

## Effect of reactive oxygen species on coral bleaching

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This study attempts to obtain quantitative information on coral metabolic changes with increased hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and to investigate antioxidant enzyme activities of zooxanthellae and host corals against increased H<sub>2</sub>O<sub>2</sub> in seawater. We used the corals *Goniastrea aspera* and *Galaxea fascicularis* for exposure to various concentrations of H<sub>2</sub>O<sub>2</sub>. Coral reef decline has recently been observed worldwide, caused by changes in the environment following natural and anthropogenic activities. Hydrogen peroxide, a strong active oxygen species, is one of the photochemically formed chemicals in both the ocean and atmosphere. Because of its strong oxidizing power, H<sub>2</sub>O<sub>2</sub> affects plants and marine organisms. Increases in seawater temperature, irradiance and UV radiation can result in the formation of harmful, reactive oxygen species within zooxanthellae and coral hosts. Coral metabolism reflect the physiological condition of a coral colony. Metabolic activities were studied using a continuous-flow, complete mixing (CFCM) experimental system.

Without the addition of H<sub>2</sub>O<sub>2</sub>, coral metabolism, including gross primary production (photosynthesis) and calcification, was relatively stable and there were no significant metabolic changes, suggesting that without H<sub>2</sub>O<sub>2</sub> added to the CFCM system, the corals did not suffer from significant stress of the experimental system over a 12-day incubation period. When H<sub>2</sub>O<sub>2</sub> was added to the seawater, clear changes were observed in coral metabolism. Higher concentrations posed more stress to the coral colonies. Within 3 days, photosynthesis and calcification decreased due to the increased H<sub>2</sub>O<sub>2</sub>, but respiration was not affected. However, the synergistic effect of high H<sub>2</sub>O<sub>2</sub> combined with high seawater temperature resulted in a 134% increase in respiration rates, which surpassed the effect of either H<sub>2</sub>O<sub>2</sub> or high seawater temperature alone. Thus, the incubation experiments suggest that higher H<sub>2</sub>O<sub>2</sub> concentrations in seawater clearly influence coral metabolism. However, the results also suggest that current seawater H<sub>2</sub>O<sub>2</sub> levels in Okinawa are not likely to pose significant acute effects on the metabolic activities of corals.

The cellular response to the formation of oxygen radicals includes many defense mechanisms such as the increased activity of free radical scavenger enzymes such as superoxide dismutase (SOD) and catalase (CAT). SOD catalyzes the dismutation of superoxide into oxygen and H<sub>2</sub>O<sub>2</sub>, and CAT is responsible for degrading H<sub>2</sub>O<sub>2</sub> into water and oxygen. In the exposure experiments, CAT activities in both coral tissue and zooxanthellae increased with increased H<sub>2</sub>O<sub>2</sub>, but SOD activities remained relatively unchanged, suggesting that increased H<sub>2</sub>O<sub>2</sub> in seawater affected the coral cytosol but did not induce superoxide formation. In contrast, elevated seawater temperature caused both SOD and CAT activities in coral tissue and zooxanthellae to increase. We indicated that coral have a defense mechanism against increased H<sub>2</sub>O<sub>2</sub> in seawater. However, coral photosynthesis and calcification system were damaged by the increased H<sub>2</sub>O<sub>2</sub>. Though CAT activities were increased, they were not sufficient to scavenge all H<sub>2</sub>O<sub>2</sub>. Coral bleaching was not observed at the levels of H<sub>2</sub>O<sub>2</sub> tested during the 5-day exposure period. Although the long-term effects of H<sub>2</sub>O<sub>2</sub> remain unknown, these results suggest that coral bleaching is likely not to occur from 5 days exposure to increased H<sub>2</sub>O<sub>2</sub> concentrations in seawater. It may be that since the oxidative stress was not due to the in situ formation of H<sub>2</sub>O<sub>2</sub> in the symbiotic algae, corals did not expel zooxanthellae.