Palmer LTER: Hydrogen peroxide in the Palmer LTER region: IV. Photochemical interactions with dissolved organic matter

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The coupled production and utilization of dissolved organic matter (DOM) through the activities of phytoplankton production, predatory consumption, and microheterotrophic growth define the "microbial loop," which constitutes an important pathway of carbon and energy flow in all marine ecosystems (Azam et al. 1983). Comprehensive results from both the AMERIEZ (Antarctic Marine Ecosystem Research at the Ice-Edge Zone) and RACER (research on antarctic coastal ecosystem rates) programs indicate, however, a dramatic uncoupling of these anticipated algal-bacterial metabolic processes (Cota et al. 1990; Karl et al. 1991) to the extent that microbial loop processes appear to be negligible in the southern oceans coastal ecosystem. Several hypotheses, including organic substrate limitation, low-temperature inhibition, and chemical antagonism have been presented to explain these fundamental differences from other marine ecosystems (Karl 1993, pp. 1–63). To date, these hypotheses have not been explicitly tested by direct field experiments.

There is a new 20th-century challenge to southern oceans microbial assemblages in the form of an increased flux of ultraviolet (UV) light caused by an erosion of the atmospheric ozone layer (Frederick and Snell 1988). Smith et al. (1992) and Helbling et al. (1992) recently documented growth inhibitory, UV-dependent effects on antarctic marine phytoplankton populations. We hypothesized that UV radiation might also play a role in microheterotrophic microbial processes by direct interactions with DOM. Two independent pathways are proposed: (1) partial photolytic degradation or alteration of refractory DOM resulting in a supply of readily available organic substrates for bacterial metabolism and (2) complete photolytic degradation of DOM. The former pathway would enhance heterotrophic bacterial processes; the latter would effectively constitute a form of competition for the availability of organic nutrients and, perhaps, provide an independent nonbiological pathway for DOM decomposition.

Our initial testing of the DOM-photolysis hypothesis was during cruise 92-09 of the R/V *Polar Duke* (November 1992).

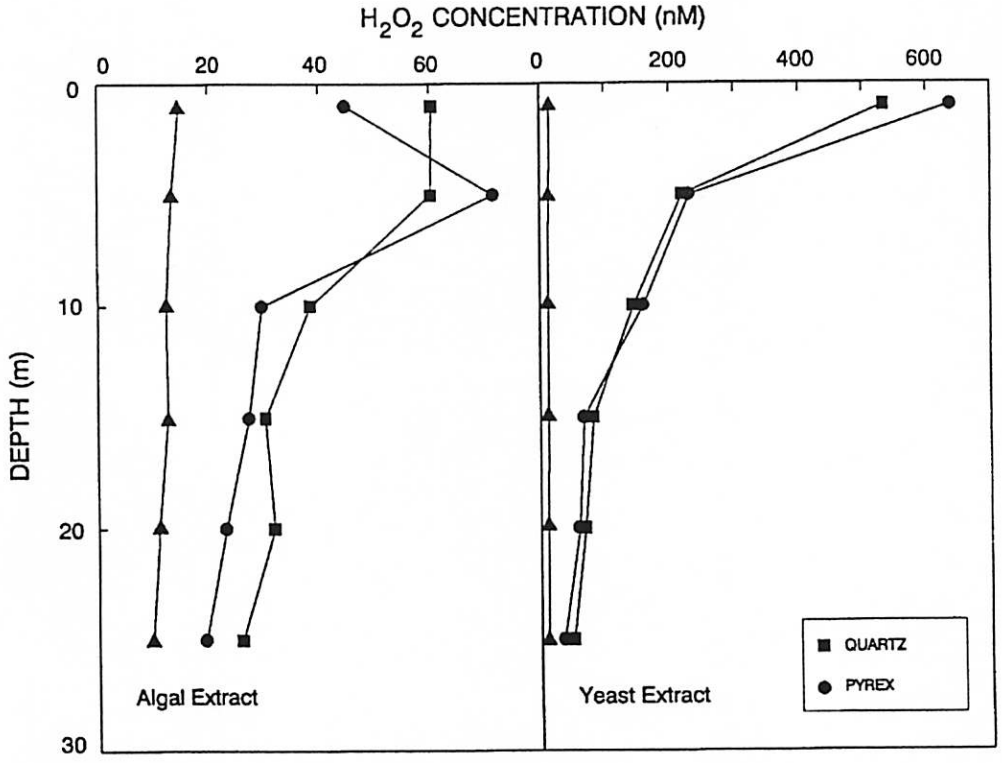


Figure 1. Yeast concentration-dependent photoproduction of H_2O_2 (nanomolar) in quartz and pyrex incubation vessels. A water sample was collected from 5 meters, spiked with Bacto yeast extract solution, and then incubated on board ship in a fully exposed flowing sea water incubator at –0.5°C for 4 hours (0900–1300 hours local). H_2O_2 concentrations were measured at the end of the 4-hour incubation period. The two stippled data points are the H_2O_2 concentrations measured in the negative control (no yeast extract added) following quartz and pyrex incubations, as described above.

Photoproduction of H_2O_2 in the presence of selected organic compounds or compound mixtures. All samples were incubated on board the ship in a fully exposed flowing sea water incubator at -0.5°C for 4–8 hours.

Organic compound(s)	Final concentration[a]	Treatment[b]	Net H_2O_2 accumulation rate[c]
None	—	Q	4.8
		P	<0.1
Glycine	500 µM	Q	6.5
		P	0.3
Proline	750 µM	Q	4.8
		P	1.3
AMP[d]	560 µM	Q	6.8
		P	<0.1
D-glucose	560 µM	Q	11.3
		P	1.6
Bacto peptone	80 mg L^{-1}	Q	85
		P	42
Bacto yeast extract	80 mg L^{-1}	Q	276
		P	275
Algal extract	1% (vol/vol)[e]	Q	37.7
		P	27.4
Sediment extract	1% (vol/vol)[f]	Q	25.2
		P	20.0

[a]µM denotes micromolar; mg L^{-1} denotes milligrams per liter.
[b]Q = quartz glass; P = pyrex glass.
[c]In nanomoles per liter per hour.
[d]AMP = adenosine monophosphate.
[e]Ice algae (10 milligrams wet weight) collected from the field and concentrated onto a glass fiber filter were extracted in 10 milliliters of boiling distilled water. The extract was filtered (0.2-micrometer filter) prior to use.
[f]Sediment (5 grams wet weight) from a depth of 150 m in Paradise Harbor was extracted in 10 milliliter of boiling distilled water. The extract was filtered (0.2-micrometer filter) prior to use.

We conducted several experiments to evaluate the effects of light on H_2O_2 production during shipboard and *in situ* incubations of sea water supplemented with a variety of exogenous organic compounds (table). The role of UV radiation was specifically investigated by comparing incubations of identical solutions in both quartz and pyrex glass containers. Quartz is essentially transparent to all UV radiation [transmits more than 85 percent of UV in the range 280–400 nanometers (nm)], whereas pyrex absorbs strongly in the range 280–305 nm (Helbling et al. 1992). We measured hydrogen peroxide (H_2O_2) concentrations (Resing et al., *Antarctic Journal*, in this issue) as one index of the extent of photochemical alteration of DOM, acknowledging the fact that H_2O_2 is an intermediate, not an end-product, in photolytic processes. Steady-state H_2O_2 concentrations, by themselves, could therefore be misleading, especially if H_2O_2 turnover is rapid; however, the rates of dark H_2O_2 decay measured for selected samples indicated that the short-term concentration changes observed in our organic perturbation experiments were probably well correlated with total H_2O_2 photoproduction.

Our results (table) indicate a large variation in photochemical H_2O_2 production from the various DOM compounds tested, ranging from negligible accumulations for glycine, proline, and adenosine monophosphate (AMP) to large accumulations for complex mixtures of organics (peptone, yeast extract, algal extract, and sediment extract). Because we cannot easily identify the specific compounds in these mixtures that are responsible for the observed photolytic effects, we are unable to compare or discuss reaction stoichiometries at this time. A comparison of the H_2O_2 photoproduction results from paired quartz vs. pyrex glass incubation containers indicates that, for most of the compounds tested, H_2O_2 photoproduction rates were enhanced in the UV-transparent quartz containers. With the possible exception of AMP, however, H_2O_2 accumulations were also observed in all pyrex incubations.

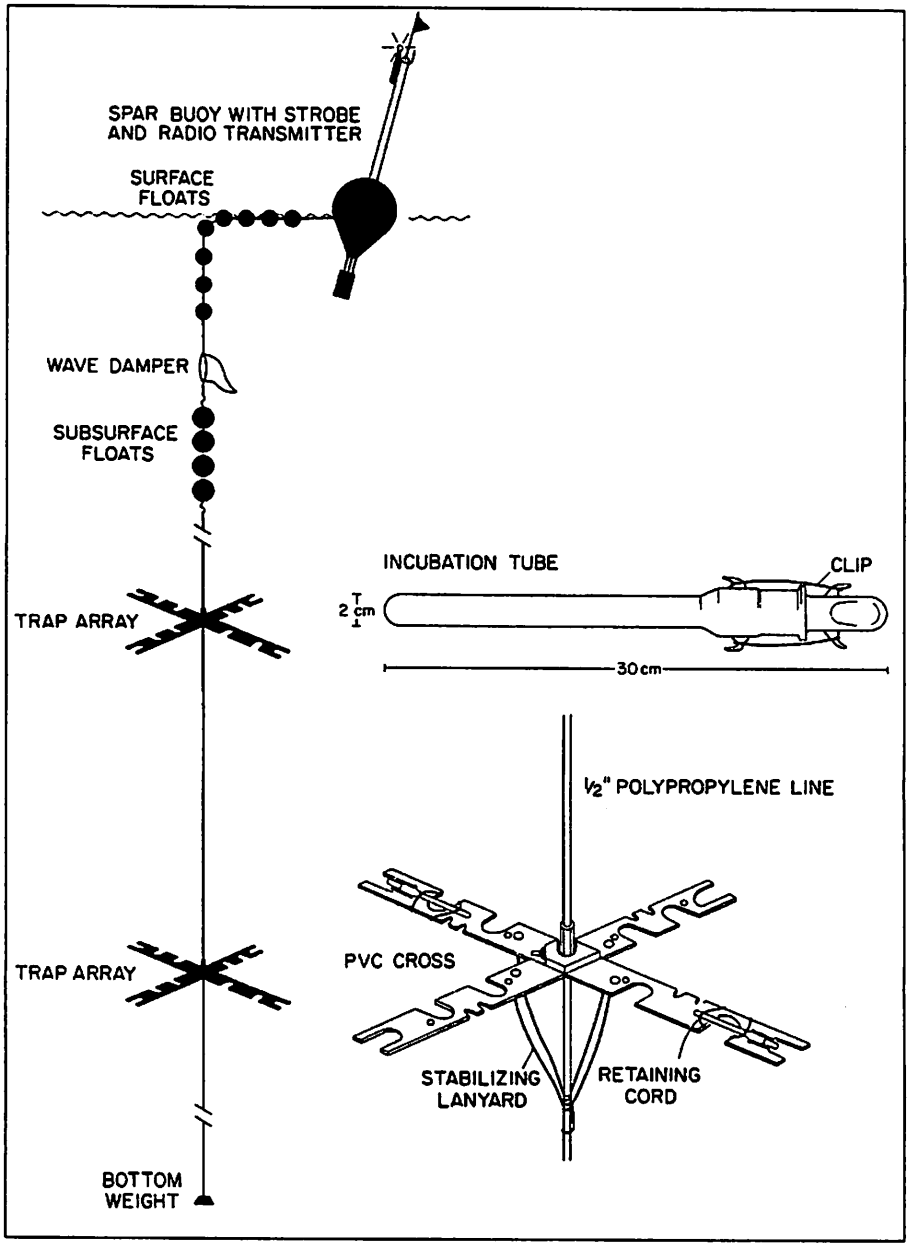
Several organic substrate "dose-response" experiments were performed to assess the concentration dependence of H_2O_2 photoproduction. For exposure of yeast extract–sea water solutions, we observed a fairly complex response over a concentration range of 0.04–40 milligrams per liter (mg L^{-1}) (figure 1). At low concentrations of organic substrate, as well as in the control with no yeast extract added, there was a significant enhancement of H_2O_2 production in quartz relative to the pyrex incubations. These differences were less evident at elevated concentrations of yeast extract and became indistinguishable at the highest concentration tested (40 mg L^{-1}). Furthermore, the addition of low concentrations of yeast extract in the quartz treatment appear to "quench" H_2O_2 production, relative to the control samples which received no addition. This may be the result of a kinetic effect caused by H_2O_2 dependent secondary reactions in the experimental solutions.

We also investigated the depth-dependence of H_2O_2 photoproduction by conducting an *in situ* incubation experiment using a modified free-drifting sediment-trap array (figures 2 and 3). In this experiment, we used yeast extract (40 mg L^{-1}) and algal extract (0.2 percent vol/vol), both in quartz and pyrex containers. As expected, results indicate a decrease in H_2O_2 photoproduction with depth. Even at a depth of 25 meters, however, we detected a net photolytic effect. Yeast extract supported a greater H_2O_2 production rate than algal extract, but again it should be emphasized that we have no information on the molar reactivities of the active components in either solution. There were no consistent differences between quartz and pyrex incubations.

In conclusion, we have documented important photochemical-DOM interactions in coastal habitats of the Antarctic Peninsula. Future studies need to focus on the composition and fate of these altered DOM compounds and to quantify the role of organic matter photolysis as an important ecological variable in southern oceans ecosystems.

We thank the scientists, officers, and crew members who participated in *PD*92-09 for their assistance and Antarctic Support Associates for expert logistical support. This research was

Figure 2. Free-drifting incubation array deployed in Paradise Bay to measure *in situ* photoproduction of H_2O_2. (cm denotes centimeter.)



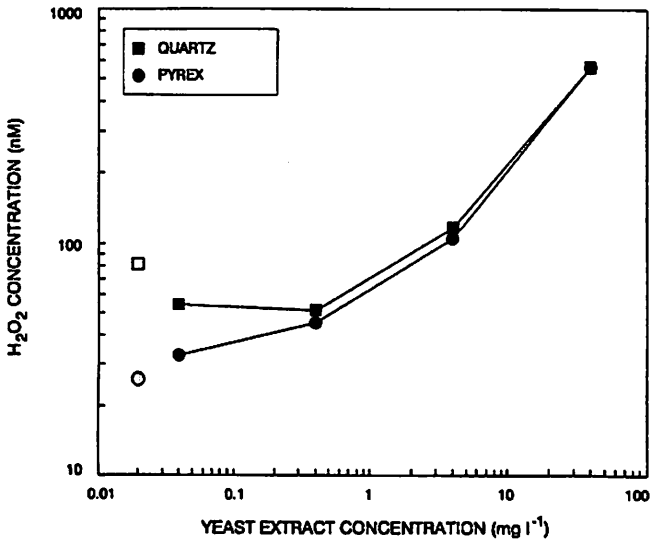


Figure 3. *In situ* net photoproduction of H_2O_2 in the presence of selected exogenous dissolved organic compounds. Water samples, collected from six depths ranging from 1 to 25 meters, were spiked with either yeast extract (40 mg L^{-1}) or algal extract (0.2 percent vol/vol), and incubated in both quartz and pyrex glass tubes attached to a free-drifting sediment trap array (see figure 2). During the 15-hour *in situ* incubation period, the glass tubes were secured to the upper surfaces of the PVC sediment-trap crosses and were thus exposed to ambient downwelling light. The 1-meter samples were incubated on board ship in a fully exposed flowing sea water incubator at –0.5°C for the duration of the experiment. After recovery, the final H_2O_2 concentrations were measured. The initial, ambient H_2O_2 concentrations (nanomolar) are shown as solid triangles

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Palmer LTER: Aquatic virus abundances near the Antarctic Peninsula

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The recent discovery of high abundances of viruses in non-polluted sea water at temperate latitudes (Bergh et al. 1989; Proctor and Fuhrman 1990) suggests that virus attack is important to the dynamics of the microbial community. Viruslike particles have been reported at abundances up to 60 times the concentration of the bacteria (Proctor and Fuhrman 1992). In addition, the occurrence of rapid increases in viral abundance during the decline of planktonic bacterial and algal blooms (Bratbak et al. 1990) indicates that virus infection might be an important source of bacterial and algal mortality in the sea.

During cruise 92-09 of the R/V *Polar Duke* (November 1992), we had an opportunity to initiate what we believe is the first study of viral ecology in Antarctica. Our objective was to determine whether viruses are present and, if so, whether they are important in the microbial ecology of the southern oceans.

Our primary goals were to count free-living viruses in the water column, to follow their dynamics through time, and to determine viral infection rate of bacteria. Additional field experiments addressed other aspects of viral population dynamics, specifically loss rates by adhesion to and sedimentation by sinking particles and damage by ultraviolet (UV) light. These studies were designed to evaluate whether viral activity could explain the lack of correlation between bacterial population size and algal biomass in antarctic coastal ecosystems (Karl et al. 1991; Karl 1993).

Sampling was carried out along the long-term ecosystem research (LTER) transect line 600 (Waters and Smith 1992) and in Paradise Harbor (64°51'S 62°54.5'W). At the latter site, water samples [0–200 meters (m)] for bacteria and virus enumeration were collected twice daily at 0400 and 2000 hours for a period of approximately 5 days. Samples were fixed with EM-grade glutaraldehyde and stored in polypropylene vials. Viruses were counted by transmission electron microscopy following direct sedimentation onto formvar-coated EM grids. The centrifuge used was the Beckman airfuge with an EM-90 rotor, which was designed especially for viral enumeration and which avoided the biases due to convection and uneven distribution that create problems when using larger ultracentrifuges. The airfuge was used on board the R/V *Polar Duke* when sea conditions were calm.

The phytoplankton community was sparse during the period of this cruise (2 November to 20 November 1992) and consisted largely of nanoflagellates with rare large diatoms. Bacteria were also relatively sparse, ranging from 9×10^4 to 3.6×10^5 cells per milliliter (mL), characteristic of pre–spring-bloom communities (Bird and Karl 1991). By contrast, however, the pack-ice algal community was visibly more abundant whenever ice was encountered.

Viral and bacterial abundances are shown for a profile at Paradise Harbor (17 November 1992, figure). Bacterial abundances declined slightly with depth. Bacteriophages were present at from 0.7 to 6 times the abundance of bacteria. The virus-to-bacteria ratio was especially low in the upper 5 m of the water column (figure) where viral abundance was minimal.

The decline in viruslike particles in the surface waters could be the result of lower production rates or to higher loss rates, or both. Bacterial production experiments using radioactive precursors were also performed on these water