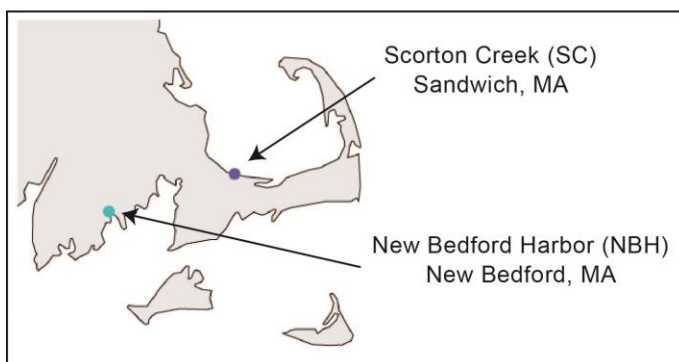


Figure 1. Map of southeastern Massachusetts showing the two study sites. New Bedford Harbor (NBH) is a highly contaminated site and Scorton Creek (SC) is a pristine environment. The sediment PCB concentration in NBH (22,666 ng/g dry weight) is several orders of magnitude higher than in SC (1 ng/g dry weight) (Data from Nacci et al., 2010)

This is the first study to characterize DNA methylation profiles and DNMT genes in an aquatic species of environmental relevance. Our results demonstrated that global methylation levels are not between sensitive (SC) and resistant (NBH) fish. However, we observed differences in the levels of hydroxymethylation, a recently discovered metabolite of 5-methylcytosine (Figure 2). The significance of differences in hydroxymethylation levels is not clear. In future, we will further characterize these differences using genome-wide sequencing approach. In addition, we also observed differences in the expression of specific DNMT genes between the two sites. In order to characterize these further, we cloned and sequenced the DNMT genes. We are using these preliminary results to obtain funding from federal funding agencies. We have presented these results at the Annual meeting of the Society of Integrative and Comparative Biology (January 2014).

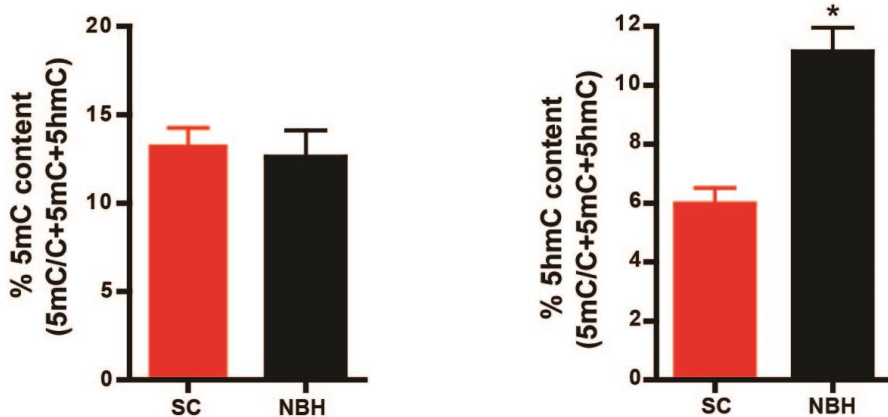


Figure 2. No differences in 5-methylcytosine levels were observed in 19 dpf embryos. However, there is significant difference in 5hmC levels between SC and NBH embryos. Future studies will focus on determining locus-specific and genome-wide DNA methylation and hydroxymethylation levels.

The results from this project are a good foundation for understanding the role of DNA methylation in environmental adaptation to stressors. Very little is known about the role of epigenetics in phenotypic plasticity or adaptation and our studies laid the foundation for using killifish as a model to study epigenetic mechanisms. The next steps of this research is to obtain funding from external sources to further characterize the role of DNA methylation in environmental adaptation. The results generated from this study will aid in obtaining funding from NSF.