

MUSSEL (*MYTILUS CALIFORNIANUS* AND *M. GALLOPROVINCIALIS/TROSSULUS* HYBRIDS) IMMUNE SYSTEMS AS BIOMARKERS OF CONTAMINANT EXPOSURE IN SAN FRANCISCO BAY, CA.

Allison Luengen (Corresponding author, allisonl@cats.ucsc.edu, Environmental Toxicology, University of California at Santa Cruz, Santa Cruz, CA 95064), Carolyn S. Friedman (Bodega Marine Laboratory, Bodega Bay, CA 94923), A. R. Flegal (Environmental Toxicology, University of California at Santa Cruz, Santa Cruz, CA 95064).

This study investigated whether the immune system responses of mussels can be used as a more sensitive and biologically relevant indicator of contaminant exposure than more standard methods. Two species of mussels (*M. californianus* and *M. galloprovincialis/trossulus* hybrids) were deployed in San Francisco Bay, California and three independent measures of variations in their immune responses were correlated with metal concentrations in the water and in the tissues. Preliminary immune system measurements indicate that mussels from relatively contaminated sites exhibit elevated immune responses when compared with the responses in control mussels deployed in relatively pristine sites. This includes variations in the following parameters: (1) number of hemocytes, (2) percentage of cells that phagocytosed particles, and (3) a phagocytic index, which gives an average number of particles engulfed by each cell. Preliminary baseline percentages of phagocytosis were also more elevated in coastal mussels, *M. californianus*, than in endemic hybrids: *M. galloprovincialis/trossulus*. Consequently, the hybrids may be a more appropriate biomonitor of contaminant effects within the estuarine system than the coastal mussels that are currently deployed as monitors of contamination in the Bay.

Introduction

San Francisco Bay has been aptly nicknamed the urbanized estuary (Conomos et al., 1979). Its watershed is home to almost 10 million people, including the major cities of San Francisco, Oakland, and San Jose (San Francisco Estuary Project, 1992). This development has had dramatic effects, resulting in loss of over 95% of San Francisco Bay's tidal marshes, a 70% reduction of freshwater input to the Bay, and on-going inputs of trace metals and organic compounds (Nichols et al., 1986). With respect to the magnitude of change that has occurred, San Francisco Bay is now considered the most modified estuary in the United States (Nichols et al., 1986).

The purpose of this study is to determine if these modifications, particularly the high contaminant concentrations, are adversely impacting the Bay's organisms. To answer this question, we evaluated mussel immune system responses as a potential biomarker of contaminant exposure. Because the immune system is highly integrated with other systems, it is likely to respond to stresses that are occurring anywhere in the organism as a result of contaminant exposure (Pipe and Coles, 1995). Also, immune system responses are meaningful because a compromised immune system renders the organism more susceptible to disease (Pipe and Coles, 1995). Immune responses have the potential to be used as part of on-going monitoring efforts if we find that they correlate with contaminant concentrations.

We hypothesize that mussels from contaminated sites will show elevated immune responses compared to mussels from more pristine sites. Fisher et al. (1999) suggest that organisms exposed to contaminants may increase their total number of hemocytes, or circulating blood cells, as well as their rates of phagocytosis, or engulfment foreign particles, in order to sequester contaminants before they cause harm. Additionally, we expect that the two different

species of mussels in the experiment will show different degrees of immune response either due to interspecies differences or because the endemic bay species, *Mytilus galloprovincialis/trossulus* hybrid, may experience less stress in the Bay than the coastal mussel. *Mytilus californianus*, the coastal mussel, has been historically deployed in the Bay for monitoring although it is not endemic.

Mussels are ideal for this immune system work because they have a long history of deployment as sentinel organisms; thus detection of stress in mussels can serve as an indicator of the overall "health" of the Bay. In mussels, phagocytosis is the centerpiece of the immune system. Hemocytes are the chief mechanism for phagocytosing and destroying microorganisms (Pipe and Coles, 1995). Past researchers have measured changes in phagocytosis following exposure to a variety of contaminants at different concentrations, including heavy metals and polycyclic aromatic hydrocarbons (Pipe and Coles, 1995).

In San Francisco Bay, contaminant concentrations and thus potential exposure to mussels vary throughout the Bay and reflect the different hydrologic regimes. In general, metal concentrations are lowest in the North and Central Bays, which are relatively well flushed, and highest in the South Bay (Figure 1). Fresh water inputs to the South Bay are relatively minor and residence times of contaminants are correspondingly long (Flegal et al., 1991). Copper, nickel, zinc, silver, and mercury have elevated concentrations in Bay waters, and they sometimes exceed state and federal water quality criteria. Although these high concentrations arouse concern, immune system measurements have the potential to show if the high concentrations are actually detrimental to organisms.

To evaluate immune responses, we measured the following parameters: (1) a phagocytic index, or average number of yeast particles engulfed by each hemocyte, (2) the number of hemocytes per mL of hemolymph, and (3) phagocytosis, or percentage of hemocytes that have engulfed yeast particles. The phagocytosis results for both mussel species are presented in this paper.

Methods

Deployment and Retrieval

Working with the San Francisco Estuary Institute's Regional Monitoring Program (RMP), we deployed mussels of both species in San Francisco Bay from June to September of 1999 as described by Gunther et al., 1999. Mussels were deployed at two stations in South San Francisco Bay (Redwood Creek and Dumbarton Bridge), which were known to have high contaminant concentrations (Figure 1). They were also deployed in Horseshoe Bay because this well-flushed station was known to have low contaminant concentrations. Finally, reference mussels were set up in tanks of clean running seawater at the Bodega Marine Labs. At the end of three months, the mussels were collected from the Bay and transported live on ice to the Bodega Marine Labs, where they were kept in tanks of clean running seawater until analysis.



Figure 1. Sampling sites in San Francisco Bay, CA. Redwood Creek and Dumbarton Bridge are the contaminated South Bay sites. Horseshoe Bay has lower contaminant concentrations. The Bodega Bay control site, located north of San Francisco Bay, is not shown.

Steps to Measure Phagocytosis

We developed a new method to measure phagocytosis in mussels, rejecting Anderson et al.'s 1995 oyster method because it damaged the more sensitive mussel cells (Luengen et al., 2000). In this new method, hemolymph is first withdrawn from the posterior adductor muscle of live mussels. Then, hemocyte densities are determined by counting live cells with a hemocytometer. Next, hemocyte densities are adjusted using the organism's own hemolymph to 2×10^5 cells/mL. Hemocytes are incubated on slides (3 replicates per individual) with yeast particles at a concentration of 1×10^7 particles/mL for 45 minutes and are then dipped in Diff-Quik stain. Finally, the slides are counted at any convenient later time to determine percentage of phagocytosis using an oil immersion lens microscope.

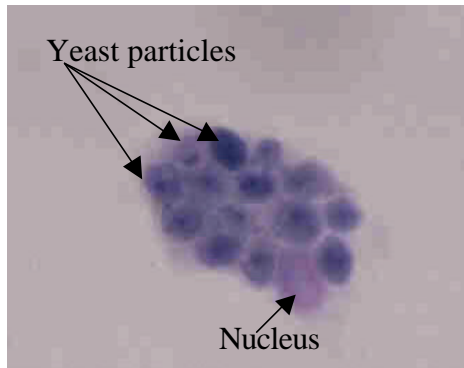


Figure 2. Hemocyte processed by new phagocytosis method. The cell has engulfed multiple yeast particles.



Figure 3. Phagocytosis in one mussel (*M. galloprovincialis/trossulus* hybrid) from each site. Each dot is the mean of the three replicates for that organism. The box shows one standard deviation above and below the mean. The bars show the range.

Preliminary Results and Discussion

The new method successfully handles hemocytes without damaging them and produces repeatable results. Figure 2 shows a healthy hemocyte processed by this method that has engulfed high numbers of particles. The results are also repeatable, meaning that the three replicates for each mussel are very consistent with each other. Figure 3 illustrates this repeatability for one mussel from each station. The relative standard deviations for this representative sampling day (Figure 3) are all less than 10%, which is equivalent to instrumental precision.

Preliminary results (Figure 4) show that organisms from the contaminated Dumbarton Bridge and Redwood Creek sites have higher levels of phagocytosis than those from the less contaminated Horseshoe and Bodega Bays. This agrees with our hypothesis that organisms from contaminated sites will show elevated immune responses.

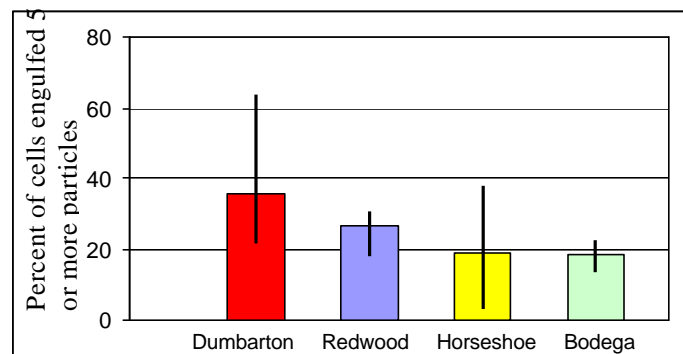


Figure 4. Phagocytosis is higher at the contaminated sites. Both species (n=4) have been included to give the averages above. The black vertical lines show the range

Preliminary results also show interspecies differences in phagocytosis rates. Whereas both species have similar percentages of phagocytosis at the contaminated sites, at the Bodega reference sites the two species show dramatically different percentages of phagocytosis (Figure 5). This suggests that, for monitoring purposes, the two species may be different.

Overall, preliminary results indicate that phagocytosis rates in mussels deployed in San Francisco Bay vary with contaminant concentrations and, therefore, immune responses can be used as a sensitive, sublethal indicator of the Bay's "health."

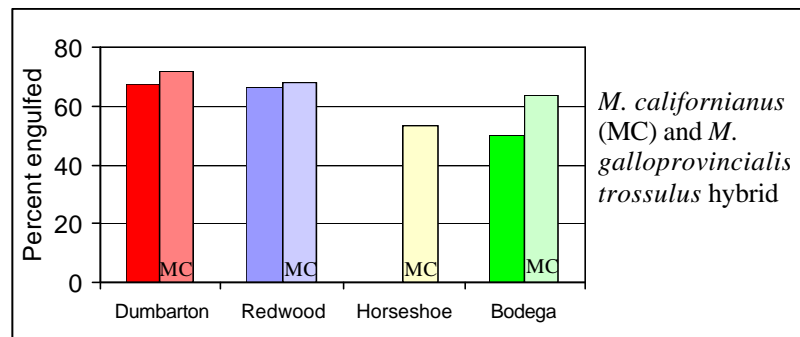


Figure 5. Preliminary results show differences in the phagocytosis capabilities of the two species at the Bodega station.

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