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Global Proteome Profiling of a Marine Copepod and the Mitigating Effect of Ocean Acidification on Mercury Toxicity after Multigenerational Exposure

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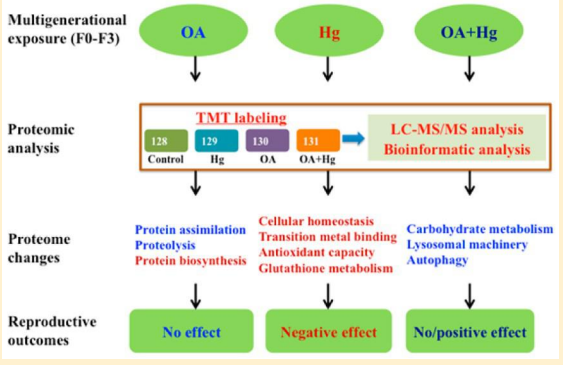
Supporting Information

ABSTRACT: Previously, we found that ocean acidification (OA) mitigates mercury (Hg) toxicity to marine copepod *Tigriopus japonicus* under multigenerational exposure (four generations, F0−F3). To determine the response mechanisms of *T. japonicus* against long-term exposure to OA and Hg pollution, we investigated the proteome of F3 copepods after multigenerational exposure to four conditions: pCO_2 400 μatm + control; pCO_2 1000 μatm + control; pCO_2 400 μatm +1.0 μg/L Hg; and pCO_2 1000 μatm +1.0 μg/L Hg. Functional enrichment analysis indicated that OA enhanced the copepod's energy production mainly by increasing protein assimilation and proteolysis as a compensatory strategy, which explained its physiological resilience to reduced pH. Conversely, Hg treatment decreased many critical processes, including ferric iron binding, antioxidant activity, cellular homeostasis, and glutathione metabolism, and these toxic events could translate into higher-level responses, i.e., restrained reproduction in copepods. Importantly, the mediation of Hg toxicity in *T. japonicus* by OA could be explained by the enhanced lysosome-autophagy pathway proteomes that are responsible for repairing and removing damaged proteins and enzymes under stress. Overall, this study provided molecular insights into the response of *T. japonicus* to long-term exposure of OA and Hg, with a particular emphasis on the mitigating impact of the CO_2-driven acidification on Hg toxicity.



INTRODUCTION

Ocean acidification (OA), a continuous decrease in pH resulting from the absorption of increased anthropogenic CO_2, has become a major global threat to the fitness of marine ecosystems. Atmospheric pCO_2 has steadily increased from a preindustrial level (i.e., approximately 280 μatm) to a present-day concentration of ~400 μatm.[1] Average ocean surface pH has been reduced by 0.1 units (a 26% increase in the hydrogen ion concentration) in comparison with levels during the industrial revolution,[2,3] and the atmospheric CO_2 level is projected to reach 1000 μatm by the end of 2100, leading to a decline of 0.3−0.5 units in seawater surface pH (7.6−7.9).[4] Also, some low-pH "hot spots" in coastal zones may already have experienced the pH values forecasted for the end of 2100 as a result of a multitude of drivers, e.g., upwelling of deeper acidified water along continental shelves[5] and high levels of heterotrophic respiration,[6,7] thereby subjecting the organisms in these zones to lower pH values than projected for the global sea surface. For instance, in the northwestern-northern near-shore areas of Bohai Sea, China, seawater pH values were 7.64−7.68 equal to that predicted for the end of 2100.[7] Elevated pCO_2 in seawater can cause hypercapnia and acidosis[8] and may subsequently result in redistribution of energy into growth and reproduction caused by the mobilization of energy-costly acid−base regulatory processes to counteract reduced pH. Accordingly, OA disturbs a multitude of physiological processes including calcification,[9] metabolism,[10] survival,[11] development,[12] and reproduction[13] in calcifying and noncalcifying organisms.

In addition to increasing global atmospheric CO_2 levels, human activities have also led to a mass of mercury (Hg) emission into the atmosphere, which is eventually deposited in marine environments especially coastal zones. Thus, OA and Hg pollution may co-occur in these environments. Indeed, Hg pollution is a severe problem for marine environments in China[14,15] because it contributes approximately 28% to the global Hg emission into the atmosphere. The maximum total Hg (T-Hg) has reached 2.7 μg/L in the seawater of Jinzhou Bay, Bohai Sea, about 3 orders of magnitude higher than the

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