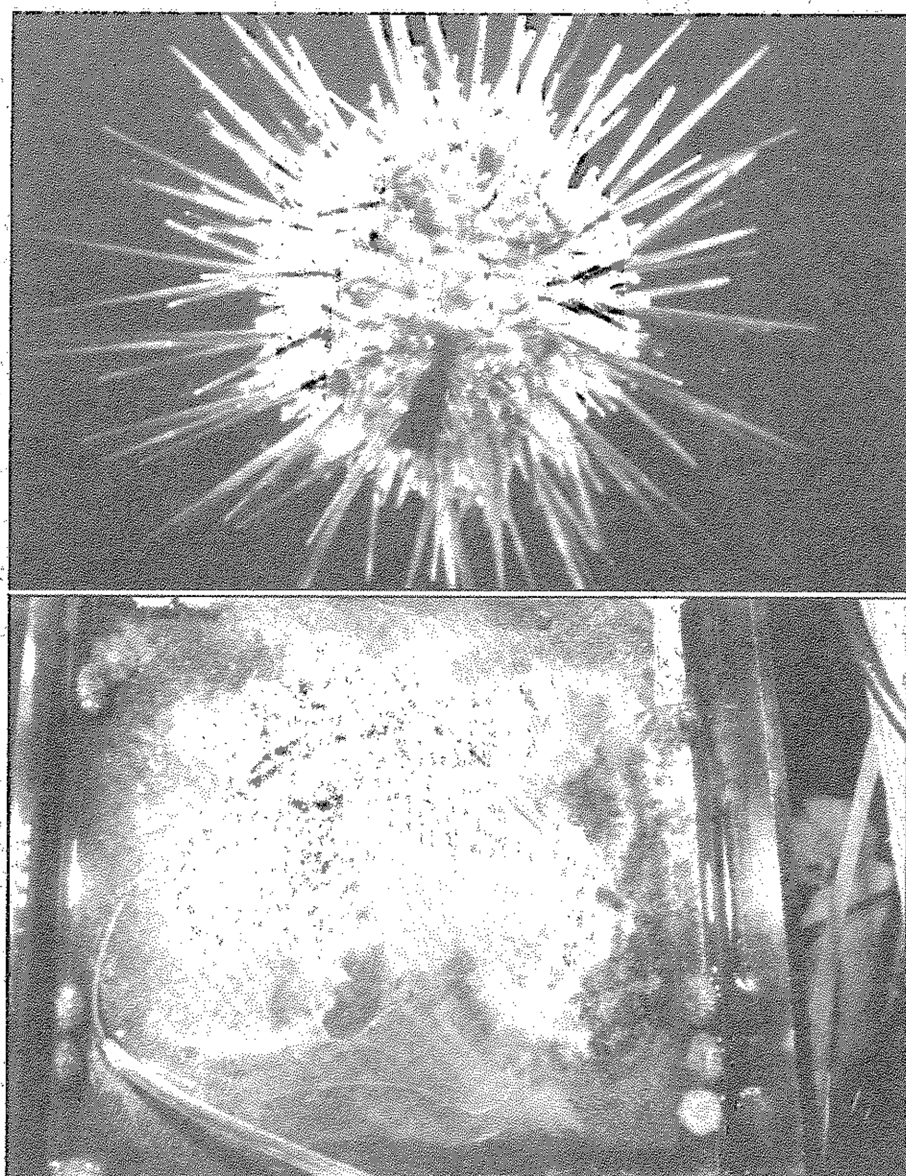
Sublethal Effects of Hydrogen Sulfide in Marine Sediments

Sediments in many marine habitats contain hydrogen sulfide (H_2S) that is highly toxic. Production of H_2S is a natural consequence of the metabolism of organic material in anoxic sediments. The organic material can come from natural deposition of marine plant and animal remains, terrestrial runoff, and discharge of sewage effluents. If oxidation of the organic material consumes all of the oxygen dissolved in the water between sediment particles (interstitial water), then bacteria may reduce dissolved sulfate and produce H_2S. Hydrogen sulfide occurs dissolved in the interstitial water where it partially dissociates and exists in equilibrium with the hydrogen sulfide (HS^-) and sulfide ($S^=$) ions (Goldhaber and Kaplan 1973).



(Above) The white sea urchin, Lytechinus pictus. (Below) Bacterial mat forms on sediment surfaces with high sulfide concentrations.

We began examining the toxicity of H_2S following studies with contaminated marine sediments from coastal southern California. Sediments with the highest H_2S concentrations had the greatest effects on the white sea urchin, *Lytechinus pictus*, and the amphipod, *Grandidierella japonica* (Thompson *et al.* 1989, Anderson *et al.* 1988). Sediments containing total sulfide concentrations ($H_2S + HS^- + S^=$) above 56 mg/l caused high mortality among test organisms. However, other potentially toxic constituents (trace metals, chlorinated hydrocarbons, and petroleum hydrocarbons) were also present in the sediments. This confounded our ability to determine which sediment constituents were responsible for toxicity.

The objectives of this study were to determine the long-term effects of hydrogen sulfide on the mortality, growth, and gonad production of *L. pictus*. We evaluated the effects of H_2S in the absence of the confounding effects of organic enrichment and other contaminants. We also estimated the concentrations of H_2S dissolved in the interstitial water of marine sediments that measurably affect this species.

Materials and Methods

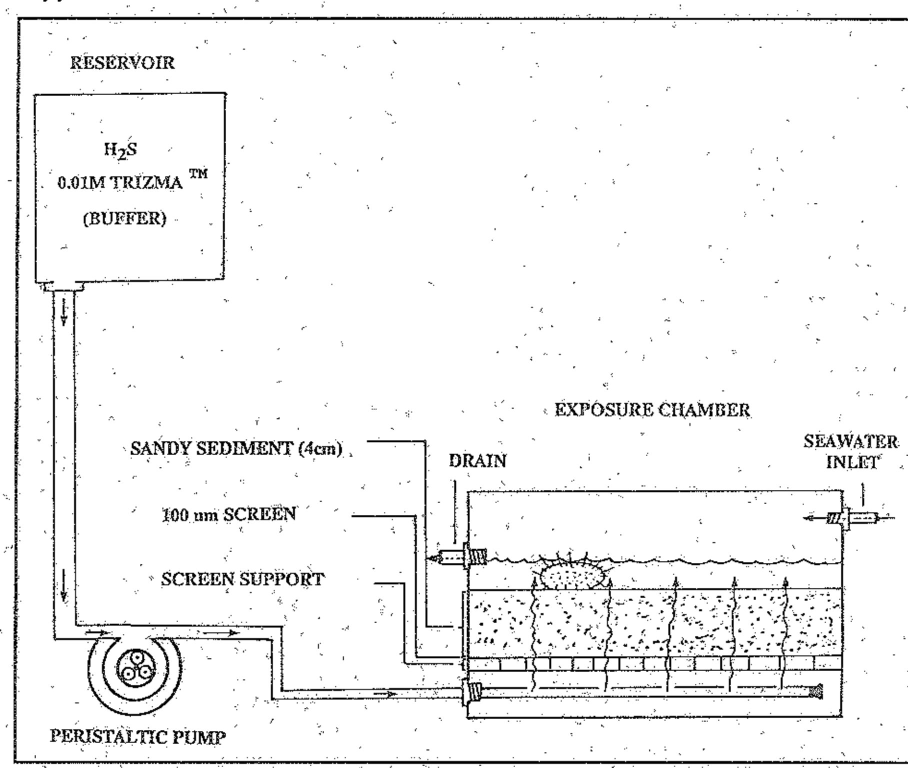
Sea urchins were exposed in the laboratory to several concentrations of dissolved sulfide in sediment. We developed an exposure system to control the concentration of dissolved sulfide in clean sediments that contained little organic material (Figure 1).

The exposure system consisted of 12 acrylic aquaria (24 cm x 16 cm), each of which was connected to a separate stock solution of sodium sulfide. The sulfide solution was pumped into a reservoir below a layer of clean sediment and slowly passed up through the sediment. A constant flow of clean seawater and aeration was supplied above the sediment to maintain suitable water quality. Temperature was maintained near 15°C throughout the experiment. The sediment was collected from a relatively uncontaminated area of Newport Bay and was 96.5% sand, 2% silt, 1.5% clay, and 0.1% total organic carbon.

The concentration of sulfide was measured in samples of interstitial and overlying water from each aquarium at the beginning and end of the 49-day experiment. A 5 ml sample of interstitial water was collected 1 cm below the sediment surface with a syringe fitted with a filter. We assumed that this sample represented the average interstitial dissolved sulfide concentration in the upper 2 cm of the sediment.

The concentration of total dissolved sulfide in the sample was measured by the methylene blue calorimetric method (APHA 1985). Interstitial water sulfide concentrations varied less than 15% during the experiment.

Figure 1.
Diagram of hydrogen sulfide exposure apparatus (not to scale). A stock solution of sodium sulfide dissolved in buffered seawater was pumped into a compartment below the sediment in each exposure chamber. Stable concentrations of sulfide in sediment interstitial water were maintained by continuously pumping sulfide solution through the sediment. Aeration and constant seawater flow were supplied to each chamber.



Undissociated H_2S concentrations were calculated based on pH, temperature, and salinity of the interstitial and overlying water in the aquaria. For interstitial water, 15-18% of total sulfide was present as H_2S, while in the overlying water, 9-11% was H_2S. Hydrogen sulfide measurements were verified by infrared spectroscopy (Applied Power Concepts, Inc., Irvine, California).

Three replicate aquaria were used for each of four sulfide treatments. Fifteen adult sea urchins (14-23 mm diameter) were placed in each aquarium. The mean interstitial water H_2S concentrations were 15.5 mg/l (high), 8.9 mg/l (medium), 2.7 mg/l (low), and 0.05 mg/l (control). Concentrations of H_2S in the overlying water were 0.010 and 0.026 mg/l in the medium and high treatments.

The sediment dosing method produced a steep sulfide concentration gradient in the upper sediment layer. Samples collected 1 cm below the sediment surface had sulfide concentrations similar to the stock solutions. Samples from the sediment-water interface had concentrations that were either undetectable (low and medium treatments) or greatly reduced (18% of interstitial water value).

The experiment was checked daily to document urchin mortality and behavior (sediment avoidance) and the urchins were fed every other day. The change in urchin test diameter, body wet weight, and gonad wet weight during the 49-day exposure were measured.

The acute toxicity of H_2S dissolved in water was measured in a separate experiment without sediment. Reservoirs containing dissolved sulfide were used to dose 1-l Erlenmeyer flasks at 0.03, 0.06, and 0.14 mg/l H_2S, and control (no sulfide). Ten urchins were placed in each flask and the flasks were sealed so that no air space was present. Mortalities were recorded after four days.

Results and Discussion

Sediments in the high and medium sulfide treatments quickly acquired a black color and the odor of sulfide. A white, filamentous bacterial mat formed on the sediment surface, a situation that was observed in previous experiments with organically enriched sediments with high sulfide concentrations.

In some treatments, sea urchins actively avoided contact with the sediment by clinging to the sides of the aquaria. Only 10-20% of the urchins were consistently in contact with the sediment in the two highest H_2S concentrations. Almost 70% of the urchins were consistently on the sediment in the controls.

Mortality

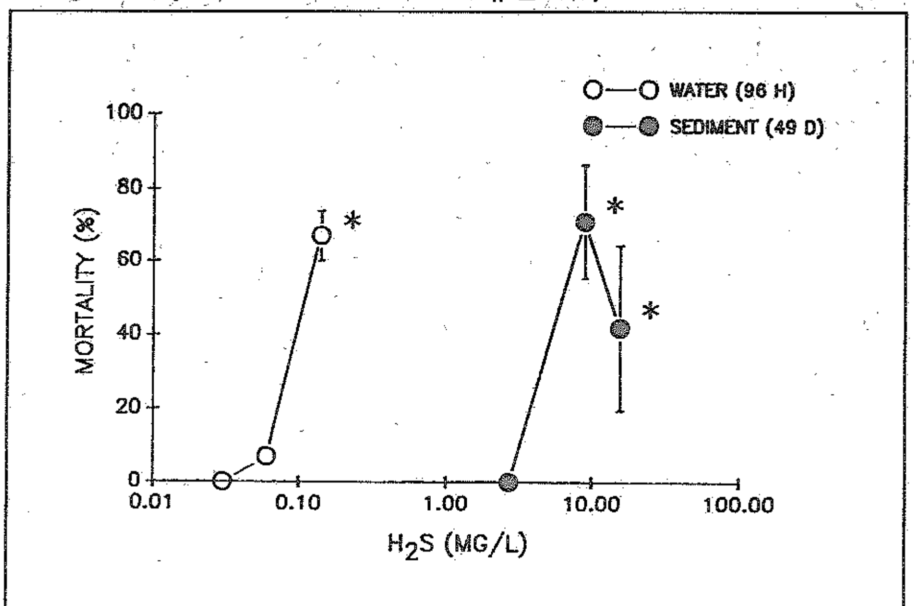
Urchins in the medium and high sulfide treatments suffered increased mortalities (Figure 2). No mortalities occurred in the low sulfide treatment or in the control. The greatest mortality occurred in the medium exposure where 71% of the urchins died by the end of the experiment.

Urchin mortality did not occur rapidly, but was most pronounced after the first few weeks of exposure. Urchins in the medium and high sulfide exposures frequently became immobilized after venturing onto the sediment. Though alive, the animals could not move and were often overgrown by the bacterial mat. Death usually followed several days later.

In the water-only sulfide exposure, H_2S concentrations as low as 0.06 mg/l caused mortality within four days (Figure 2). The estimated four-day LC50 (concentration lethal to 50% of the urchins) was 0.10 mg/l. This estimate of the sensitivity of *L. pictus* to sulfide can be compared to other organisms. Unfortunately, there has been little research on the toxicity of hydrogen sulfide to marine animals under controlled conditions. Compared to freshwater invertebrates, *L. pictus* is moderately sensitive to sulfide. The four-day LC50 of H_2S to a variety of freshwater crustaceans ranges from 0.02 to 1.07 mg/l (Oseid and Smith 1974).

No sea urchin mortality was observed at an interstitial water H_2S concentration of 2.7 mg/l, even though this concentration was nearly 30 times greater than the four-day LC50 estimated in the water-only test. While urchins were in contact with sediments that contained high sulfide concentrations, they probably received a relatively small dose of the toxicant. First, most of the animal's body was not in contact with the sediment and therefore was exposed to the much lower levels of sulfide in the overlying water. Second, interstitial water H_2S concentrations were measured 1 cm below the sediment surface. Concentrations in the upper few millimeters of sediment (in contact with the urchin)

Figure 2.
Mortality of Lytechinus pictus following exposure to hydrogen sulfide in sediment for 49 d and in water for 96 hr. Values (mean ± standard error) with an asterisk (*) are significantly different from the control ($p \leq 0.05$).



were much lower because of dilution and oxidation processes at the sediment surface.

Growth

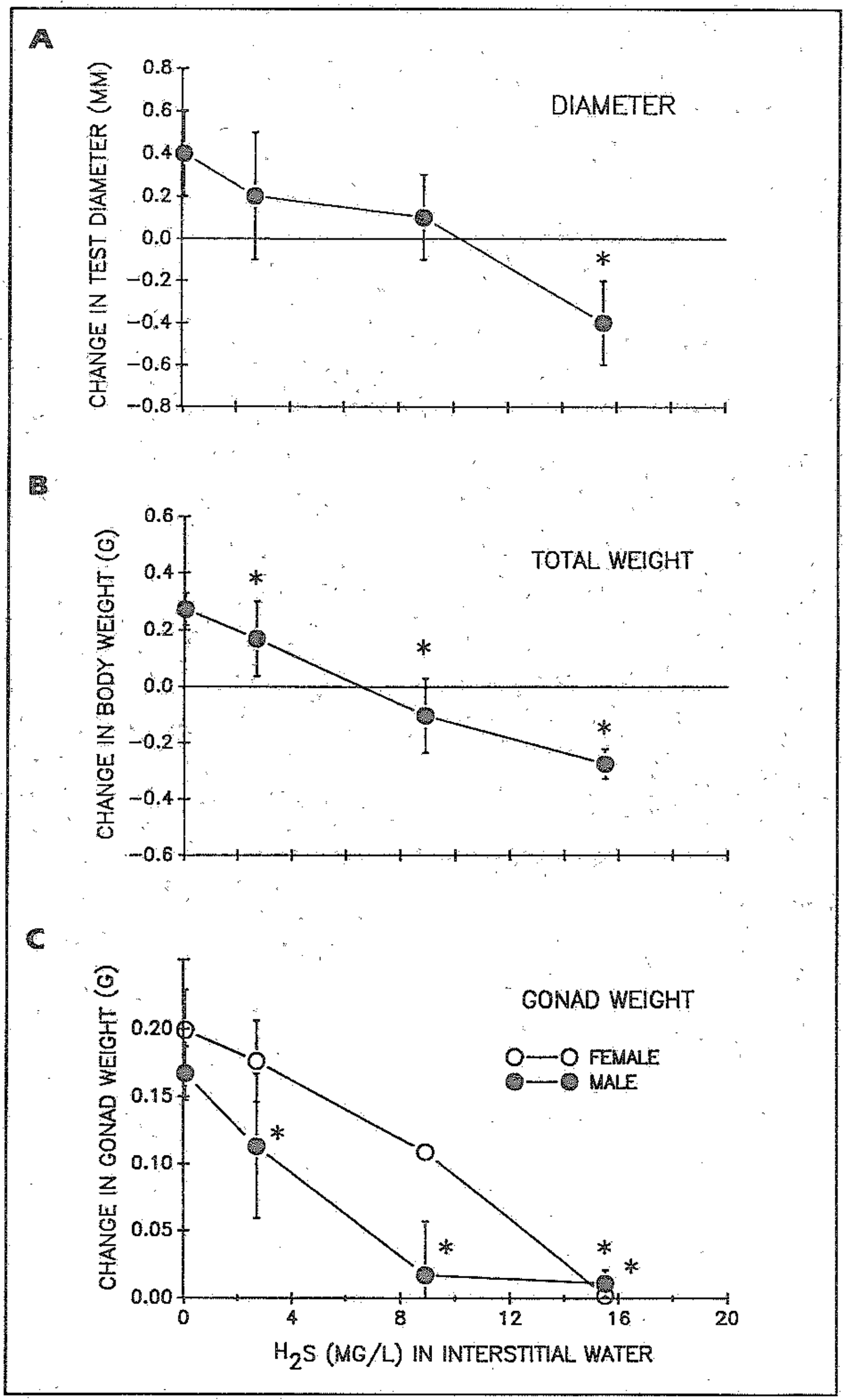
Urchin growth rates (change in test diameter and wet weight) were inversely related to sulfide concentration (Figure 3). Control sea urchins grew an average of 0.4 mm in diameter and 0.27 g in wet weight during the 49-day exposure. Reductions in growth were significant at all three exposure levels for wet weight and at the highest level for test diameter (Figure 3).

The size and weight of urchins in the medium and high sulfide exposures declined. Much of the weight loss may have been loss of spines, a toxic response observed during this experiment. Reductions in test diameter occurred in previous experiments with urchins and contaminated sediments (Thompson *et al.* 1989), but the causal mechanism is not understood.

Gonad Production

During the sediment exposure, the average gonad weight of male urchins in the control chambers increased 0.17 g and the average gonad weight of female urchins increased 0.20 g, an increase of about 2.5 times the initial measurements. Urchin gonad production in the experimental treatments was inversely related to sulfide concentration (Figure 3). Compared to the control, testis size was significantly reduced at all sulfide levels. Ovary size was significantly reduced only at the highest concentration. Too few

Figure 3.
Growth and gonad production of Lytechinus pictus during the 49-day sediment exposure to hydrogen sulfide. A) Change in sea urchin test diameter. B) Change in total wet weight. C) Change in gonad wet weight. Negative values indicate that final size was less than initial size. Initial mean diameter was 17.3 mm, mean wet weight was 2.02 g, and mean gonad weight was 0.07 g. Values (mean ± standard error) with an asterisk (*) are significantly different from the control ($p \leq 0.05$).



female urchins survived at the medium sulfide concentration to permit statistical testing.

Discussion

Elevated H_2S concentrations produced adverse effects on sea urchin behavior, survival, growth, and gonad production. Some endpoints were more sensitive to sulfide than others; wet weight and male gonad production were affected at the lowest concentration tested (2.7 mg/l). We do not know what the threshold toxic concentration is for *L. pictus* in long-term hydrogen sulfide exposure.

We could not determine if the sublethal effects of sulfide exposure were produced by inhibition of respiratory enzymes. Samples of sea urchin gonad and intestine were analyzed for cytochrome oxidase activity, but did not show a consistent pattern of inhibition by hydrogen sulfide. Sulfide effects on enzyme activity are transient; enzyme inhibition patterns may have been obscured by handling the urchins (activity tests and size measurement) prior to dissection, or by storage of samples for several weeks before analysis.

Adverse effects on growth and gonad production may have been caused by changes in sea urchin behavior. *L. pictus* were reluctant to venture onto sulfide-contaminated sediments to feed and perhaps did not eat enough to sustain normal metabolic rates. Growth reductions may represent malnutrition rather than direct biochemical effects of hydrogen sulfide.

Sediments from Santa Monica Canyon and the Palos Verdes Shelf contain high concentrations of organic matter from sewage discharge and plankton production. In laboratory experiments with *L. pictus*, exposure to the sediments resulted in decreased survival and growth (Anderson *et al.* 1988). Hydrogen sulfide concentrations in these sediments were within the range identified as toxic in this experiment. When comparisons are based on dissolved H_2S concentrations, the reduction of sea urchin growth and survival is similar between studies with sediment collected near outfalls and the results reported herein. However, a sample of Long Beach Harbor sediment with similar sulfide concentrations did not produce toxic effects.

The major source of hydrogen sulfide in sediments near sewage outfalls is buried organic matter from historical discharges. Sulfide produced from the decay of this material diffuses upwards into surface sediments. This process continues for many years until the organic matter is exhausted. Hydrogen sulfide may be an important factor in the toxicity of sediments contaminated with organic material and may be responsible for some of the changes in benthic infaunal populations observed near wastewater outfalls. The applicability of our results to the coastal environment is limited by the artificial nature of the experimental system and the fact that we do not know how sediment H_2S concentrations vary in time and space in the field. Other contaminants and sediment constituents are also altered near outfalls and may contribute to population effects.

The mobility of sea urchins may allow them to avoid areas with undesirable sediment characteristics, and may have a greater influence on their distribution than toxic effects of H_2S have on growth and survival. Additional experimental research is needed on the importance of the various aspects of sediment quality to benthic organisms. ■

References

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