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Quantitative Shotgun Proteomics Associates Molecular-Level Cadmium Toxicity Responses with Compromised Growth and Reproduction in a Marine Copepod under Multigenerational Exposure

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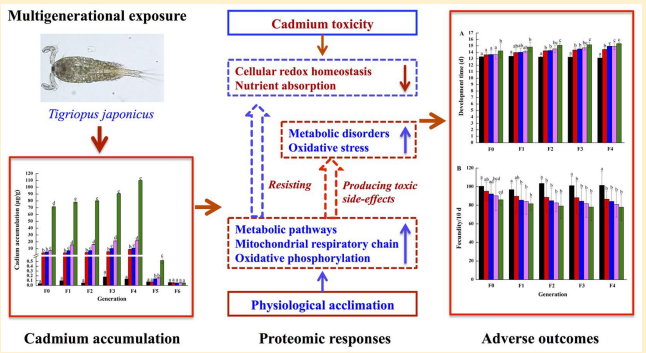
Supporting Information



ABSTRACT: In this study, the copepod *Tigriopus japonicus* was exposed to different cadmium (Cd) treatments (0, 2.5, 5, 10, and 50 μg/L in seawater) for five generations (F0−F4), followed by a two-generation (F5−F6) recovery period in clean seawater. Six life-history traits (survival, developmental time of nauplius phase, developmental time to maturation, number of clutches, number of nauplii/clutch, and fecundity) were examined for each generation. Metal accumulation was also analyzed for generations F0−F6. Additionally, proteome profiling was performed for the control and 50 μg/L Cd-treated F4 copepods. In F0−F4 copepods, Cd accumulated in a concentration-dependent manner, prolonging the development of the nauplius phase and maturation and reducing the number of nauplii/clutch and fecundity. However, during F5−F6, Cd accumulation decreased rapidly, and significant but subtle effects on growth and reproduction were observed only for the highest metal treatment at F5. Proteomic analysis revealed that Cd treatment had several toxic effects including depressed nutrient absorption, dysfunction in cellular redox homeostasis and metabolism, and oxidative stress, resulting in growth retardation and reproduction limitation in this copepod species. Taken together, our results demonstrate the relationship between molecular toxicity responses and population-level adverse outcomes in *T. japonicus* under multigenerational Cd exposure.

INTRODUCTION

Cadmium (Cd) is a metal that is nonessential in most organisms (with the exception of marine diatom)[1] and that can be toxic at even low doses. Cd toxicity has been attributed to the induction of oxidative stress,[2] dysfunction in calcium homeostasis,[3] depletion of cellular sulfhydryl groups,[4] and substitution for functional essential metals (e.g., zinc),[4,5] leading to cytotoxic events (e.g., lipid peroxidation and DNA damage). Cd thus produces multiple adverse effects in living organisms, including humans. In the planktonic marine copepod *Centropages ponticus*, 0.2 μg/L Cd exposure can cause oxidative stress by inducing lipid peroxidation, which significantly affects the enzyme activity and protein synthesis.[6]

Anthropogenic activities have led to Cd pollution becoming a severe environmental problem in many estuarine and coastal waters. In 2005 and 2006, water samples collected from 30 sites to explore the spatial distribution and temporal changes in dissolved metals in the seawater of Jinzhou Bay (China) revealed Cd concentrations from 1.65 to 2.01 μg/L.[7] Indeed, Cd concentrations have reached 73.8 mg/L in some heavily polluted coastal areas (e.g., the Dardanelles).[8] In such a seriously contaminated environment, the marine biota could be exposed to Cd pollution through many generations. Although many studies have examined the long-term effects of Cd pollution on freshwater organisms under multigenerational exposure,[9−11] very little information has been presented concerning its

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