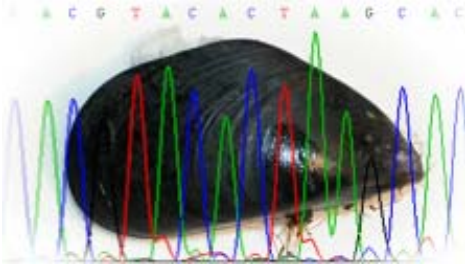


Marine Biotechnology and Bioinformatics



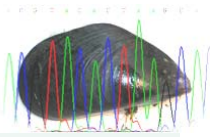
A program of ITEST (Information Technology Experiences for Students and Teachers) funded by the National Science Foundation



Preliminary Scientific Results

Dr. Simona Bartl

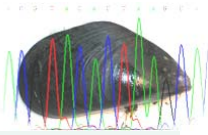
Moss Landing Marine Laboratories
Moss Landing, CA



Introduction

- ▶ <1988 all “bay mussels” = *Mytilus edulis*
 - ▶▶ Found in temperate areas world-wide
- ▶ Genetic studies found 3 morphologically indistinguishable species (refs)
 - ▶▶ *Mytilus edulis*, *M. trossulus*, *M. galloprovincialis*
- ▶ Only one = successful invader (*M. galloprovincialis*)
- ▶ Genetic markers essential for species identification
 - ▶▶ Historic, native species ranges identified
 - ▶▶ Invasion events mapped; areas of hybridization found

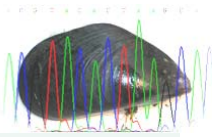
References: Hilbish, et al., 2000, Marine Biology; Riginos & Cunningham, 2005, Molecular Ecology



Introduction

- ▶ West coast US native = *M. trossulus*
 - ▶▶ *M. galloprovincialis* arrived in Southern CA in 1930's (ref)
 - ▶▶ Progressively spread northward and displaced *M. trossulus*
- ▶ A mixed population of *M. trossulus* & *M. galloprovincialis* exists in sheltered areas of Monterey Bay
 - ▶▶ Another native, *Mytilus californianus* found on exposed coast
 - Morphologically and genetically distinct
 - Used as an outgroup

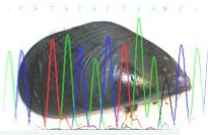
Reference: Geller, 1999, Conservation Biology



Introduction

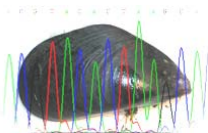
- ▶ Hypothesis: As *Mytilus galloprovincialis* spreads northward it displaces *Mytilus trossulus*
 - ▶▶ This will be seen as a greater percentage of invaders and hybrids in Monterey Bay over time.
 - ▶▶ Potential refuges for the native in Moss Landing and Palo Alto may also see a greater percent of invaders (ref).

Reference: Braby & Somero, 2005, Marine Biology

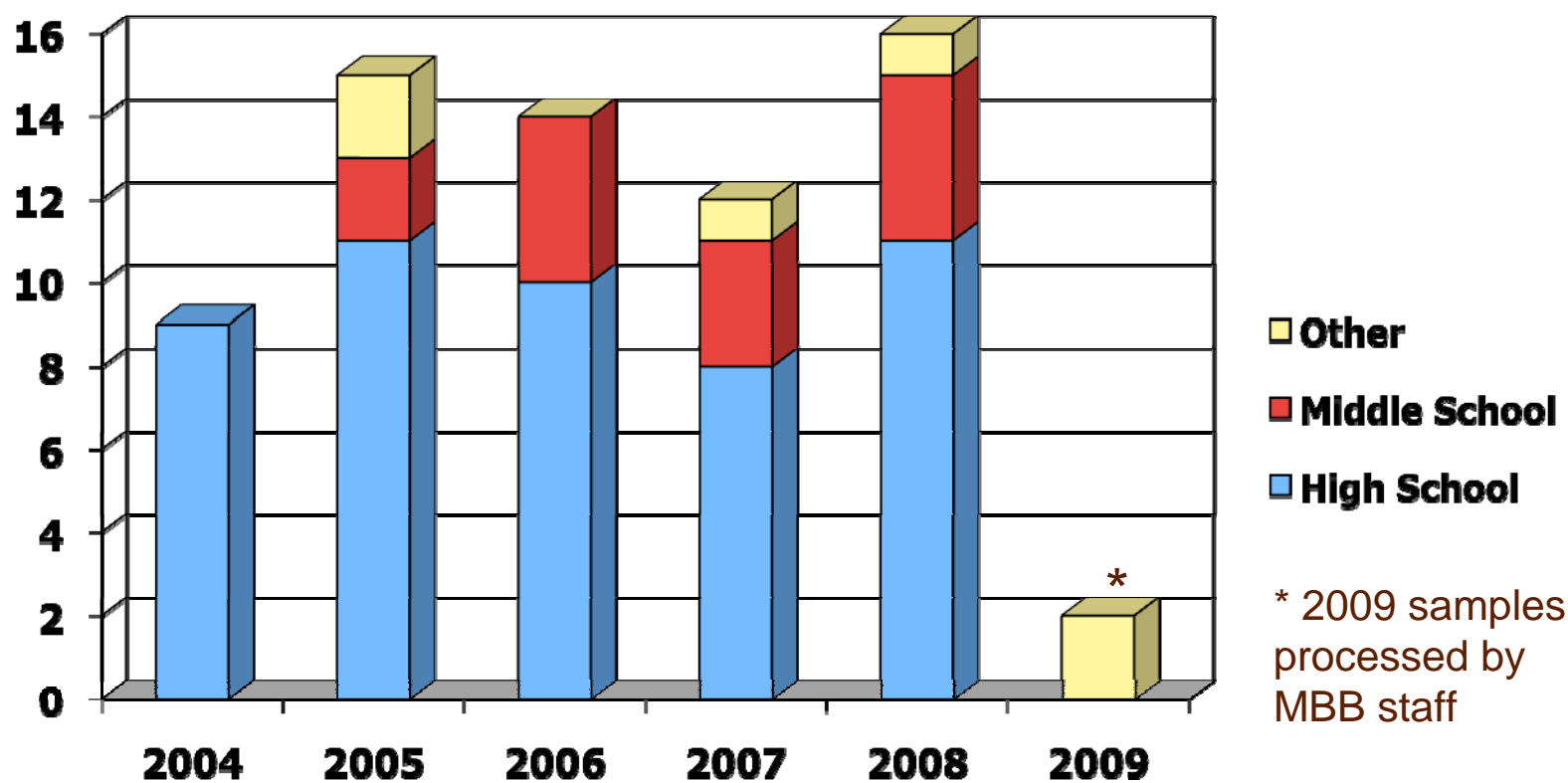


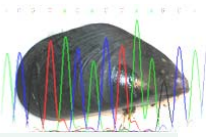
Materials & Methods

- ▶ **Materials:** Mussels from Monterey Bay (Harbor, Moss Landing) & San Francisco Bay (Boat Launch, Palo Alto)
- ▶ **Methods:**
 - ▶▶ **Biotechnology:**
 - DNA extraction -> PCR -> Analysis
 - Restriction digestion, gel electrophoresis, DNA sequencing
 - Genetic markers: ITS, Glu, CO3
 - ▶▶ **Bioinformatics:**
 - DNA sequences -> Edit
 - Align, search Genbank, build phylogenetic trees
 - ▶▶ **Work performed by:** MBB staff & Workshop Participants (mostly Middle & High School science teachers)

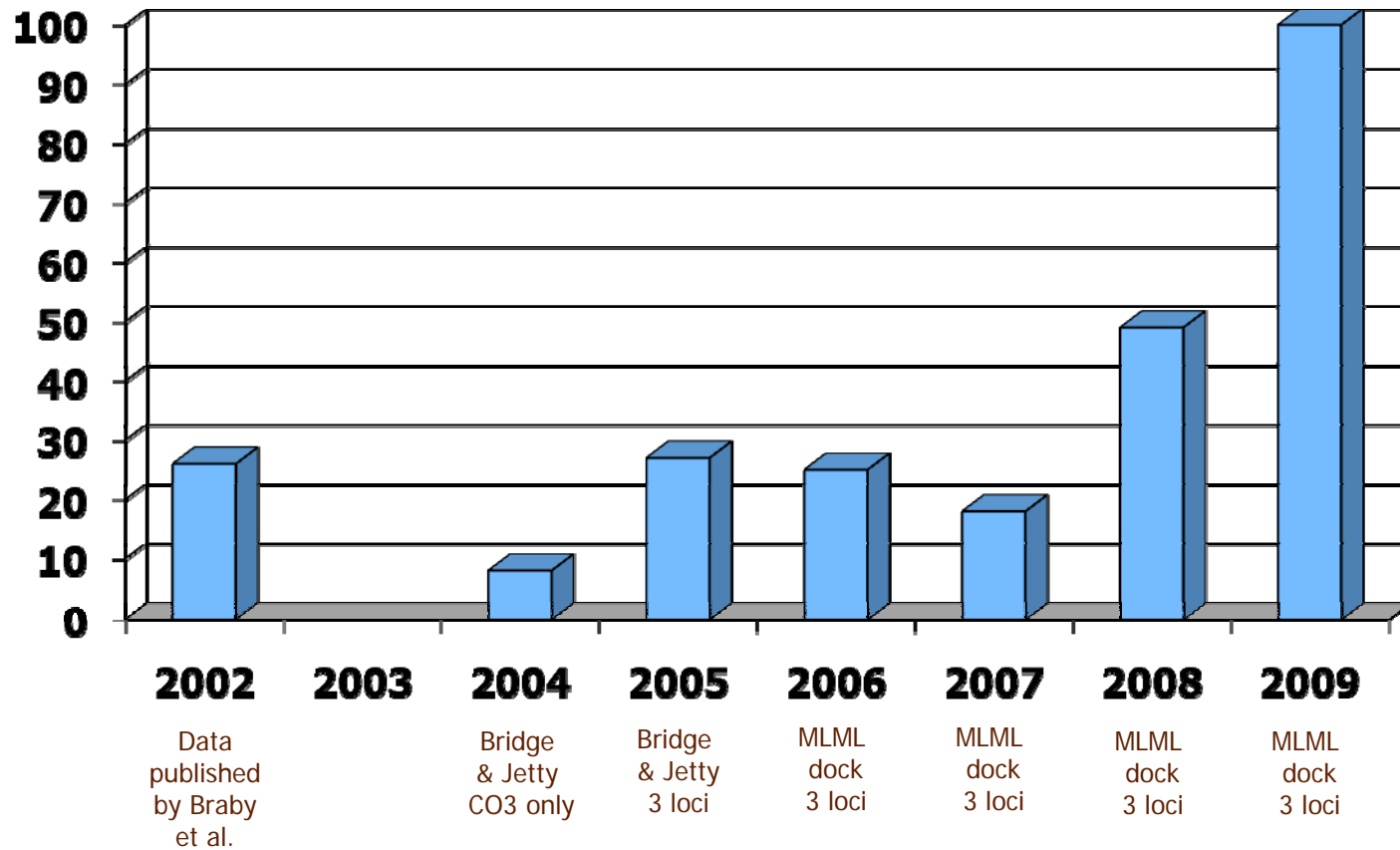


Workshop participants per year

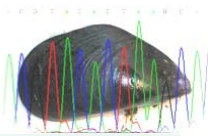




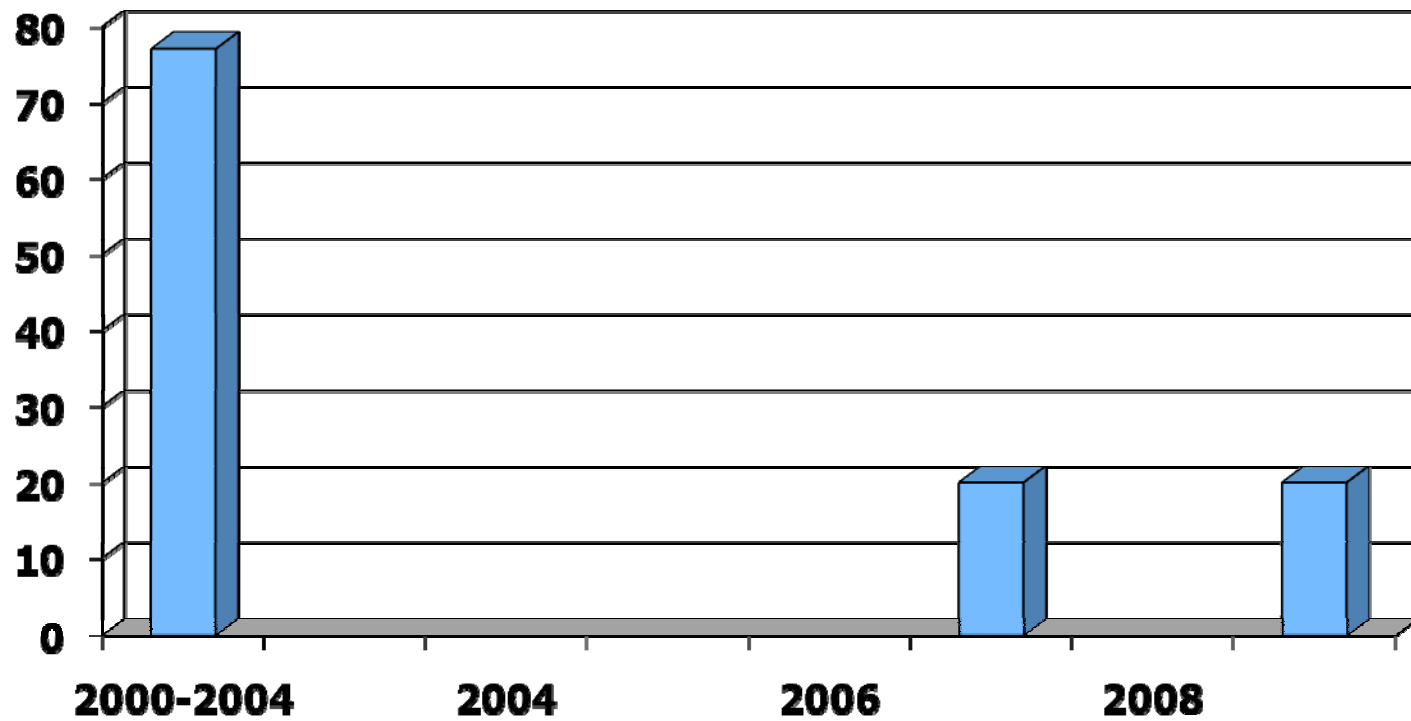
No. mussels sampled per year: Moss Landing



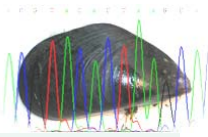
2004-2008: N = 127



No. mussels sampled per year: Palo Alto

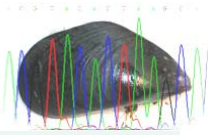


Data
published
by Braby
et al.



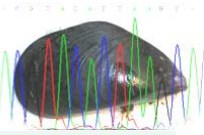
Species Identification

- ▶ Nuclear loci (ITS & Glu) were analyzed by gel electrophoresis
 - ▶▶ ITS PCR products showed a species-specific banding pattern after digestion with *Hha I*
 - ▶▶ Glu primers amplified different-sized products in a species specific manner
 - ▶▶ The previous study (Brady & Somero) used these two loci

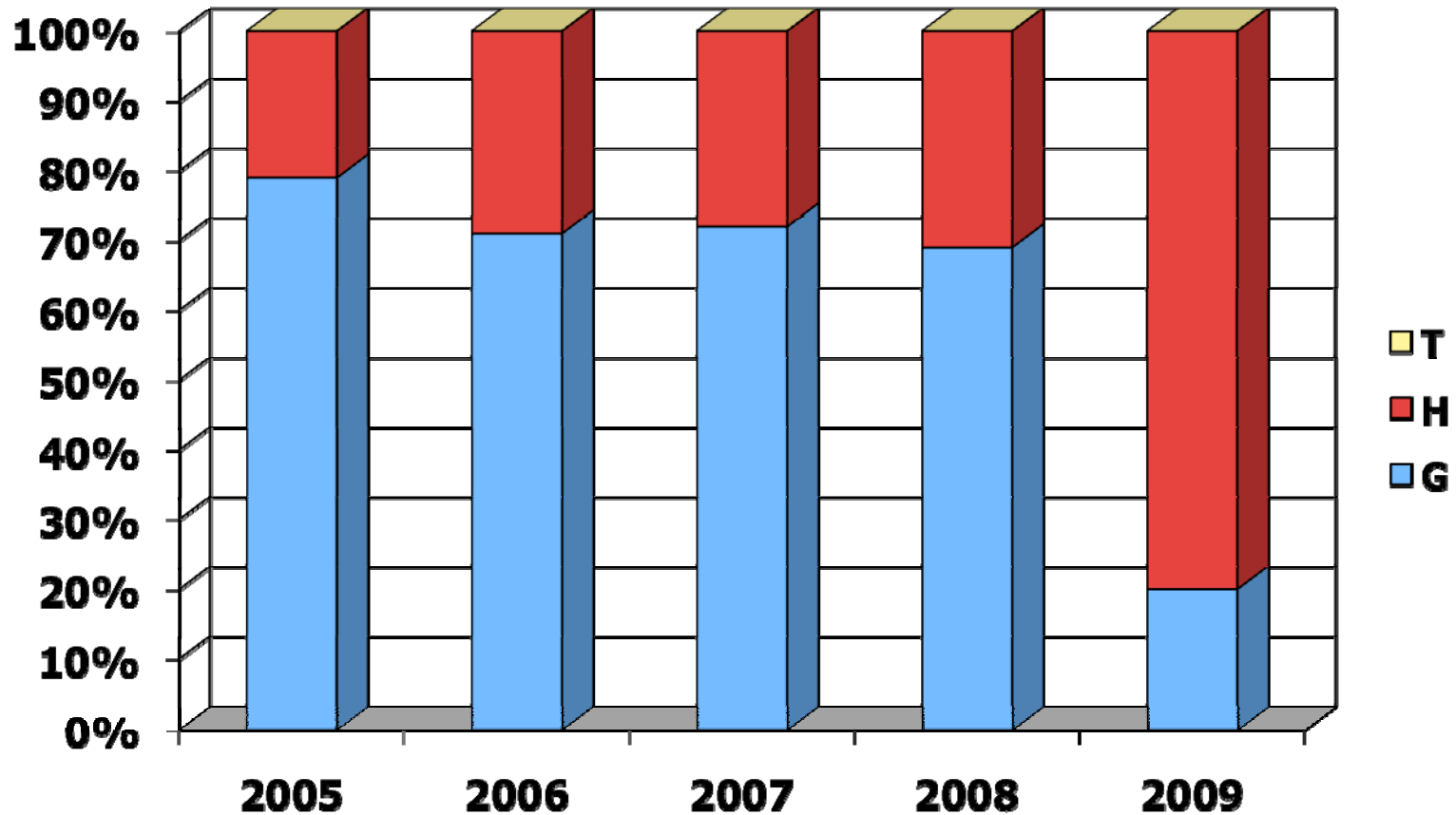


Species Identification

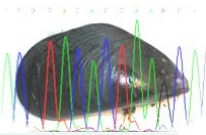
- ▶ This study also used a mitochondrial locus (CO3)
 - ▶▶ This locus was sequenced and analyzed at the species level on phylogenetic trees
 - ▶▶ *Mytilus* spp. exhibit unique bi-parental inheritance of mitochondria resulting in gender-specific lineages of mitochondrial DNA



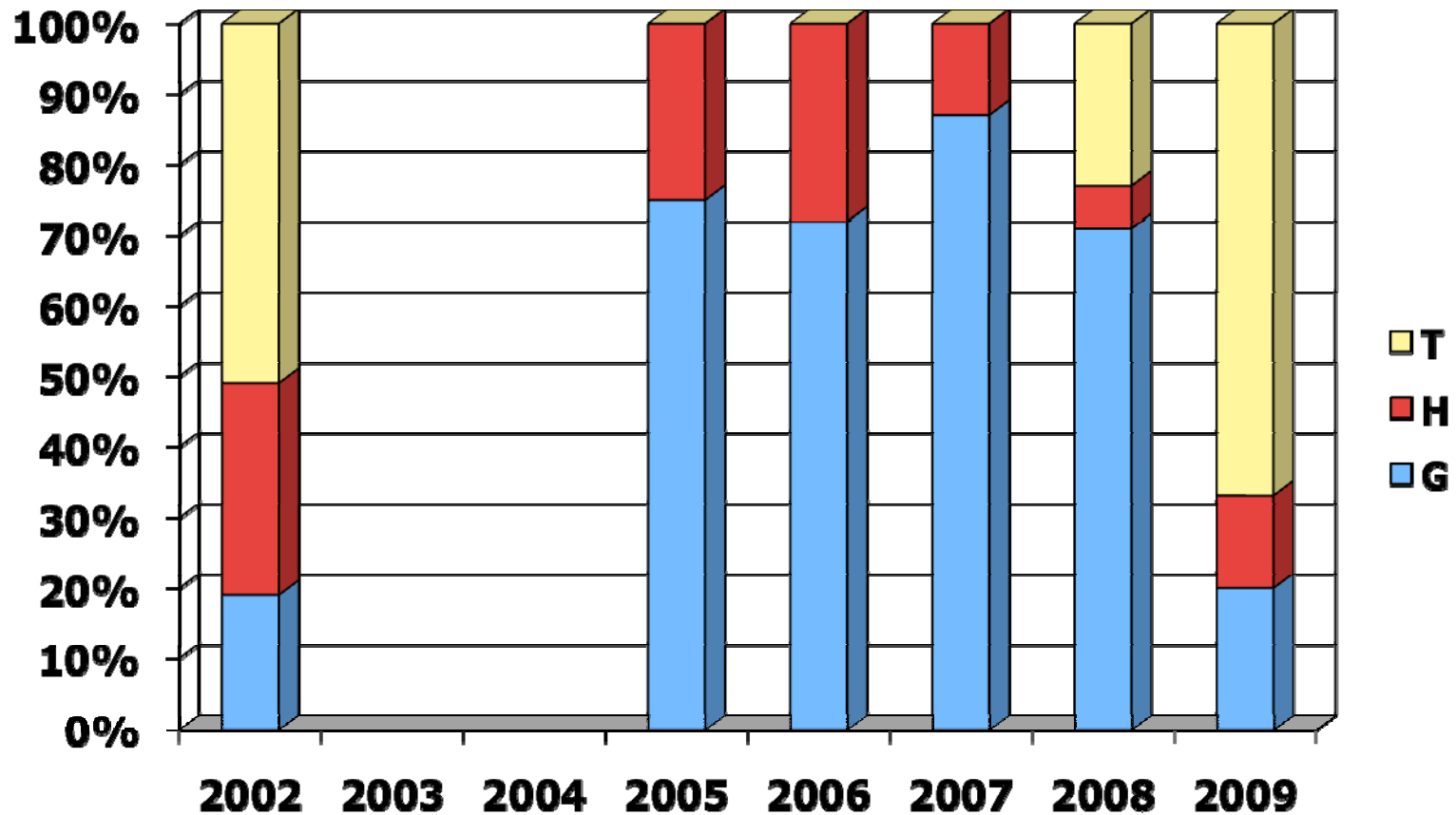
% each species per year at Moss Landing (data from 3 loci – ITS, Glu, CO3)



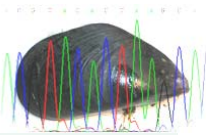
T = *M. trossulus*; H = hybrid; G = *M. galloprovincialis*



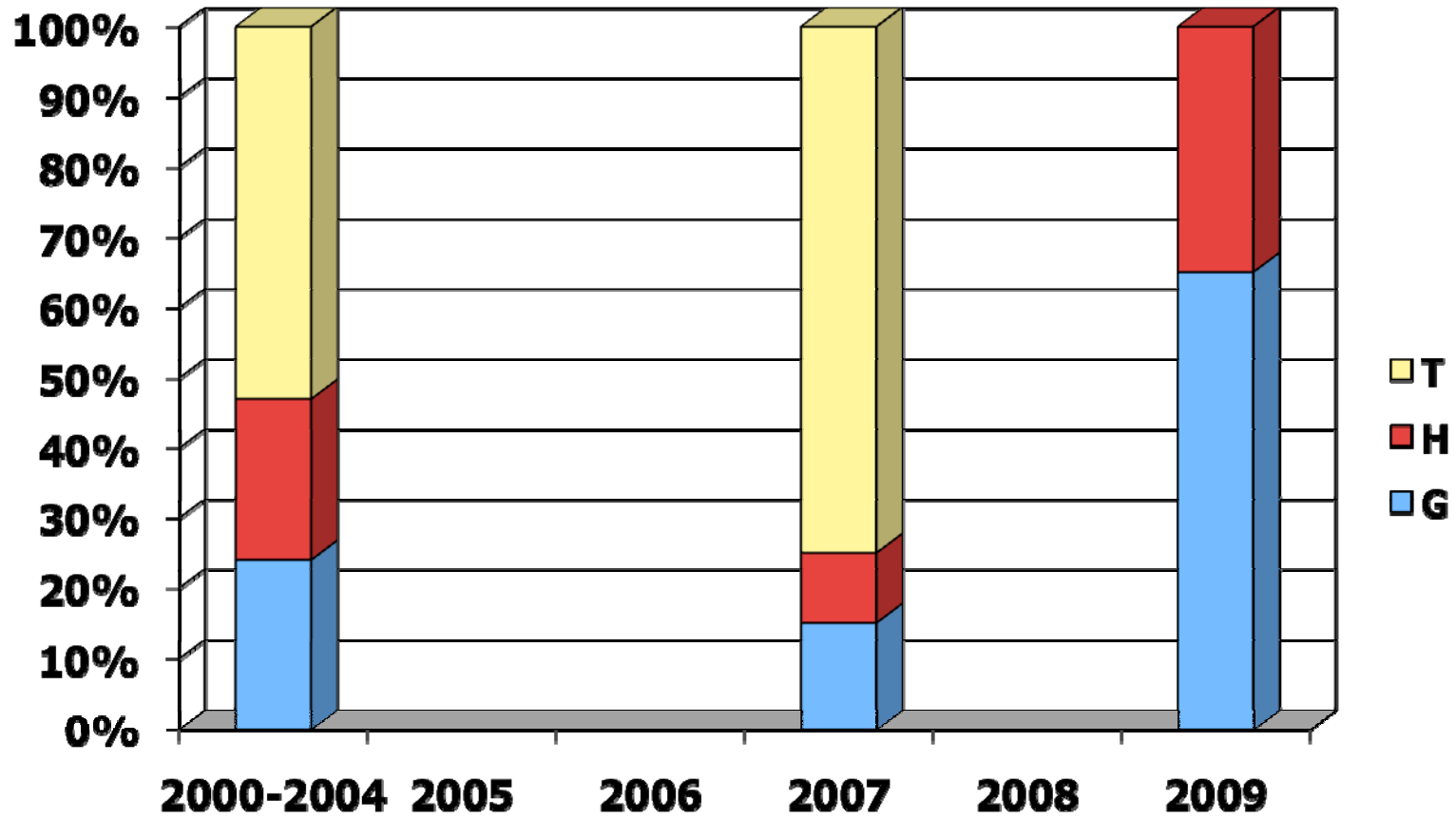
% each species per year at Moss Landing (data from 2 loci – ITS, Glu)



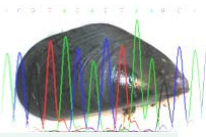
Data published by Braby et al.



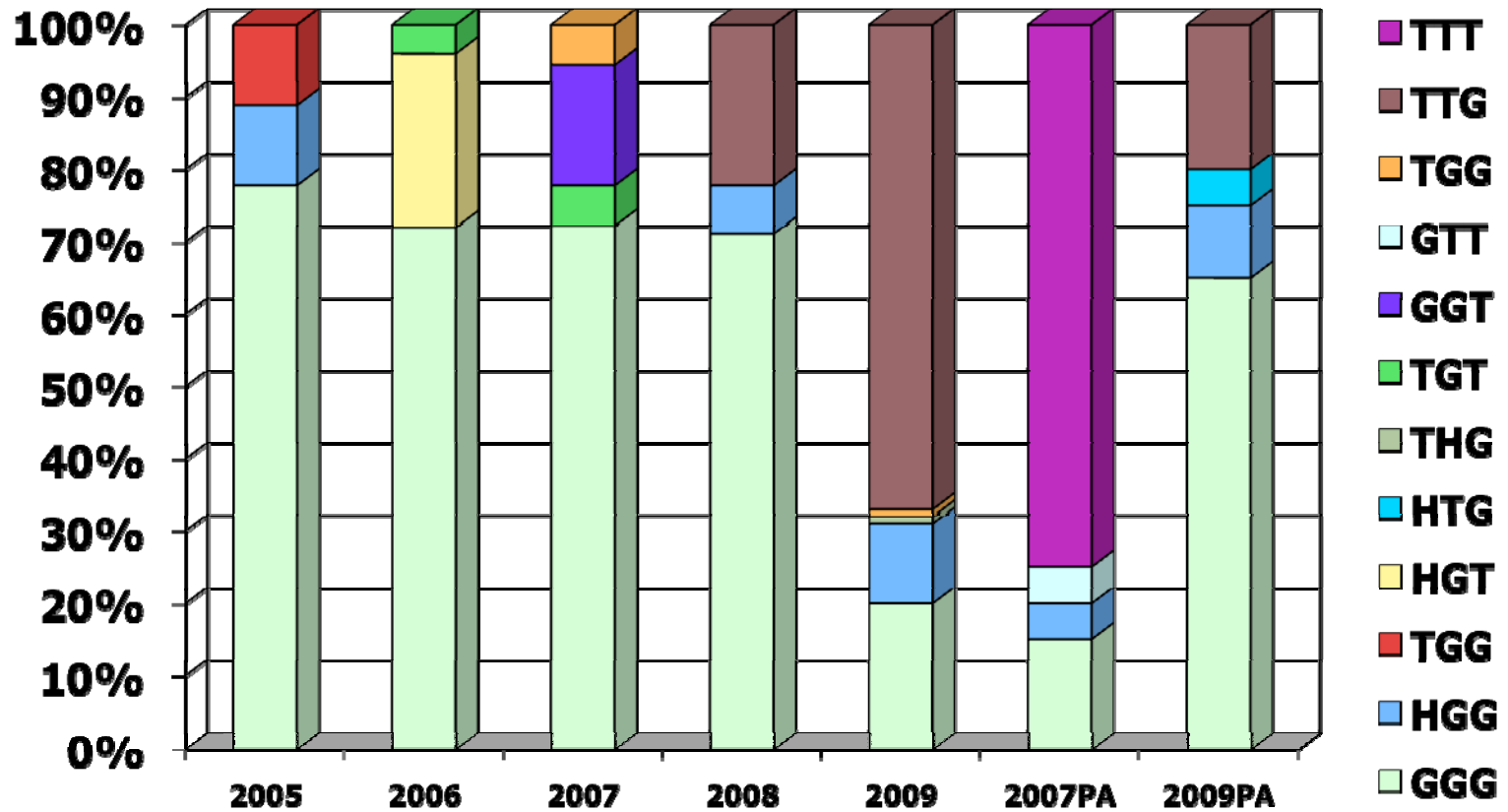
% each species per year at Palo Alto (data from 2 loci – ITS, Glu)



Data published by Braby et al.

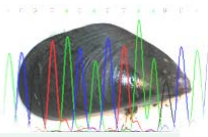


Occurrence of genotypes per year



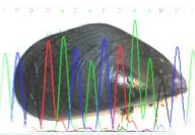
Genotypes denoted as species pattern indentified for ITS-Glu-CO3.

(PA = Palo Alto site)

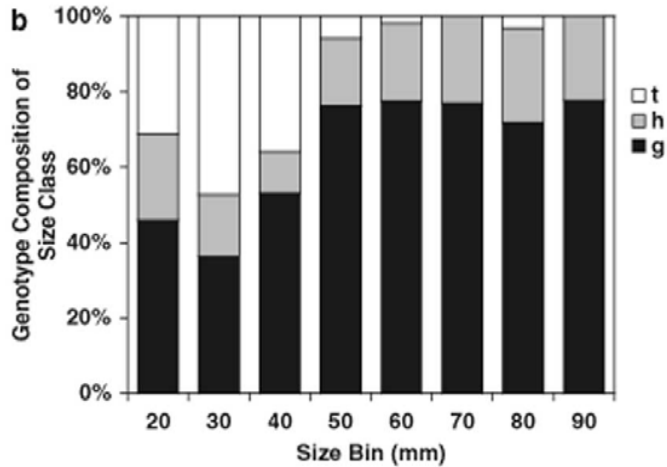
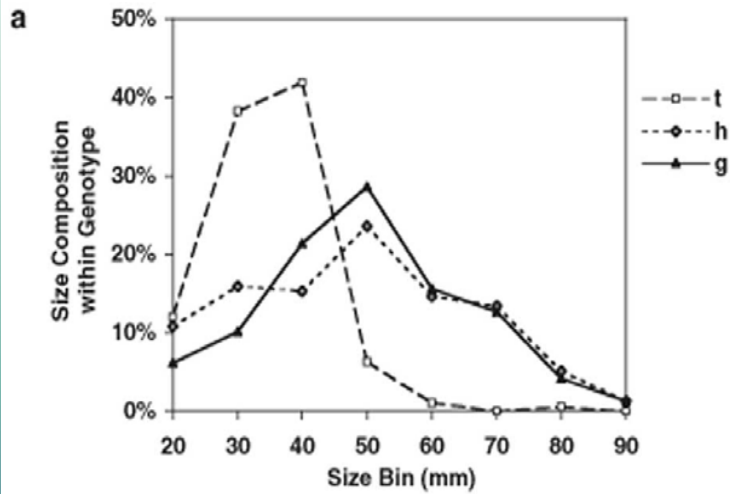


Results

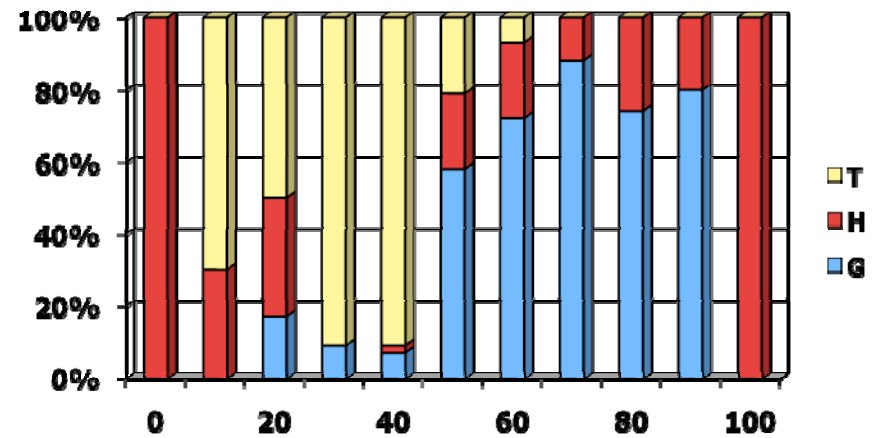
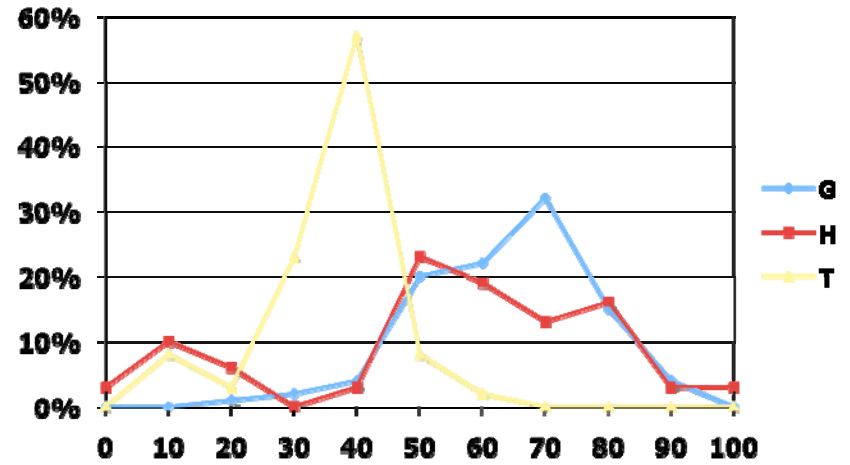
- ▶ Analysis at 3 loci finds only hybrids and *M. galloprovincialis* in Moss Landing
 - ▶▶ Analysis of only nuclear loci (2 loci) would have concluded the presence of some *M. trossulus*
 - ▶▶ The relative ratios of each species varies over time
- ▶ Genotypes (species level at 3 loci) changes over time
- ▶ Previous evidence showed that smaller mussels in the population tended to be natives (see next slide) (Braby & Somero 2005, Marine Biology)

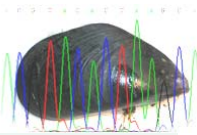


Braby & Somero 2000-04

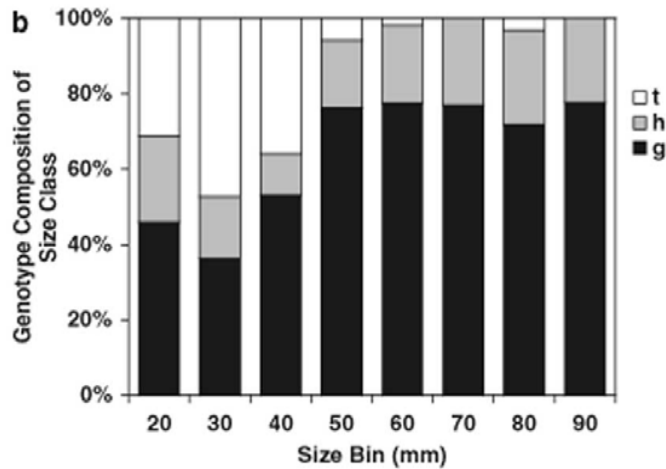
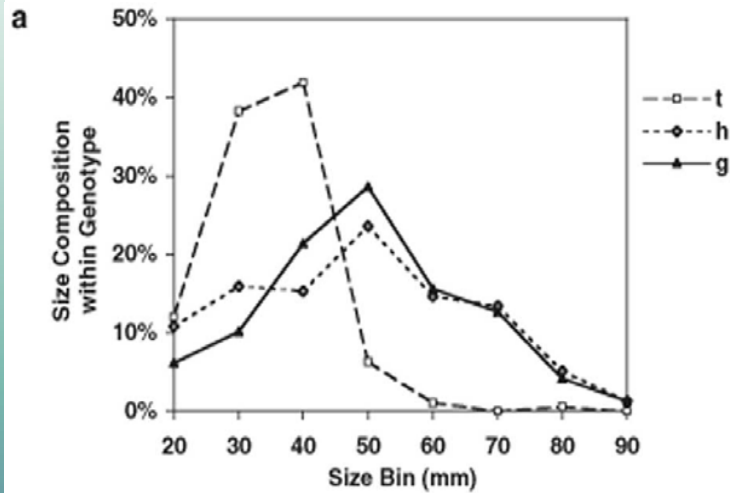


MBB 2006-09, 2 Loci

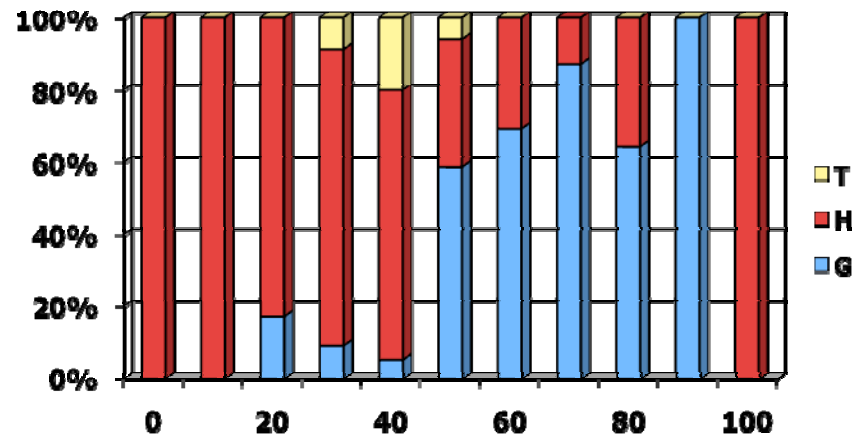
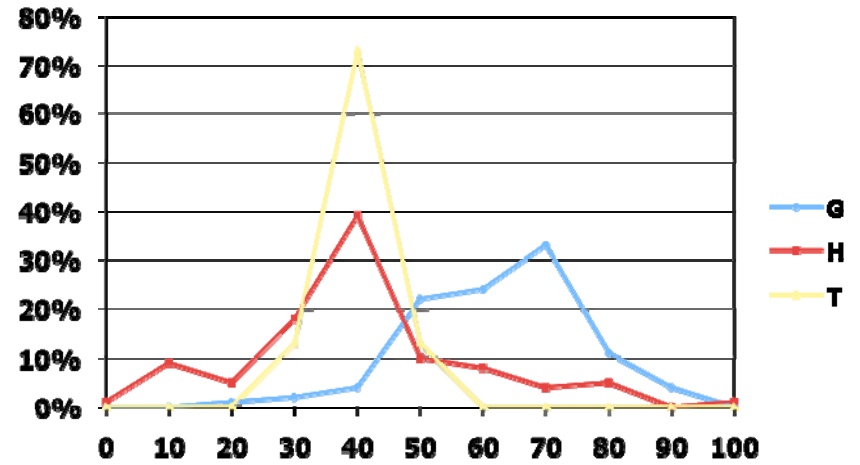


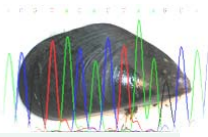


Braby & Somero 2000-04 (2 loci)



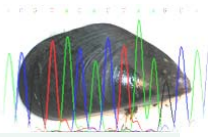
MBB 2006-09 (3 loci)





Preliminary Conclusions

- ▶ *Mytilus galloprovincialis* appears to be spreading northward, hybridizing with AND displacing *Mytilus trossulus*
 - ▶▶ In Moss Landing Harbor (2006-09) only hybrids and *M. galloprovincialis* were detected
 - ▶▶ In potential refuges for the native (Moss Landing & Palo Alto) only hybrids and *M. galloprovincialis* were detected
 - ▶▶ Though the trends are supported, the actual percentages vary greatly from year to year



Preliminary Conclusions

- ▶ Using 3 loci rather than 2 loci, more hybrids and fewer natives are detected.
- ▶ As found previously, smaller mussels may be more “native”
 - ▶▶ In this study, there was a greater representation of both hybrids and *M. trossulus* in the smaller size groups
- ▶ *M. californianus* is distinguishable at all 3 loci and by morphology
 - ▶▶ The first C03 sequence was reported in this study