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# THE COMBINED EFFECTS OF CHEMICAL AND NATURAL STRESSORS ON PHOSPHAGENS AND NONSPECIFIC IMMUNITY IN TWO SPECIES OF ABALONE

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# THE COMBINED EFFECTS OF CHEMICAL AND NATURAL STRESSORS ON PHOSPHAGENS AND NONSPECIFIC IMMUNITY IN TWO SPECIES OF ABALONE

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## ABSTRACT

Whereas there is considerable information on the toxicity of xenobiotics for marine invertebrates, as measured in terms of lethality, comparatively little is known of the more subtle physiological effects of chronic exposure to sublethal doses of toxicants. Environmental pollutants can have acute effects on organisms exposed in that the concentration of toxicant may be sufficiently high to cause injury or death in a short period of time. It is more likely, however, that toxicants are present in low concentrations and have sublethal effects on aquatic organisms which predispose them to debilitating disease. By examining phosphagen concentrations after exposure to sublethal levels of a model chlorinated phenol, pentachlorophenol (PCP), it is possible to elucidate the subtle alterations in energy regulation that are vital for the survival of any organism. PCP is a universally toxic compound which is used as a general biocide and because of its fungicidal properties, as a wood preservative. PCP induced reduction in energy production might effect an organism's ability to forage, reproduce, and defend itself from predators as well as endure adverse environmental conditions. This thesis

initially addresses how exposure to pentachlorophenol combined with salinity stress effects concentrations of phosphoarginine (PA), adenosine triphosphate (ATP), intracellular pH (pHi), and inorganic phosphate (Pi) among both red (*Haliotis rufescens*) and black abalone (*H. cracherodii*) using in vivo $^{31}$P NMR spectroscopy. Black abalone appear to have a greater overall resistance to PCP exposure and salinity variations as affirmed by $^{14}$C PCP uptake analysis demonstrating their slower uptake of the biocide.

The study continues by examining how sublethal exposure to this general biocide might effect an organism's ability to defend itself against microorganisms, thereby representing an experimental approach to describing functional differences between hemocytes that have been exposed to a toxin and hemocytes that are unexposed. Using the same flow-through exposure system as that developed for NMR spectroscopy, abalone were exposed to sublethal concentrations of PCP in combination with salinity variations. A poorly functioning immune system in animals inevitably exposed to a number of invading microorganisms can cause their death. It is therefore important to describe how toxins effect these defense mechanisms. Because the timing of the decline in various species of abalone have shown tremendous variation the defense capabilities in hemocytes of red versus black abalone after exposure to PCP was compared. A tool for comparison used with NMR was adopted for assessment of host defense mechanisms. The metabolic endpoint (MEP) served as a gauge for the level of stress that the abalone were

enduring. It is defined as the *time* for the spectral peak area of inorganic phosphate (Pi) to reach one-half that of phosphoarginine (PA) and was demonstrated to be the maximum amount of time abalone could be exposed and still consistently recover. These exposure periods were employed for assessment of hemocyte defense mechanisms to determine if the effects seen using NMR could be correlated with impaired immune function.

Phagocytic and chemotactic function after *in vivo* exposure of abalone to PCP and salinity stress was examined. Most noteworthy was the dramatic effect that high salinity, particularly in combination with PCP, had on both red and black abalone; red abalone demonstrating the most profound effects. As was previously demonstrated using $^{31}P$ NMR, it took black abalone nearly twice as long to manifest toxic effects of PCP on phagocytic and chemotactic ability as that for red abalone. Furthermore, phagocytic function proved to be significantly more robust overall among black abalone.

Finally, mechanisms employed to kill microorganisms among red and black abalone were examined. These included the quantification of reactive oxygen intermediates as well as lysozyme. While exposure of red abalone to salinity variations plus PCP caused a stimulatory effect on the production of microbicidal reactive oxygen intermediates (ROIs) the same exposure parameters caused an inhibitory effect on ROI production among black abalone. Furthermore, no lysozyme-like activity was discernable for any of the exposure parameters even upon stimulation with a variety of antigens.

As aquatic organisms are rarely exposed to one stress at a time the research performed in this dissertation has contributed to the understanding of the impacts of multiple environmental stresses. Those responsible for managing threatened aquatic environments will hopefully find the information herein useful to the understanding of the processes and mechanisms that govern such effects as stress.

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