Hemocyte responses of the oyster *Crassostrea hongkongensis* exposed to diel-cycling hypoxia and salinity change

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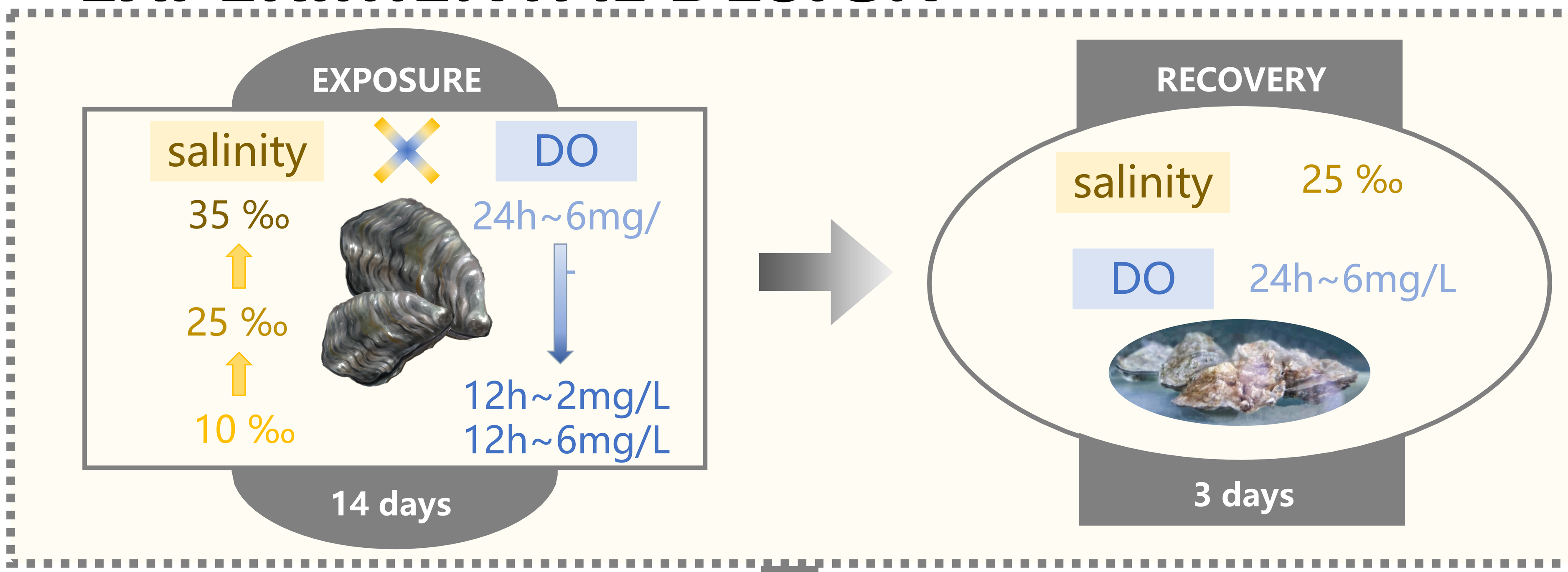
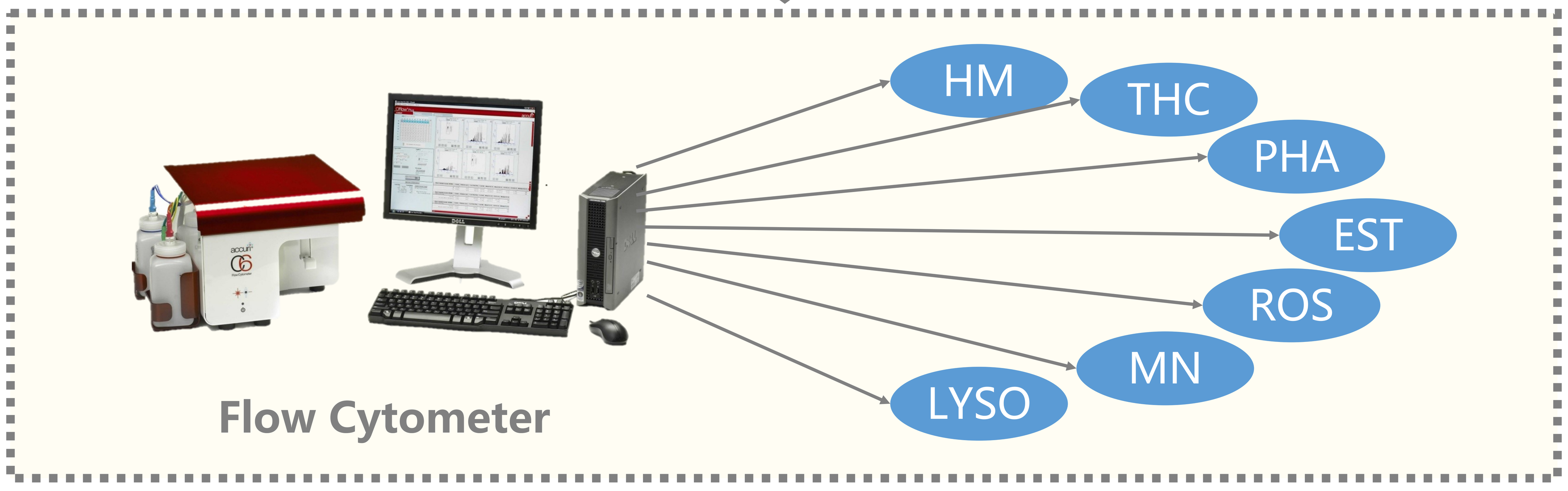
INTRODUCTION

Given the global climate change, increasingly frequent rains and typhoons have occurred in recent years, and the salinity in seawater is incrementally in a periodical change. Therefore, the fauna in coastal and estuarine waters, like bivalves which have weak migration, are facing the survival challenges from severe and frequent salinity change. Salinity in coastal and estuarine waters always varies from near-zero low salinity to high salinity depending on the amount of freshwater input. Also, anthropogenic activity impacts coasts and estuaries, causing high frequency of eutrophication, and forming hypoxic waters. Low DO can cause mortality, high migration, high risk of predation and infection, change of food resources and decrease of immune responses in fish and aquatic invertebrates, especially when DO is less than 2 mg/L the immune function and growth can be impeded in aquatic fauna significantly, and individual mortality may increase. We studied the changes in various immune parameters of Hong Kong oysters under the conditions of diel-cycling hypoxia and salinity change and flow cytometry was used to determine the hemocyte parameters.

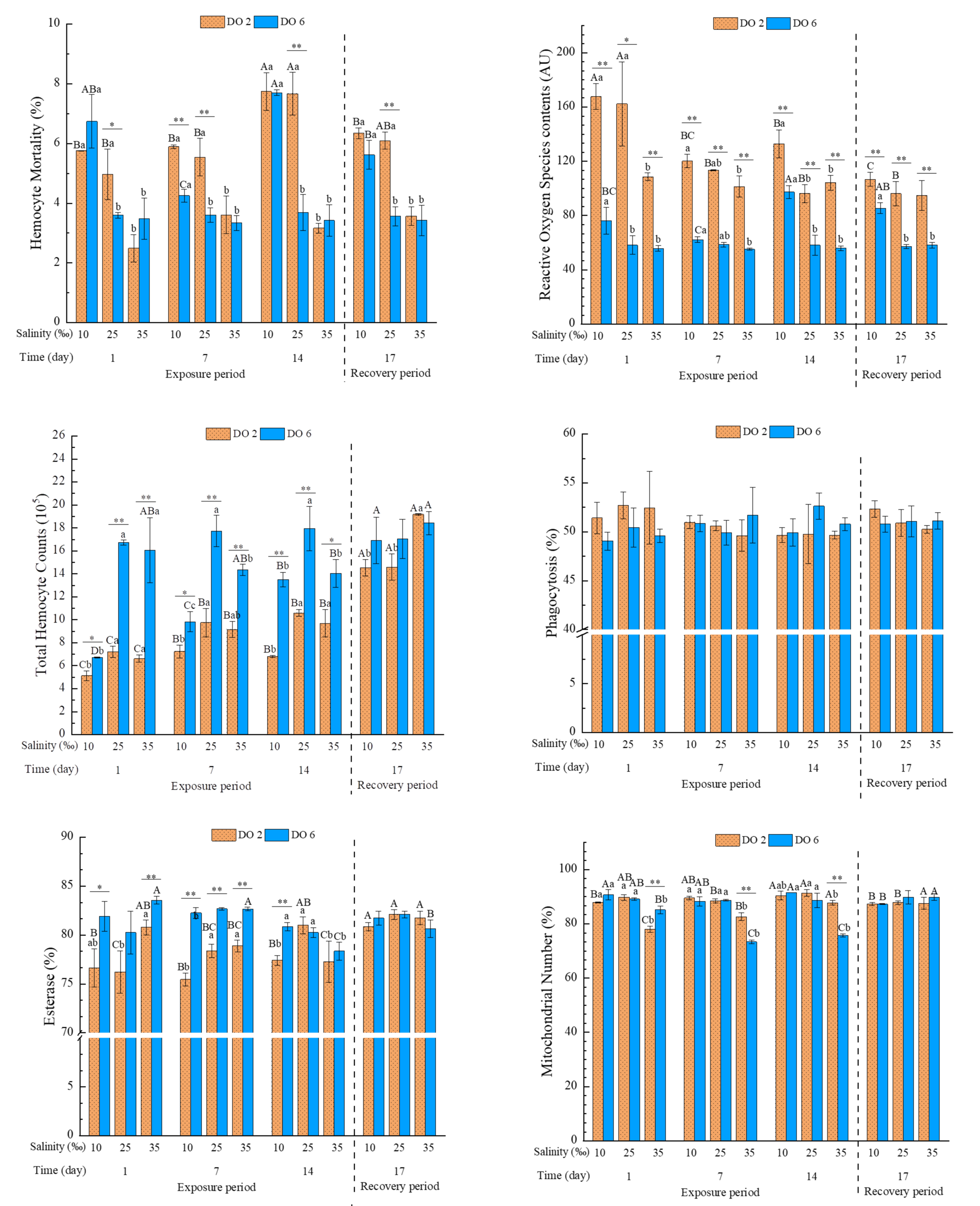
EXPERIMENTAL DESIGN

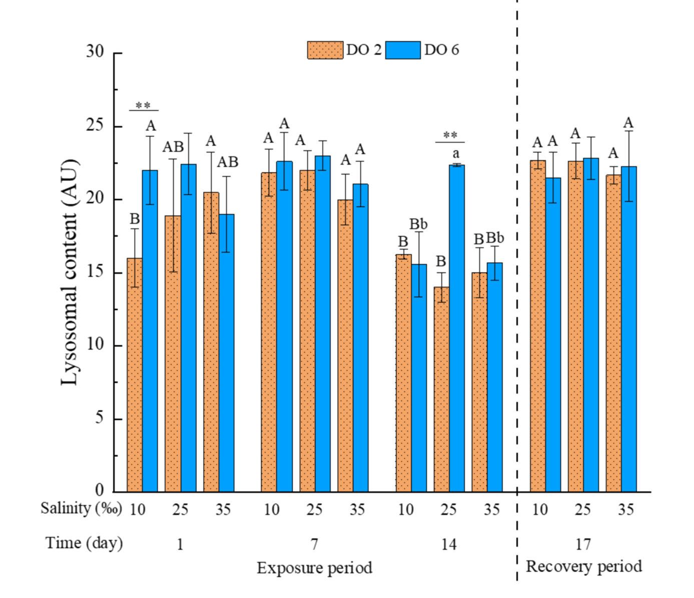




RESULTS

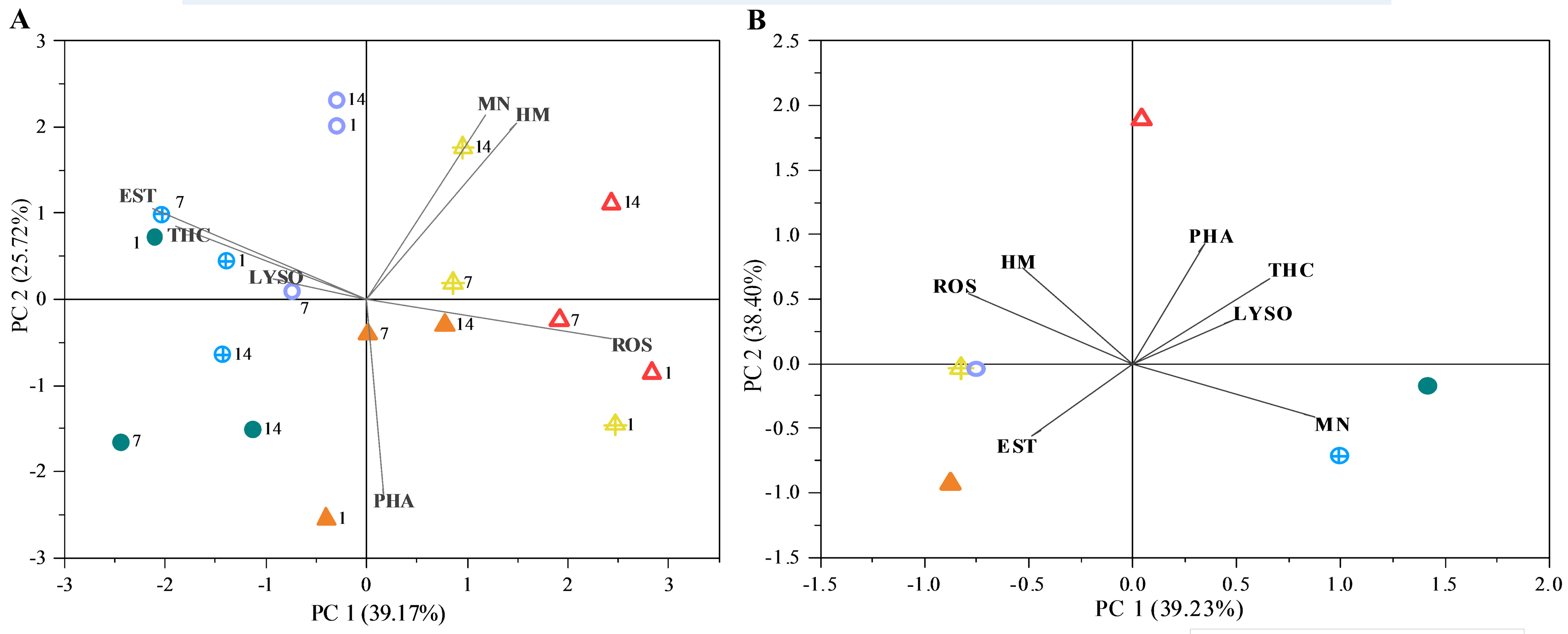
PART 1 Result of hemocyte parameters





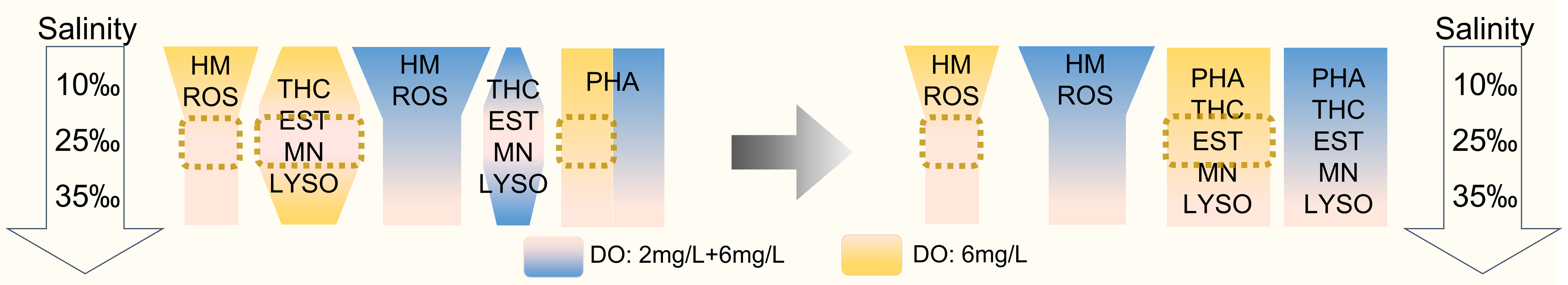
Hemocyte mortality (HM), Reactive oxygen species (ROS), Total hemocyte counts (THC), Phagocytosis (PHA), Esterase (EST), Mitochondrial number (MN) and Lysosomal content (LYSO) of *C. hongkongensis* exposed to six combinations of salinity (10, 25, and 35‰) and DO (2 and 6 mg/L) at 1, 7 and 14 days during exposure period and 3 days for recovery period. Different capital letters indicate significant differences among time points within each salinity level in hypoxia level (2 mg/L) or control group (6 mg/L) ($p < 0.05$). Different small letters indicate significant differences between salinity within each time point in salinity level in hypoxia level (2 mg/L) or control group (6 mg/L) ($p < 0.05$). Asterisk indicates significant differences between DO within each time point and fixed salinity treatment, in which * represents significant difference ($p < 0.05$) and ** represents highly significant difference ($p < 0.01$).

PART 2 Principal Components Analysis



A, Biplot originating from PCA integrating all measured variables (HM, PHA, EST, ROS, THC, LYSO and MN) and three time points (days: 1, 7 and 14) at six different treatments during the exposure period. B, Biplot originating from principal component analysis integrating all measured variables (HM, PHA, EST, ROS, THC, LYSO and MN) at six different treatments after three-day recovery. The scores of the experimental conditions were shown.

CONCLUSION



Variable salinity and hypoxia are typical stressors for marine organisms inhabiting estuaries, coastal and intertidal zones where natural or anthropogenic activity may result in large changes of environmental salinity and DO. Under the stress of salinity changes and diel-cycling hypoxia, hemocyte functions of Hong-Kong oyster *C. hongkongensis* were impaired. In addition, low salinity did massive harm to immunity of oysters than high salinity. Meanwhile hypoxia at night shaved immune functions substantially, which was synergistic with low salinity. Though long-term exposure to salinity changes with diel-cycling hypoxia was adverse, short-term recovery could be helpful for oysters to recover, apart from hemocyte mortality and ROS. Our results provide useful information for health assessment of oysters and bring new insights to oyster aquaculture industries.

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