# HYDROGEN SULFIDE EFFECTS ON SELECTED LARVAL AND ADULT MARINE INVERTEBRATES

by

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## ABSTRACT

Six species of marine invertebrates including larval and juvenile Dungeness crab, Cancer magister, and Pacific oyster, Crassostrea gigas, were examined for their tolerance to dissolved hydrogen sulfide in seawater. In tests lasting up to 4 days, the sulfide tolerances or organisms ranged from a low of 0.2 mg/l for Anisogammarus confervicola to 6.0 mg/l for Macoma balthica. The range of tolerances appeared to correlate with the expected degree of anoxic conditions that would be encountered by each species in its natural habitat. Early embryos of C. gigas were found to be very sensitive to hydrogen sulfide since an exposure to 0.32 mg/l for only 2 h drastically affected the normal development of this stage. Seven day old veliger larvae of this species were not affected by 2 h exposures of up to 0.56 mg/l hydrogen sulfide but were inactivated by 1.0 and 3.2 mg/l. However, recovery, even after exposure to the highest concentration, was complete after 24 h. Dungeness crab zoeae exposed for 74 h to 0.56 mg/l sulfide were less able to tolerate a 15 or 90 min period of heat shock at 29.0° C than were control organisms or those exposed to 0.18 mg/l. However, zoeae exposed for 48 h to either 0.5 or 1.0 mg/l sulfide survived as well as controls at temperatures of 25.0 - 28.0° C. It is suggested that estuarine organisms live very close to their tolerance limits for hydrogen sulfide.

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## INTRODUCTION

Dissolved hydrogen sulfide is a constituent of nearly all marine sediments (Fenchel and Riedl, 1970). Where conditions of high temperature and poor circulation of overlying water occur adjacent to sediments high in organic content, the anerobic layer may rise to the sediment water interface and release free hydrogen sulfide into the overlying water. Bella et al. (1972) have found that hydrogen sulfide concentrations of 1 mg/l are not uncommon in water overlying sediments in Oregon estuaries. Where poor flushing characteristics were combined with high organic content of sediments as found in Isthmus Slough, Coos Bay, Oregon, sulfide concentrations in the water as high as 12 - 16 mg/l were reported (Bella et al., 1972). Much higher concentrations exist in interstitial water of marine sediments. Here, concentrations of 700 mg/l may occur, and values of 300 mg/l may be common (Fenchel and Riedl, 1970).

Normally, sulfide production in sediments is not detrimental to marine organisms. Epifaunal and pelagic organisms generally do not encounter high levels of hydrogen sulfide and infaunal animals often communicate with surface water using siphons or irrigated burrows. Additionally, the benthic infauna of softer muddy sediments appear to be slightly more tolerant of sulfide than epifaunal forms (Theede et al., 1969). However, in regions where water stagnation or high organic pollution occurs or when sediments are displaced as in channel dredging operations, the concentrations of hydrogen sulfide in the water column

may reach lethal levels. In this study we have examined the tolerance of selected estuarine organisms to hydrogen sulfide and have included work on larval forms of a bivalve mollusc and a decapod crustacean.

## METHODS AND MATERIALS

Adults of Gnorimosphaeroma oregonensis (Isopoda), Anisogammarus confervicola (Amphipoda), Corophium salmonis (Amphipoda), and Macoma balthica (Eulamellibranchiata) were collected in Yaquina Bay immediately prior to use in the toxicity studies. One to two month old juvenile oysters, Crassostrea gigas (Eulamellibranchiata) were reared in the laboratory from commercial stock. Dungeness crabs, Cancer magister (Decapoda), were collected as megalopae and allowed to metamorphose in the laboratory to the first postlarval crab instar prior to use in tests. First stage zoeae of the Dungeness crab were obtained from spawning ovigerous females in the laboratory and collected within 5 - 15 h of hatching for use in the bioassays (Buchanan et al., 1970). Mature adult oysters, Crassostrea gigas, were conditioned, spawned, and the gametes fertilized in the laboratory (Loosanoff and Davis, 1963) to obtain embryos and 7 day old veliger larvae. In some experiments embryos were exposed to sulfide within 2 h after fertilization of the freshly spawned eggs. Before testing, the 7 day old larvae were held at 26° C and fed with the alga Pseudoisochrysis.

Test solutions containing hydrogen sulfide were obtained by pipetting or metering appropriate volumes of a stock solution of sodium sulfide into seawater. The sodium sulfide stock solutions were prepared in oxygen depleted water immediately prior to use. A microwinkler technique was used for the measurement of the dissolved oxygen in test water. Free dissolved sulfide was measured by a micro adaptation of the method of Cline (1969). The concentration was expressed as milligrams per liter of hydrogen sulfide.

Ninety-six hour acute toxicity studies against all adult and juvenile stages and crab zoeae were conducted in a flowing exposure apparatus. The exposure chambers consisted of rubber stoppered 125 ml erlenmeyer flasks. The overflow was fitted with nylon screen of 360 μ mesh to prevent escape of the test organisms. Before the seawater entered the test containers, sodium sulfide stock solutions were metered into the test water with a peristaltic pump and mixed through a glass bead column. The flasks were supplied with seawater, previously sand filtered and UV sterilized, at a rate of 4 ml/min which was sufficient to keep sulfide oxidation and oxygen depletion to a minimum except at the highest sulfide concentrations tested. Three to five sulfide concentrations and a control were employed for each species with a minimum of ten animals per concentration. Deaths were recorded either continuously or on a 24-h basis. The toxicity data are expressed as the $LC_{50}$ determined graphically (American Public Health Association, 1971).

The small size and delicate nature of oyster larvae precluded the testing of these organisms in the dosing apparatus described previously. Therefore, the embryos and larvae were exposed to sulfide by adding the solutions of the sodium salt directly to static-water cultures in 125 ml stoppered erlenmeyer flasks. In these experiments sulfide exposures were limited to 2 h to minimize sulfide oxidation and oxygen depletion. Careful monitoring of the flasks confirmed that the residual sulfide concentration after 2 h always exceeded 80 % of the initial value. No corrections were made for this depletion in reporting the results. Oxygen depletion was minimal; the lowest oxygen level found in one of the high sulfide exposure flasks was 3.2 mg/l. In the embryo tests, 50 embryos were cultured per milliliter of seawater. After the 2 h

sulfide exposure period the flasks were vigorously aerated to remove the remaining sulfide, and the larvae were held for an additional 22 h before examination. By the end of the incubation period normal embryos had developed to straight-hinge or D-shaped veliger larvae. The criteria of toxic effect used in these experiments was to determine the percentage of embryos which developed normally as opposed to non-shelled or imperfectly shelled larvae (Dimick and Breese, 1965). Exposures of 7 day old veliger larvae were conducted in the same manner and effects on activity were observed immediately following the exposure period, and again 24 h later. Throughout these tests embryos and veliger larvae were held at 26° C in full strength filtered seawater.

Two approaches were employed to evaluate the effects of sublethal sulfide exposure on thermal tolerances of Dungeness crab zoeae. In one experiment zoeae were exposed for 72 h to 0, 0.18, and 0.56 mg/l sulfide at 13° C and the survivors were then exposed to sulfide free seawater at 29.0° C for 15 or 90 min. The survival of heat shocked zoeae after a 15-h recovery period at 13° C was then tabulated. In a second study, newly hatched crab zoeae were dosed with sulfide at levels of 0.5 and 1.0 mg/l for a 48-h period. A control group received no sulfide in the water. Death of some of the zoeae occurred, particularly at the highest sulfide exposure and the surviving zoeae were isolated for use in the tolerance tests. Control and treatment groups of zoeae were then exposed to temperatures of 25.0, 26.0, 27.0 and 28.0° C and deaths recorded as a function of time over a 30-h period. The criteria of death used in the latter experiment was complete absence of movement of appendages and the development of an opaque appearance of the larvae.

# RESULTS

The concentrations of hydrogen sulfide tested against Dungeness crab zoeae in 96-h tests ranged from 0.1 to 1.0 mg/l (Table 1). The 96-h median lethal concentration of sulfide for crab zoeae was found to be 0.5 mg/l (Table 2). Furthermore, zoeae exposed to concentrations as low as 0.1 mg/l were seen to exhibit abnormal swimming behaviors. First instar postlarval C. magister exhibited sulfide tolerance only slightly greater than the zoeal stage. The 96-h $LC_{50}$ for these crabs was 1.0 mg/l hydrogen sulfide.

In addition to crabs, hydrogen sulfide toxicity was examined in three other crustaceans, the isopod, Gnorimosphaeroma oregonensis, and the amphipods Anisogammarus confervicola, and Corophium salmonis. In 96-h tests G. oregonensis was substantially more tolerant of hydrogen sulfide than A. confervicola. Median lethal concentrations of hydrogen sulfide for these two species in 96 h were 5.2 and 0.2 mg/l, respectively (Table 2). Since we did not test hydrogen sulfide concentrations less than 1.0 mg/l against C. salmonis, a 4 day comparison between A. confervicola and C. salmonis was not possible. However, C. salmonis was more sensitive to hydrogen sulfide than A. confervicola in a 24-h exposure period; the respective 24-h $LC_{50}$'s were 1.4 and 3.2 mg/l (Table 2).

The two bivalve molluscs studied, Macoma balthica and Crassostrea gigas, exhibited slightly greater resistance to hydrogen sulfide than did the amphipods A. confervicola and C. salmonis, and resistance similar to G. oregonensis and postlarval C. magister (Table 2). The 96-h $LC_{50}$'s for these two bivalve species were 6.0 and 1.4 mg/l, respectively.

Table 1. Conditions observed in 96-h tolerance tests with six marine invertebrate species.

| Test Description | Measured Conditions[a] | | | |
|---|---|---|---|---|
| | Hydrogen sulfide (mg/l) | Dissolved oxygen (mg/l) | pH | Temperature (°C) |
| *Cancer magister* | | | | |
| Zoeae | | | | |
| control | - | 7.5 ± 0.5 | 8.2 ± 0.1 | 14.0 ± 0.0 |
| 0.10 | 0.10 ± 0.02 | 7.0 | 8.2 ± 0.1 | 14.5 ± 0.6 |
| 0.32 | 0.32 ± 0.04 | 6.9 ± 0.2 | 8.0 ± 0.2 | 14.5 ± 0.6 |
| 0.56 | 0.61 ± 0.01 | 7.1 ± 0.6 | 8.0 ± 0.3 | 14.0 ± 0.0 |
| 1.0 | 1.1 ± 0.1 | 6.4 ± 0.2 | 8.2 ± 0.1 | 14.5 ± 0.5 |
| Postlarval Crab | | | | |
| control | - | 6.2 ± 0.5 | - | 12.0 |
| 0.18 | 0.10 ± 0.07 | 7.0 ± 0.4 | - | 12.0 |
| 0.56 | 0.35 ± 0.14 | 7.1 ± 1.6 | - | 12.0 |
| 1.8 | 1.4 ± 0.2 | 6.8 ± 0.4 | - | 12.0 |
| 5.6 | 6.1 ± 1.1 | 2.0 ± 0.4 | - | 12.0 |
| *Gnorimosphaeroma oregonensis* | | | | |
| control | - | 8.4 ± 0.7 | 7.8 ± 0.0 | 17.3 ± 0.5 |
| 0.10 | 0.15 ± 0.02 | 7.2 ± 0.2 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 0.33 | 0.35 ± 0.08 | 6.8 ± 0.5 | 7.8 ± 0.1 | 17.3 ± 0.5 |
| 1.0 | 1.4 ± 0.3 | 6.1 ± 0.1 | 7.9 ± 0.2 | 17.3 ± 0.6 |
| 3.3 | 3.2 ± 0.2 | 2.7 ± 1.3 | 8.1 ± 0.3 | 17.3 ± 0.5 |
| 10.0 | 8.7 ± 0.3 | 0.5 ± 0.6 | 8.6 ± 0.1 | 17.3 ± 0.5 |
| *Anisogammarus confervicola* | | | | |
| control | - | 8.4 ± 0.7 | 7.8 ± 0.0 | 17.3 ± 0.5 |
| 0.10 | 0.15 ± 0.02 | 7.2 ± 0.2 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 0.33 | 0.38 ± 0.11 | 7.1 ± 0.1 | 7.8 ± 0.0 | 17.5 ± 0.7 |
| 1.0 | 1.5 ± 0.1 | 6.1 ± 0.1 | 7.9 ± 0.2 | 17.3 ± 0.6 |
| 3.3 | 3.2 ± 0.2 | 3.0 ± 1.4 | 8.1 ± 0.3 | 17.3 ± 0.6 |
| 10.0 | 8.8 ± 0.4 | 1.2 | 8.7 | 18.0 |
| *Corophium salmonis* | | | | |
| control | - | 8.4 ± 0.7 | 7.8 ± 0.0 | 17.3 ± 0.5 |
| 1.0 | 1.3 ± 0.3 | 6.1 ± 0.1 | 8.0 ± 0.1 | 17.5 ± 0.7 |
| 3.3 | 3.2 ± 0.2 | 3.8 ± 0.6 | 8.3 ± 0.2 | 17.5 ± 0.7 |
| 10.0 | 8.8 ± 0.4 | 1.2 | 8.7 | 18.0 |

Table 1. (continued)

| Test Description | Measured Conditions | | | |
|---|---|---|---|---|
| | Hydrogen sulfide (mg/l) | Dissolved oxygen (mg/l) | pH | Temperature (°C) |
| *Macoma balthica* | | | | |
| control | - | 8.4 ± 0.7 | 7.8 ± 0.0 | 17.3 ± 0.5 |
| 0.10 | 0.14 ± 0.03 | 7.2 ± 0.2 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 0.33 | 0.37 ± 0.08 | 6.8 ± 0.5 | 7.8 ± 0.1 | 17.3 ± 0.5 |
| 1.0 | 1.4 ± 0.2 | 6.1 ± 0.1 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 3.3 | 3.2 ± 0.2 | 2.7 ± 1.3 | 8.1 ± 0.3 | 17.3 ± 0.5 |
| 10.0 | 8.8 ± 0.3 | 0.5 ± 0.6 | 8.6 ± 0.1 | 17.3 ± 0.5 |
| *Crassostrea gigas* | | | | |
| control | - | 8.4 ± 0.7 | 7.8 ± 0.0 | 17.3 ± 0.5 |
| 0.10 | 0.15 ± 0.02 | 7.2 ± 0.2 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 0.33 | 0.38 ± 0.08 | 6.8 ± 0.5 | 7.8 ± 0.1 | 17.3 ± 0.5 |
| 1.0 | 1.4 ± 0.2 | 6.1 ± 0.1 | 7.9 ± 0.1 | 17.3 ± 0.5 |
| 3.3 | 3.2 ± 0.2 | 3.0 ± 1.4 | 8.1 ± 0.3 | 17.3 ± 0.6 |
| 10.0 | 8.8 ± 0.3 | 1.0 ± 0.3 | 8.7 ± 0.0 | 17.5 ± 0.7 |

[a]Mean ± standard deviation.

Table 2. Toxicity of hydrogen sulfide to six marine invertebrate species.

| Organism | $LC_{50}$ (mg/l) | | |
|---|---|---|---|
| | 24-h | 48-h | 96-h |
| Cancer magister | | | |
| Zoeae | 0.7 | 0.6 | 0.5 |
| Postlarval crab | 1.0 | 1.0 | 1.0 |
| Gnorimosphaeroma oregonensis | 6.8 | 6.0 | 5.2 |
| Anisogammarus confervicola | 3.2 | 0.8 | 0.2 |
| Corophium salmonis | 1.4 | <1.0 | <1.0 |
| Macoma balthica | >10.0 | 8.0 | 6.0 |
| Crassostrea gigas | 3.3 | 2.6 | 1.4 |

The percentage of oyster larvae exhibiting normal development 22 h after a 2-h exposure to 0.056 and 0.1 mg/l hydrogen sulfide was essentially the same as that found in control tests; about 90 % (Table 3). With a 0.32 mg/l exposure the percentage of normal development ranged widely from 18 - 92 % in replicate experiments, averaging 35 %. Many of the abnormal larvae at this concentration were shelled but had not attained the normal D-shape configuration. Similar observations were made on larvae exposed to 0.56 mg/l hydrogen sulfide, but the percentage of normally developing larvae was much lower, not exceeding 36 % in any of the tests. At 1.0 mg/l 21 % normal development was the maximum observed in any test. At this concentration many of the abnormal larvae were rounded and unshelled (Fig. 1), but were actively swimming. Normally developed larvae were observed in only a single test at 3.2 mg/l. Most of the abnormal larvae were unshelled at this concentration. Even the deformed larvae, however, were still found to be actively swimming. Essentially similar observations were made on larvae exposed to the highest hydrogen sulfide concentration, 5.6 mg/l, but even at this concentration a few normally developed veligers were seen.

Following the initial 24-h observations, culture flasks were held at 26° C for an additional 42 - 48 h. In general, after this period of time, control larvae and larvae exposed to the two lowest sulfide concentrations (0.056 and 0.1 mg/l) appeared normal in all respects. In larval cultures that had been exposed to higher sulfide concentrations a large majority of abnormal larvae were observed to be dead or to be inactive. A few shelled larvae were seen swimming in some of these cultures, but these often exhibited abnormal morphological features, such as unusual lobular or filamentous structures extending beyond the shell margin and deformed shells (Fig. 1).

Table 3. Percentage of oyster larvae (C. gigas) developing normally after a 2 h exposure of fertilized gametes to several concentrations of hydrogen sulfide.[a]

| Sulfide Concentration (mg/l) | Percent Normally Developed[b] | Number of Tests |
|---|---|---|
| 0 (control) | 85 ± 4 | 3 |
| 0.056 | 94 ± 4 | 2 |
| 0.10 | 92 ± 2 | 2 |
| 0.32 | 35 ± 39 | 4 |
| 0.56 | 27 ± 6 | 4 |
| 1.0 | 11 ± 8 | 4 |
| 3.2 | 6 ± 13 | 4 |
| 5.6 | 18 ± 21 | 2 |

[a]Counts of 100 larvae per replicate test were made after 24 h of development.

[b]Mean ± standard deviation.

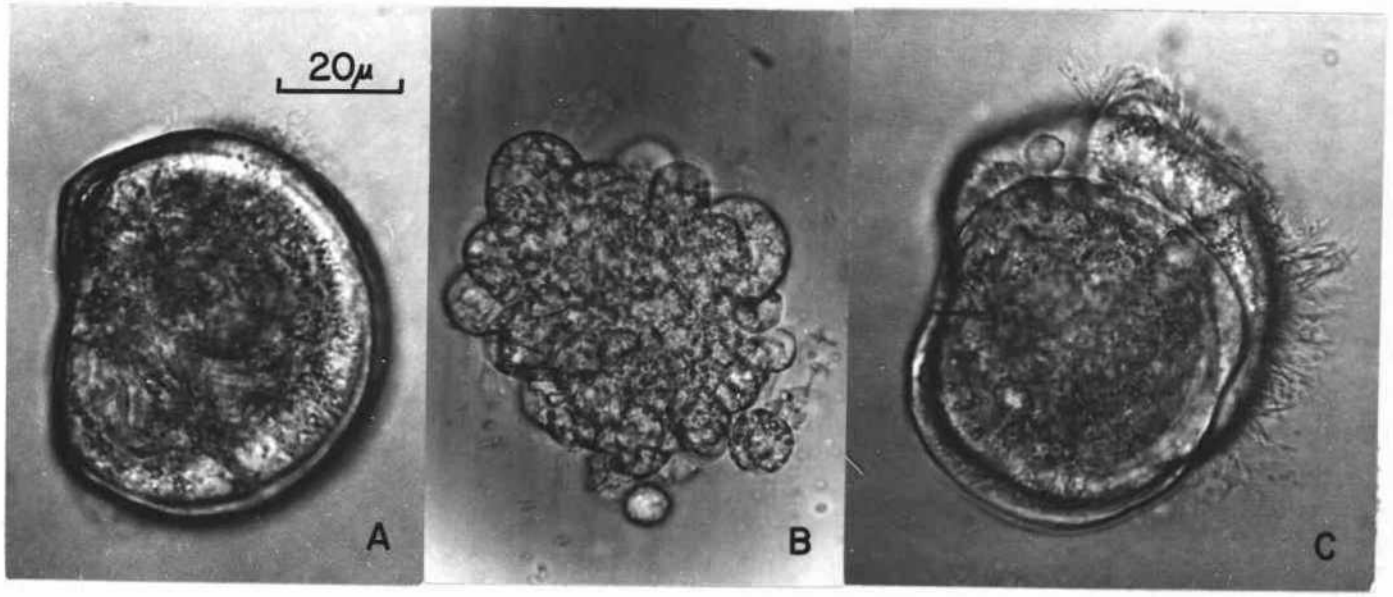


Figure 1. Larvae of Crassostrea gigas. (A) A normally shelled veliger larva from a control treatment photographed after 72 h of development. (B) Unshelled abnormal larvae, 24 h old, exposed during the first 2 h of development to 3.2 mg/l of hydrogen sulfide. (C) Abnormally shelled larva, 72 h old, exposed during the first 2 h of development to 1.0 mg/l of hydrogen sulfide. All photographs to the same scale.

Seven day old veligers of Crassostrea gigas were exposed to hydrogen sulfide concentrations ranging from 0.32 to 3.2 mg/l in the same manner as were embryos. Larvae in the control flasks and the two lower sulfide concentrations (0.32 and 0.56 mg/l) were actively swimming in the water column following a 2-h exposure period. At 1.0 mg/l hydrogen sulfide the majority of the larvae were on the bottom of the flasks after exposure, while at 3.2 mg/l all the animals were on the bottom and were moving very slowly or not at all. Following the sulfide exposures the culture containers were held for an additional 24-h period, after which the larvae in all flasks were found actively swimming in the water column. At this time there were no detectable differences between the control larvae and those exposed to the highest levels of hydrogen sulfide.

In the heat shock experiment, all of the zoeae were nearly immobilized and resting on the bottom of the test vessels within minutes of exposure to 29.0° C. Upon return to 13° C many of the larvae revived and were actively swimming 30 - 60 min later. The survival of control and sulfide treated groups following the 15-h recovery period is shown in Table 4. The difference between a 15 min and 90 min heat treatment seemed to have little or no effect on survival but zoeae that had first been exposed to 0.56 mg/l hydrogen sulfide exhibited a substantially poorer survival (35 - 48 %) than control larvae (97 %) and larvae exposed to only 0.18 mg/l hydrogen sulfide (88 - 97 %).

However, in a second experiment in which control zoeae and survivors of zoeae treated with 0.5 and 1.0 mg/l hydrogen sulfide for 48 h were tested for thermal tolerance at 25.0, 26.0, 27.0 and 28.0° C no effect of the prior sulfide exposure was observed (Fig. 2). In this experiment the time to 50 % mortality for all treatment groups at 28.0° C was about 9 h and extended to about 24 h at 25.0° C.

Table 4. Percentage survival of hydrogen sulfide exposed crab zoeae (*C. magister*) subjected to heat shock at 29.0° C.

| Hydrogen sulfide exposure level[a] (mg/l) | Period of heat exposure (min) | Number of zoeae per treatment | Percent survival after a 15 h recovery period at 13° C |
|---|---|---|---|
| 0 (control) | 15 | 35 | 97 |
| 0.18 | 15 | 38 | 97 |
| 0.56 | 15 | 43 | 35 |
| 0 (control) | 90 | 37 | 97 |
| 0.18 | 90 | 41 | 88 |
| 0.56 | 90 | 61 | 48 |

[a]Newly hatched zoeae were continuously exposed to the indicated level of hydrogen sulfide for 74 h prior to administering heat shock treatment.

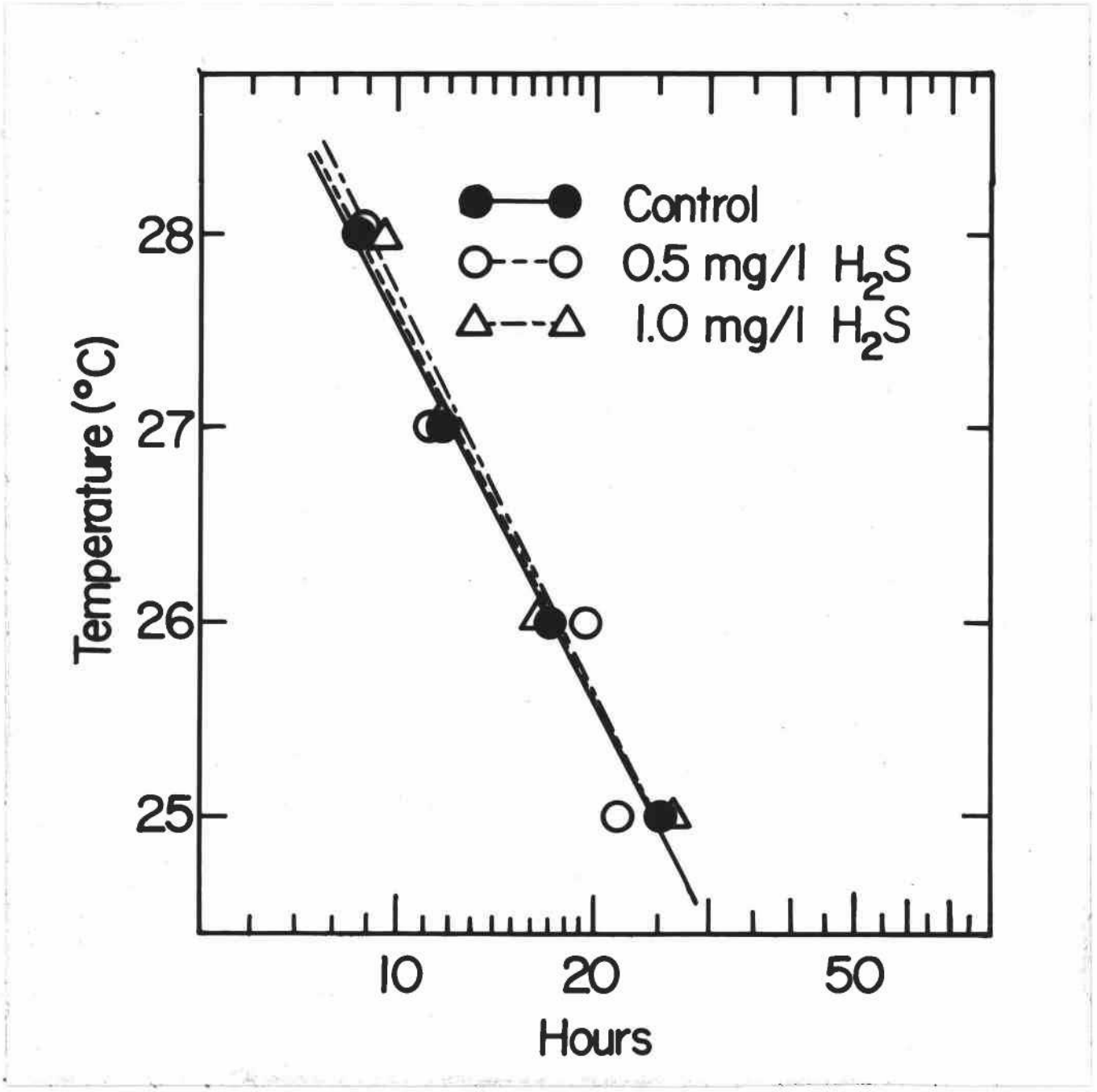


Figure 2. Time to 50 % mortality as a function of temperature for first instar zoeae of Cancer magister previously exposed for 48 h to 0, 0.5 and 1.0 mg/l hydrogen sulfide.

## DISCUSSION

Among the adult stages of invertebrates examined in this study, the most tolerant to hydrogen sulfide was the bivalve mollusc M. balthica followed closely by the isopod G. oregonensis. In declining order of tolerance these were followed by C. gigas, the postlarval stage of C. magister and the amphipods A. confervicola and C. salmonis. Of the larval forms studied, the tolerance of the first zoeal stage of C. magister was essentially similar to most of the adult forms; the 96-h $LC_{50}$ for this stage was 0.5 mg/l. The embryos of C. gigas appeared considerably more sensitive than any of the other forms examined since an exposure of 0.32 mg/l for only 2 h inhibited the further development of over half of these organisms. The high sensitivity of these embryos may have been due, in part, to the elevated temperature under which the tests were made. Oyster embryos were incubated at 26° C while incubation temperatures employed in the other tests ranged from 12° C for postlarval crabs to about 17.5° C for most of the other adult and juvenile forms. Adelman and Smith (1972) found that high temperatures raised the sensitivity of goldfish to hydrogen sulfide although increased sensitivity was only 3 - 4 times over the temperature range of 12 - 25° C. By the time of development to 7 day old veligers, larvae were more tolerant to hydrogen sulfide. Veligers exposed for 2 h to 3.2 mg/l became inactive but after a 24-h recovery period all of the larvae appeared to be fully recovered.

The relationship of hydrogen sulfide tolerance between phylogenetically distinct groups is of considerable interest. In this regard, Theede et al. (1969) found a distinct correlation in their studies with 14 marine invertebrate species. In their work, animals were exposed,

in oxygen deficient water, to a single hydrogen sulfide concentration, 6.67 mg/l sulfide sulfur, and the time to 50 % mortality for each species was reported. The four most resistant organisms were bivalve molluscs followed by a gastropod mollusc. The sixth and seventh most tolerant organisms were the annelid Neresis diversicolor and the bivalve Cardium edule which exhibited equal tolerances. These were followed in order of declining resistance by an additional gastropod mollusc, an echinoderm, a brachyuran crab, another echinoderm, and three additional crustaceans. The range of tolerance times to this concentration of hydrogen sulfide in the absence of dissolved oxygen was from 2 - 1,000 h. Although we examined fewer species, over which the range of tolerances was considerably less, a similar relationship was evident; the tolerance of bivalve molluscs being greater than the majority of the crustaceans examined.

The principal effect of hydrogen sulfide at low concentrations is believed to be inhibition of the iron containing oxidative enzymes (Smith and Gosselin, 1964, 1966). Although the comparative inhibition kinetics of sulfide for these enzymes is not known, it would not be unreasonable to suppose that differences between taxa would be minimal. It is likely, therefore, that differences in toxicity of hydrogen sulfide that are seen are attributable to other factors. The most plausible explanation would appear to be the degree of facultative anaerobiosis of which an organism is capable. Organisms able to accumulate an oxygen debt or to maintain basic life support processes indefinitely, using the energy derived through anerobic metabolism, should be most resistant to hydrogen sulfide since inhibition of the oxidative enzymes would be of little or no consequence. The available data supports this hypothesis.

Theede, et al. (1969) have shown a strong correlation between the ability of various invertebrates to tolerate oxygen deficiency and their ability to tolerate hydrogen sulfide. Many molluscs, particularly the bivalves, are known to be facultative anaerobes (Hochachka, 1973) and Theede et al. (1969) found that the bivalves were the most tolerant of both oxygen deficiency and hydrogen sulfide.

Although we have not examined the ability of organisms used in the present study to tolerate oxygen deficiency, a gross estimate of their ability to tolerate anoxic conditions may be inferred from knowledge of their habitat requirements. The species we found most tolerant of hydrogen sulfide, M. balthica, is an infaunal inhabitant of muddy sediments in the upper reaches of estuaries. The most tolerant crustacean G. oregonensis is frequently found in the lower portions of estuaries under stones imbedded in sediments. It is conceivable that either of these organisms could be faced with periods when anoxic conditions prevail and may have evolved a tolerance to this condition. With the exception of C. salmonis, the benthic organisms studied which are less tolerant of sulfide are epifaunal forms directly exposed to currents in the overlying water. The zoeae of C. magister and oyster larvae are both planktonic forms that are unlikely to experience anoxic water containing sulfides. Although C. salmonis is a burrowing amphipod found in muddy sediments, this organism lives within a centimeter of the surface in well aerated burrows and, therefore, may not normally encounter oxygen deficient water.

The hydrogen sulfide tolerances of marine invertebrates observed in this study and by Theede et al. (1969) are of a similar order of magnitude, but are considerably higher than found in studies of freshwater fish. The 96-h $TL_{50}$'s for hydrogen sulfide for eggs and fry of northern

pike (Esox lucius) are 0.037 and 0.008 mg/l, respectively (Adelman and Smith, 1970). Colby and Smith (1967) found that the 96-h median tolerance level of hydrogen sulfide to walleye fry (Stizotedion vitreum) was 0.05 mg/l. The 96-h $TL_{50}$'s for adult goldfish ranged from 0.004 mg/l at 25° C to 0.530 mg/l at 6° C (Adelman and Smith, 1972). The median tolerance concentration for this species at 15° C was about 0.100 mg/l. The slightly greater tolerance of this species to hydrogen sulfide compared with the other teleosts examined may be related to the ability of this species to undergo partial anaerobiosis (Kutty, 1968). These low tolerances are not limited to teleost fish. Oseid and Smith (1974) reported that the 96-h $LC_{50}$ of hydrogen sulfide for the freshwater amphipod Gammarus pseudolimnaeus at 18° C was 0.022 mg/l, and in chronic studies at the same temperature the threshold of toxic effect was noted at 0.002 mg/l. The greater sensitivity of these freshwater forms to hydrogen sulfide may be related to the lesser incidence of sulfides in freshwater habitats as compared to marine environments.

Sublethal exposures of Dungeness crab zoeae to hydrogen sulfide appeared to have only minimal effect on the thermal tolerance of this organism. Zoeae surviving 48 h exposure to 0.5 and 1.0 mg/l were as tolerant of exposures to 25.0 - 28.0° C as were untreated zoeae. However, in another experiment zoeae that had survived a 22-h exposure to 0.56 mg/l were less able than controls to resist an acute thermal shock treatment at 29.0° C. The causes of thermal death are not clearly understood but may involve physiological functions at several loci. In addition, different loci or mechanisms may be involved at different temperatures even though all temperatures would ultimately be lethal. It is conceivable, therefore, that sulfide poisoning could potentiate thermal

damage at temperatures above 28° C in crab zoeae, but not influence the thermal response at lower temperatures. Regardless of the mechanisms involved, the influence of hydrogen sulfide on thermal tolerance appears to be so slight as to have no ecological significance.

Hydrogen sulfide tolerance is a property of considerable significance to marine organisms. Concentrations of 1 mg/l of sulfide are not uncommon in water overlying sediments in estuaries even though oxygen may be present and may range to 10 mg/l or higher under certain conditions (Bella et al., 1972). Yet such common members of the estuarine benthos as C. salmonis and A. confervicola and postlarval crabs C. magister are unable or only barely able to tolerate 1.0 mg/l hydrogen sulfide during several days of continuous exposure. These observations clearly demonstrate the fragile nature of the relationship between estuarine organisms and their environment with respect to sulfide and suggests the danger that may be associated with such perturbations of the environment as may occur during channel dredging operations.

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