



# Genetic variability of *Melampus bidentatus* populations in Connecticut salt marshes

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## Introduction

The goal of this project was to assess genetic variation in localized CT populations of *Melampus bidentatus*, a small amphibious gastropod resident in salt marshes along the Atlantic coast (Dennis & Hellberg, 2010). Certain species may be sensitive to environmental stressors and will therefore show a decline in the presence of said stressors. Part of this decline may be due to decreased genetic diversity in the earlier stages of the collapse or decline of an ecosystem's biodiversity (Halme, Mönkkönen, Katiaho, Ylissirniö, & Markkanen, 2009). By evaluating the genetic diversity of such species, it is hoped that a better understanding of the genetic underpinnings of population maintenance can be obtained. *Melampus* is a good candidate for an indicator species to signal overall health of the salt marshes in which it resides. The species has a specific range, residing almost exclusively in the high marsh, meaning it is sensitive to changes in water level in the marsh (Schaeffer, Keller, & Buroker, 1985). It is also likely vulnerable to changes in the marsh flora, as it feeds and lives virtually exclusively on marsh grass (Dennis & Hellberg, 2010).



Figure 2: Two *M. bidentatus* specimens on Pleasant Point Front.



Figure 1: Satellite images of (a) Stony Creek salt marsh area of Branford, CT and (b) Chaffinch Point salt marsh area of Guilford, CT with collection sites indicated by green markers

## Materials and Methods

### Collection

Specimens of *M. bidentatus* were collected from several salt marshes in Branford, CT—Banca Front, Banca Back, Peasant Point Front, and Pleasant Point Back—as well as a single marsh in Guilford, CT, Chaffinch Point (Figure 1). A total of 11 collection sites within the marshes sampled. From each collection site, 3 to 5 patches were designated within an approximately 3 meter radius using a 15cmx15cm square; all *M. bidentatus* specimens within each designated patch were collected.

### Tissue/DNA Extraction

20 specimens were selected from Pleasant Point Front, Banca Front, and Banca Back each; 30 were selected from Pleasant Point Back and 10 from Chaffinch Point. A total of 100 specimens were processed. A sample of foot tissue was collected from the specimens as detailed by Dennis and Hellberg (2010) or by cutting away the back of the foot off the specimen. Tissue was preserved in 0.5 mL 95% EtOH and held at -20°C until DNA extraction, which was carried out using QIAamp DNA Mini Kit based on instructions from the kit manufacturer, Qiagen (2015).

### Polymerase Chain Reaction (PCR) and Sequencing

PCR was carried out using the procedures described by Dennis and Hellberg (2010) for the amplification of the mitochondrial cytochrome oxidase subunit I (MCOI) gene. The resulting PCR product was then sent to the DNA Analysis Facility on Science Hill in New Haven, CT for sequencing.

### Data Analysis

Sequence data was aligned using a MUSCLE alignment via Geneious version 9.1 and the sequences were trimmed to a length of 648 bp. From this alignment, a neighbor-joining tree was also constructed using Geneious software (Figure 3). A second tree was built using bootstrapping (Figure 5). A rarefaction curve based on the number of unique haplotypes was constructed using Past3 software (Figure 4).

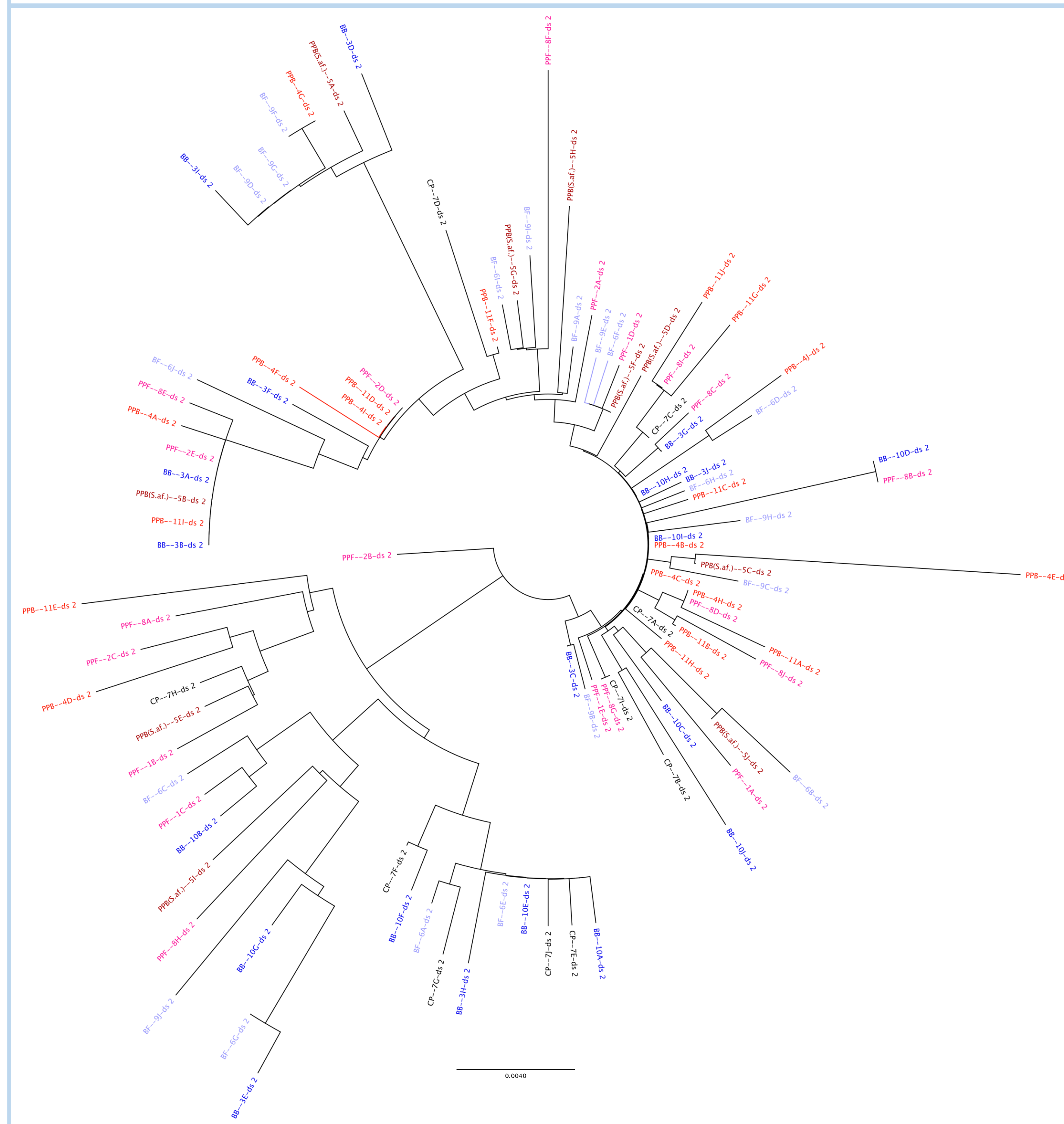


Figure 3: Neighbor-joining tree showing relationships of collected specimens based on MCOI gene. Abbreviations are as follows: Banca Front (BF); Banca Back (BB); Pleasant Point Front (PPF); Pleasant Point Back (PPB); Pleasant Point Back, *S. alterniflora* (PPB(S.af.)); Chaffinch Point (CP). Image generated using Geneious version 9.1.

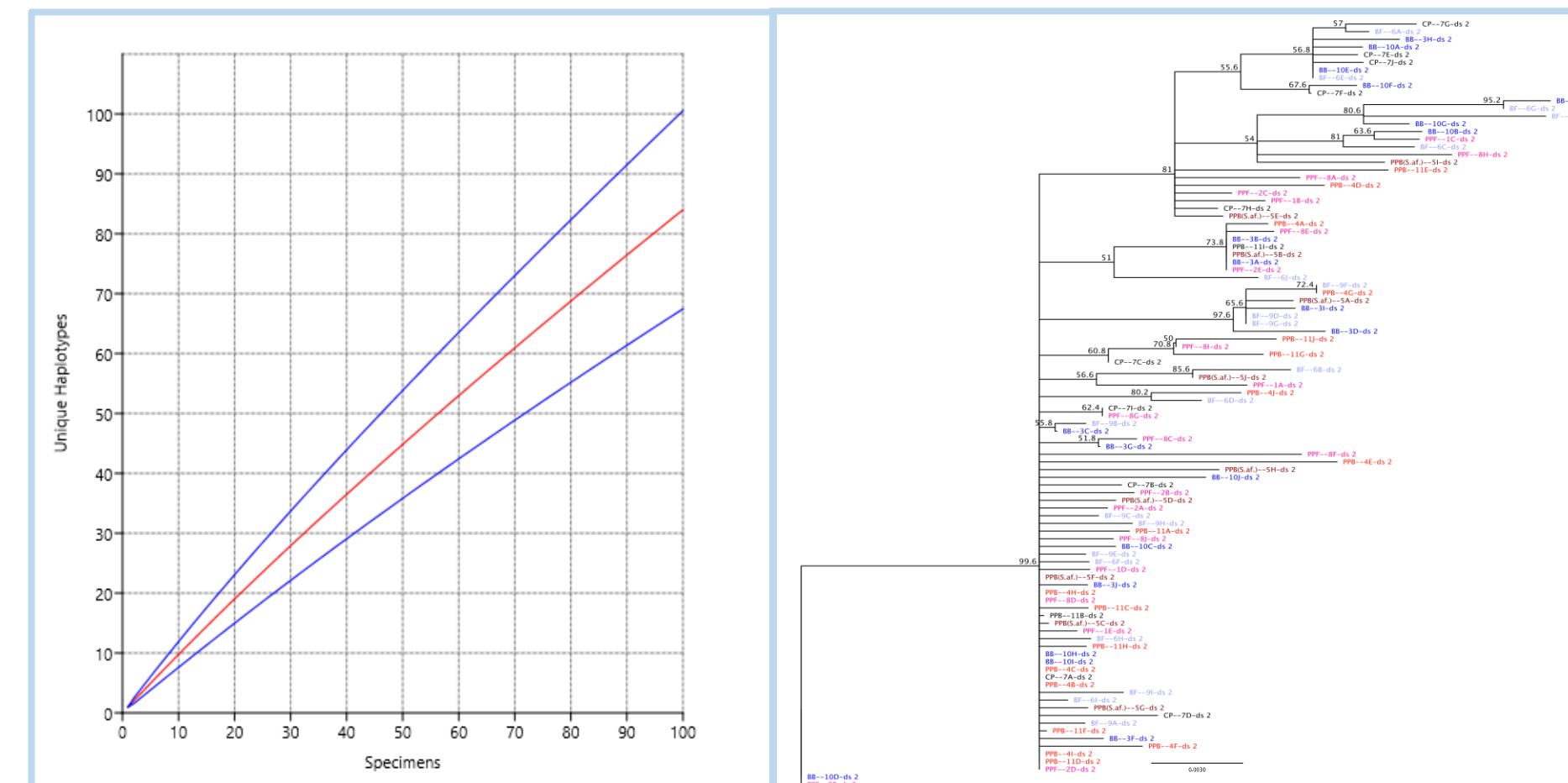


Figure 4(L): Rarefaction curve (red) displaying the projected number of unique haplotypes that would be found for a given number of specimens; 95% confidence bounds represented above and below the curve (blue). Chart generated using Past3 software. Figure 5(R): Bootstrap analysis of aligned sequences. Image generated using Geneious version 9.1.

## Results

Of the 100 total sequences, there were 84 unique haplotypes. Rarefaction analysis indicates that these samples do not represent all of the diversity of haplotypes present, with a 95% confidence interval suggesting that 100 specimens could yield between 67 and 100 unique haplotypes (Figure 4). Phylogenetic tree modeling and bootstrap analysis suggest there are no major discernable patterns of relatedness among the specimens in the context of collection location, with most branches being relatively unsupported by the bootstrap analysis (Figures 3 and 5). Alignments with published sequences revealed that sequences from the current study represent a diverse set of lineages from the "North" clade as defined by Dennis and Hellberg (2010).

## Discussion

This study failed to capture the full extent of the genetic diversity of the *M. bidentatus* populations in the surveyed marshes. The bootstrap analysis of the sequences yielding few well-supported branches and the rarefaction curve showing no signs of plateauing suggest that there are many unobserved haplotypes in the populations. As a result, more sampling is required before further conclusions can be drawn regarding the populations.

A possible explanation for this high degree of genetic diversity, as suggested by Dennis and Hellberg (2010), is the 7-14 day period *M. bidentatus* spends in a planktonic larval form combined with the over 33,000 eggs a female can lay each year. This could result in a large volume of larvae entering open bodies of water and potentially being carried great distances before being deposited once again on a marsh. This could lead to the lack of population structure observed in the sampled populations. Increased levels of salt marsh inundation could also benefit populations by allowing for more eggs to be swept out by high tides. Further research should be conducted to better understand the effects of these factors on population structure.

## References

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## Acknowledgements

I would like to thank the Summer Undergraduate Research Fellowship for generously funding and supporting this project throughout, specifically the incredible efforts of Carol Withers and her team. I would also like to thank Mr. & Mrs. Carrubba for generously supporting the SURF program. My thanks also go to Joseph Eigenberg for his help in the lab and Gabrielle Hartley for her help in the field.