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Antioxidant defenses and biochemical changes in pacu in response to single and combined copper and hypercapnia exposure

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A range of compounds and environmental changes can influence fish welfare e.g. copper compounds and carbon dioxide concentration. Copper sulfate is a compound widely used in aquaculture as algaecide and high CO_2 concentration (hypercapnia) frequently occurs in aquaculture systems as a result of high fish density and low water exchange rates. Exposure to copper and/or to environmental hypercapnia can be stressful for certain fish species. This study investigated the potentially detrimental effects of CO_2 and its combination with copper on pacu. Fish (Wt = 40.20 ± 1.05 g) were distributed in experimental glass aquaria (n = 10; 180 L static system, 2 - 6 replicates for each group) and exposed for 48 h to control (without copper supply; normocapnic medium) (0Cu), 0.4 mg Cu^{2+} L^{-1} (0.4Cu), to hypercapnic medium (Hyp) (0CuHyp) and to 0.4 mg Cu^{2+} L^{-1} + Hypercapnic medium (Hyp) (0.4CuHyp). Results were tested by analysis of variance in a General Linear Model (GLM). If interaction effects were significant ($p < 0.05$), an F test was applied. The *t* test was performed to determine which individual groups differed from the control ($p < 0.05$). The results are presented as mean ± standard deviation. Mortality was not observed in any experimental group. Pacu was resistant to the exposure to both isolated and associated conditions. In liver the effect of the increase in lipid hydroperoxide concentration in response to the 0.4Cu and 0CuHpy was abolished in 0.4CuHyp. The response pattern of the superoxide dismutase (SOD) hepatic activity in response to 0.4Cu was the same from that of 0.4CuHyp. The increased SOD hepatic activity in response to copper exposure was independent on the aquatic CO_2 level. The decrease in glutathione peroxidase hepatic activity in response to the exposure of 0.4Cu and 0CuHyp was abolished in the 0.4CuHyp. Catalase (CAT) hepatic activity decreased in response to 0.4Cu and 0CuHyp, but did not change in response to 0.4CuHyp. This indicates that the combined effects of copper and hypercapnia exposure do not change the CAT hepatic activity. In relation to 0Cu the exposure to 0.4Cu did not change the plasmatic glucose concentration (Gluc), and the exposure of fish to 0CuHyp decreased the Gluc. However, fish exposed to 0.4CuHyp increased the Gluc. This suggests that the effect of the decrease in Gluc in response to the hypercapnic water is abolished in higher water copper concentration. The plasmatic lactate levels (Lact) did not change in response to the 0.4Cu, decreased in fish exposed to 0CuHyp and increased in fish exposed to 0.4CuHyp. There was no interaction between copper and CO_2 levels on the plasmatic concentration of pyruvate, ammonia and protein. These plasmatic variables displayed the same pattern to copper exposure in both normocapnic and hypercapnia waters. In pacu, regardless of the water CO_2, the exposure to copper decreased the branchial Na^+/K^+-ATPase (Na/K_b) activity in fish from group 0.4Cu and 0.4CuHyp. This suggests that the Na/K_b activity in response to aquatic copper is independent of the CO_2 water. Fish exposed to 0.4Cu and 0.4CuHyp increased the branchial metallothionein (MT_b) concentration and when exposed to 0CuHyp there was no difference from 0Cu. As a consequence, the increase in MT_b concentration occurs exclusively in response to water copper enhancement, regardless of the water CO_2. MT_b and Na/K_b were effective biomarkers, responding to copper in different CO_2 levels. Combined-factors, copper + hypercapnia, caused more effective disturbance in the biomarkers than single factors alone.