

# Different Habitat Use May Skew Abundance Measurements of Native and Invader Blue Mussels in the California Hybrid Zone

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## Abstract

An cryptic invasion of the Mediterranean mussel *Mytilus galloprovincialis* over the Pacific blue mussel *M. trossulus* started in southern California about 100 years ago and has continued to spread up the coast. Although *M. trossulus* and *M. galloprovincialis* are indistinguishable morphologically, all southern California bay mussels are now known to be the Mediterranean invader, *M. galloprovincialis*, on the basis of genetic markers. While mussels north of Cape Mendocino appear to still be the native *M. trossulus*. Along the central coast of California there is a mixed zone containing both species along with hybrids. Current sampling protocol of adult mussels in this zone is primarily from subtidal floating docks although mussels are found on multiple substrate within harbors that vary in aerial exposure. This raises questions regarding whether this protocol is unintentionally biased in favoring of sampling one species and if there is a difference in the distribution patterns of these mussel species between the intertidal rock and subtidal dock habitats. In order to distinguish between the two species, DNA from mussels collected at both subtidal floating dock and rocky intertidal habitats at Moss Landing, California was extracted and agarose gel electrophoresis of the PCR-amplified Internal Transcribed Spacer (ITS) region between the 18S and 28S ribosomal genes was used to discriminate between species. Preliminary results initially revealed striking species differences between the two habitats in which the mussels on the floating docks are exclusively *M. galloprovincialis* while the nearby rocky intertidal contained an equal distribution of both species. However species identification of a subset of the sampled mussels did not show a significant association between habitat type and the distribution of the bay mussel species. These results suggest that there is not a difference in the distribution of the two species across different habitats within the same geographic location.

## Introduction

Marine biological invasions of non-native species are known to alter the structure and function of nearshore benthic and pelagic marine communities (Geller 1999). The extensive unplanned introduction of such marine species is primarily through the transport and discharge of pelagic larvae in ballast water from transport ships (Heather et al. 1995). In the case of *Mytilus*, it is hypothesized that the Mediterranean mussel *M.*



Figure 1: *Mytilus galloprovincialis*.

*galloprovincialis* (Figure 1) was first transported to Los Angeles Harbor and has continued to spread up the California coast. *M. galloprovincialis* is morphologically identical to the native Pacific blue mussel *M. trossulus* so this cryptic invasion was unnoticed until DNA sequence analysis of genetic markers revealed that all of the bay mussels in southern California are *M. galloprovincialis* while bay mussels north of Cape Mendocino are *M. trossulus* (Geller 1999). Given this distribution of the bay mussel species, there is a hybrid zone in central California in which both *M. galloprovincialis* and *M. trossulus* are found. Extensive studies of the interactions between and distribution of both bay mussel species within the hybrid zone have collected bay mussels from only subtidal floating docks (Figure 2) (Braby & Somero 2006). Since mussels are found on most substrates within harbors, including intertidal areas (Figure 3), we questioned whether current protocol of only sampling docks is accurately representing the current distribution of bay mussel species within the hybrid zone.

**Research Question:** Is the sampling protocol of floating docks unintentionally biased and is there a difference in the distribution patterns of *M. trossulus* and *M. galloprovincialis* between the intertidal rock and subtidal dock habitats?

**Hypothesis:** The distribution of bay mussel species (*M. galloprovincialis* and *M. trossulus*) will be different between the subtidal floating dock and the rocky intertidal habitats.



Figure 2: Subtidal floating dock habitat.

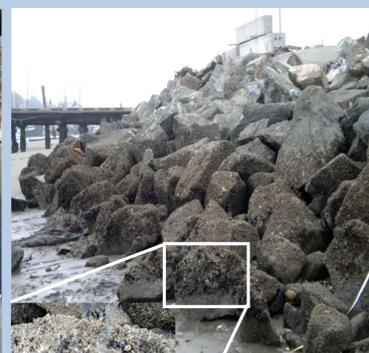


Figure 3: Intertidal rock habitat.

## Methods

### Field Methods

- Mussels were collected at subtidal dock and intertidal rock habitats within the hybrid zone at Moss Landing Harbor (Figure 4 & 5) in central California for a total sample size of 100 mussels
- Moss Landing (ML) Dock sampling** (n = 50 mussels)
  - Transect tapes were laid along the edge of a subtidal floating dock
  - Mussels were then randomly chosen at various points along each transect using a random number table and the first mussel touched at each point was collected
- Moss Landing (ML) Rock sampling** (n = 50 mussels)
  - A 10 m transect tape was laid across intertidal rock habitat
  - Transect tapes of 5 m were laid perpendicular to the 10 m transect starting at 0 m
  - The highest and lowest mussels along with three mussels randomly chosen in between were collected at each perpendicular transect



Figure 4: Hybrid zone of bay mussel species at Moss Landing Harbor.



Figure 5: Dock and rock collection sites at Moss Landing Harbor.

### Laboratory Methods

- Following collecting, all mussel samples from dock and rock habitats were frozen
- Frozen mussels were cut along the adductor muscles and pried open
- DNA was then extracted from a 25 mg piece of gill tissue from each frozen mussel (Figure 6) using a Qiagen DNeasy Blood and Tissue Kit
- Internal Transcriber Spacer (ITS) region between the 18S and 28S ribosomal genes (Figure 7) was amplified by PCR, using GoTaq® Green Master Mix and the following primers
  - F 5'-GTTCCGCTAGGTGAACCT-3',
  - R 5'-CTCGTCTGATCTGAGGTC - 3' (Braby & Somero 2006)
  - PCR Conditions: 3 min at 94°C then 34 cycles of 94°C for 30 s, 55°C for 20 s, 72°C for 1 min 5 min at 72°C (adapted from Heath et al. 1995)
- Restriction Fragment Length Polymorphism (RFLP) of the resulting PCR product was then cut using Hha1 restriction enzyme
- Gel electrophoresis of the PCR product and corresponding restriction enzyme digests were run on a 1.5% agarose gel and visualized to distinguish between mussel species
  - Resulting banding patterns (Heath et al. 1995):
    - Mytilus galloprovincialis* Two band visible Segments at 450 bp and 180 bp
    - Mytilus trossulus* One band visible Segments at 280 bp and 180 bp

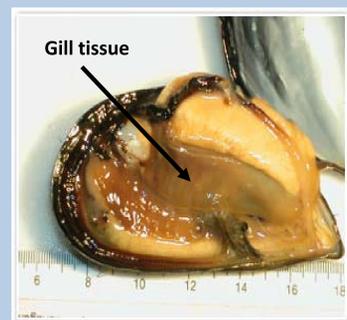


Figure 6: Gill tissue from a collected mussel.

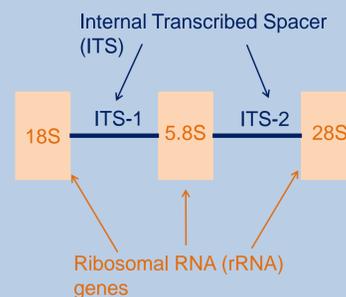


Figure 7: ITS region between 18S and 28S genes.

## Results

### RFLP Mussel Species Identification

ML Rock had **23 *M. galloprovincialis*** and **10 *M. trossulus***  
 ML Dock had **17 *M. galloprovincialis*** and **6 *M. trossulus***

### Chi-Squared Analysis

From a subset of the sampled mussels, there is no association between habitat type and the distribution of the bay mussel species ( $\chi^2 = 0.118$ ,  $p = 0.731$ ,  $df = 1$ ).

Table 1: Output of Chi-squared cross-tabulation of habitat and bay mussel species.

		Habitat * Species Crosstabulation			
		Species		Total	
		Gal	Tross		
Habitat	Rock	Count	23	10	33
		Expected Count	23.6	9.4	33.0
Dock	Count	17	6	23	
		Expected Count	16.4	6.6	23.0
Total	Count	40	16	56	
		Expected Count	40.0	16.0	56.0

## Discussion & Future Work

The non-significant association between habitat type (subtidal dock and intertidal rock) and the distribution of the bay mussel species (*M. galloprovincialis* and *M. trossulus*) implies that the current sampling protocol of only docks is not biased in representing one species over another. Despite the difference in size of intertidal mussels found at the high zone and the low zone, the distribution of the bay mussel species is similar to the distribution collected at the subtidal dock. Thus docks in the hybrid zone can more conveniently be sampled while confidently representing the distribution and interaction of the bay mussel species.

### Future Work

As a continuation of our findings, we plan on sampling a larger number of mussels from subtidal dock and intertidal rock habitats within Moss Landing Harbor and Elkhorn Slough as well as nearby harbors, such as Santa Cruz, San Francisco Bay, and Monterey Harbor. There are plans to develop a map of Elkhorn Slough that shows the location of mussels on various substrate. It was noted during sampling of the dock that there was a sea otter foraging on the mussels beneath the dock, which could have selected for a particular mussel species and therefore altered the distribution of sampled mussels. The large mussels found in the high zone of the rocky intertidal were reminiscent of the California mussels. As an outgroup of the bay mussels, the California mussel *M. californianus*, which is found in highly exposed areas of the rocky intertidal, may also be involved in the hybrid interactions. Our next step is to subclone and sequence ITS regions from *M. galloprovincialis*, *M. trossulus*, and *M. californianus* to look at which species compose the hybrids. We also plan on looking at other gene loci (CO1 and Glu) to further distinguish between *M. galloprovincialis*, *M. trossulus*, and hybrids.

## Acknowledgments

This project was supported in part by the Department of Education under CCRAA and the Ronald E. McNair Postbaccalaureate Achievement Program. The authors would like to thank Drs. Simona Bartl and Aparna Sreenivasan for reagents along with steadfast support.

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