

Sulfide exposure accelerates hypoxia-driven mortality

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Abstract

The effect of the presence of sulfide on the survival of benthic organisms under hypoxia was tested using a meta-analysis of published experimental results evaluating the effects of the presence of hydrogen sulfide on the median survival time of benthic macrofauna under hypoxia. The meta-analysis confirmed that survival times under hypoxia are reduced by an average of 30% in marine benthic communities exposed to hydrogen sulfide. The effect of sulfide on survival was higher for egg forms than for juvenile or adult stages. The aggravation of the negative effects of spreading hypoxia in the presence of sulfide suggests that the threats derived from hypoxia to marine biodiversity are greater than anticipated on the basis of the direct effects of low oxygen concentration alone.

Dissolved oxygen is a property that has changed drastically in a short period of time in the marine environment (Diaz and Rosenberg 1995; Diaz 2001). Oxygen deficiencies have increased in frequency, duration, and severity in the world's coastal areas during recent decades (Diaz and Rosenberg 2008), and as a consequence hypoxia is emerging as a major threat to marine biodiversity (Vaquer-Sunyer and Duarte 2008). Hypoxia reduces the abundance and diversity of the benthic macrofauna (Josefson and Widbom 1988; Rosenberg et al. 1991; Gray et al. 2002) and affects the structure and function of marine ecosystems (Wu 2002). Changes in the benthic macrofauna community observed after episodes of severe oxygen deficiency indicate differential tolerance to oxygen concentrations (Diaz and Rosenberg 1995), with mollusks and polychaetes being typically more tolerant to oxygen deficiency than echinoderms, fishes, and crustaceans (Theede et al. 1969; Taylor and Spicer 1987; Levitt and Arp 1991).

These differences in tolerance are reflected also in the length of time organisms survive under hypoxia (Vaquer-Sunyer and Duarte 2008). However, the onset of hypoxia is followed by a number of changes in the ecosystem that significantly affect the conditions for further organismal survival (Conley et al. 2009). In particular, as hypoxia progresses, benthic microbial communities shift to sulfate reduction, and sulfide concentrations increase in the environment (Conley et al. 2009). When oxygen is not available to oxidize organic matter, prokaryotes utilize alternative electron acceptors such as sulfate, nitrate, nitrite, or metal oxides. Sulfate is normally the most abundant, and its reduction has sulfide as an end product (Berner 1984). Sulfide is always present in the anoxic layer of marine sediments (Fenchel and Jorgensen 1977) and its diffusion into bottom water is controlled by the oxic sediment depth, dictated by the rate of oxygen diffusion and the oxygen consumption in the sediment (Vistisen and Vismann 1997). During hypoxic events, the anoxic layer of the sediments migrates upwards and can reach the water

column, with sulfide intrusion into the bottom water. Sulfide is very toxic to most aerobic organisms because of its inhibition of cytochrome *c* oxidase activity at micromolar ($\mu\text{mol L}^{-1}$) concentrations (Nicholls 1975*b*; Petersen 1977; Nicholls and Kim 1982). Sulfide binds with high affinity to the ferric iron in the heme site of cytochrome aa_3 (Nicholls 1975*a*). The interaction of sulfide with blood proteins such as hemoglobin, binding to the hemoglobin porphyrin ring (Berzofsky et al. 1971), reduces oxygen delivery to mitochondria in some species (Evans 1967). As sulfide increases during hypoxia, the macrofauna is also exposed to sulfide, so that benthic mass mortalities during hypoxic events may be a consequence of both sulfide toxicity and hypoxia rather than low oxygen concentration alone (Vistisen and Vismann 1997). Environmental stressors can have additive effects in shortening survival time of marine organisms under hypoxia, such as increasing temperature, increased $p\text{CO}_2$ levels in the ambient waters (Portner and Farrell 2008), and the presence of hydrogen sulfide and contaminants, among others.

Here we test whether the presence of sulfide affects survival of benthic animals under hypoxia. More specifically, we use a meta-analysis of published experimental results examining the survival of benthic organisms under both hypoxia and the presence of sulfide to evaluate the effects of the presence of hydrogen sulfide on the median survival time of benthic macrofauna under hypoxia.

Methods

We searched for reports of hypoxia on the Web of Science and Scholar Google using the keywords “hypoxia,” “marine,” “benthic,” “sea,” and “sulfide” and their combinations to guide the search. This search delivered more than 6000 published reports of responses of benthic marine organisms to hypoxia, which were then examined further for the availability of experimental assessments of responses to reduced oxygen concentration that included treatments with addition of sulfide. This more restricted search delivered a total of 68 experimental assessments examining the median lethal time (LT_{50}), representing the

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Table 1. Median lethal time in presence of sulfide ($LT_{50} H_2S$) and median lethal time without sulfide (LT_{50}) reported in experimental assessments.

Taxon	Group	Species	Life stage	$LT_{50} H_2S$ (h)	Sulfide content ($\mu\text{mol L}^{-1}$)	LT_{50} (h)	Source
Annelida	Polychaeta	<i>Nereis diversicolor</i>	Adult	96	196.2	120	Theede et al. 1969
Annelida	Polychaeta	<i>Streblospio benedicti</i>	Adult	25	20	27	Llansó 1991
Annelida	Polychaeta	<i>N. diversicolor</i>	Adult	99.732	196.2	123.3	Diaz and Rosenberg 1995
Annelida	Polychaeta	<i>Scoloplos armiger</i>	Adult	39	220	46	Kruse et al. 2004
Annelida	Polychaeta	<i>S. armiger</i>	Adult	32	220	36.5	Kruse et al. 2004
Mollusca	Bivalva	<i>Cyprina islandica</i>	Adult	900	196.2	1320	Theede et al. 1969
Mollusca	Bivalva	<i>Scrobicularia plana</i>	Adult	425	196.2	550	Theede et al. 1969
Mollusca	Bivalva	<i>Mya arenaria</i>	Adult	408	196.2	504	Theede et al. 1969
Mollusca	Bivalva	<i>Mytilus edulis</i>	Adult	600	196.2	840	Theede et al. 1969
Mollusca	Bivalva	<i>Cardium edule</i>	Adult	96	196.2	102	Theede et al. 1969
Mollusca	Bivalva	<i>Donax serra</i>	Juvenile	80	100	110	Laudien et al. 2002
Mollusca	Bivalva	<i>Macoma secta</i>	Adult	120	100	312	Levitt and Arp 1991
Mollusca	Bivalva	<i>Macoma nasuta</i>	Adult	168	100	432	Levitt and Arp 1991
Mollusca	Bivalva	<i>Scapharca inaequivalvis</i>	Adult	208.8	100	672	De Zwaan et al. 1993
Mollusca	Bivalva	<i>S. inaequivalvis</i>	Adult	228	200	364.8	De Zwaan et al. 1993
Mollusca	Gastropoda	<i>Littorina littorea</i>	Adult	180	196.2	365	Theede et al. 1969
Mollusca	Gastropoda	<i>Littorina saxatilis</i>	Adult	72	196.2	144	Theede et al. 1969
Mollusca	Bivalva	<i>Macoma balthica</i>	Adult	528	100	194.6	Jahn and Theede 1997
Mollusca	Bivalva	<i>M. balthica</i>	Adult	528	100	251.3	Jahn and Theede 1997
Mollusca	Bivalva	<i>M. balthica</i>	Adult	216	100	268.5	Jahn and Theede 1997
Mollusca	Bivalva	<i>M. balthica</i>	Adult	216	100	280.5	Jahn and Theede 1997
Mollusca	Bivalva	<i>Mulinia lateralis</i>	Adult	91.5	18,941.2	259	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	115.8	13,617.6	259	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	119.7	9470.6	259	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	132.8	4735.3	259	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	146	1470.6	259	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	100	18,941.2	251	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	100	13,617.6	251	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	107	9470.6	251	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	119.5	4735.3	251	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	127	1470.6	251	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	54.6	18,941.2	107	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	54.6	13,617.6	107	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	56	9470.6	107	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	77.5	4735.3	107	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Adult	87	1470.6	107	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	52.8	18,941.2	181	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	52.8	13,617.6	181	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	55.4	9470.6	181	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	57.2	4735.3	181	Shumway et al. 1983
Mollusca	Bivalva	<i>M. lateralis</i>	Juvenile	91.6	1470.6	181	Shumway et al. 1983
Mollusca	Gastropoda	<i>L. saxatilis</i>	Adult	72.8		162.1	Diaz and Rosenberg 1995
Mollusca	Bivalva	<i>Cerastoderma edule</i>	Adult	99.1		110.0	Diaz and Rosenberg 1995
Mollusca	Gastropoda	<i>L. littorea</i>	Adult	192.4		406.0	Diaz and Rosenberg 1995
Mollusca	Bivalva	<i>M. arenaria</i>	Adult	438.5		541.9	Diaz and Rosenberg 1995
Mollusca	Bivalva	<i>S. plana</i>	Adult	508.6		699.4	Diaz and Rosenberg 1995
Mollusca	Bivalva	<i>M. edulis</i>	Adult	651.5		971.5	Diaz and Rosenberg 1995

Table 1. Continued.

Taxon	Group	Species	Life stage	LT ₅₀ H ₂ S (h)	Sulfide content (μmol L ⁻¹)	LT ₅₀ (h)	Source
Mollusca	Bivalva	<i>Arctica islandica</i>	Adult	1128.5		1394.7	Diaz and Rosenberg 1995
Crustacea	Decapoda	<i>Carcinus maenas</i>	Adult	32	196.2	48	Theede et al. 1969
Crustacea	Amphipoda	<i>Gammarus oceanicus</i>	Adult	8	196.2	15	Theede et al. 1969
Crustacea	Isopoda	<i>I. balthica</i>	Adult	6	196.2	6	Theede et al. 1969
Crustacea	Decapoda	<i>Crangon crangon</i>	Adult	2	196.2	2	Theede et al. 1969
Crustacea	Thalassinidea	<i>Calocaris macandreae</i>	Adult	29	1002.9	30	Johns et al. 1997
Crustacea	Copepoda	<i>Acartia tonsa</i>	Eggs	9.5	278.2	29.0	Marcus et al. 1997
Crustacea	Copepoda	<i>A. tonsa</i>	Eggs	9.0	264.1	7.51	Marcus et al. 1997
Crustacea	Copepoda	<i>Centropages hamatus</i>	Eggs	11.8	337.6	16.9	Marcus et al. 1997
Crustacea	Copepoda	<i>C. hamatus</i>	Eggs	8.7	352.2	32	Marcus et al. 1997
Crustacea	Copepoda	<i>Labidocera aestiva</i>	Eggs	32	282.9	32	Marcus et al. 1997
Crustacea	Copepoda	<i>L. aestiva</i>	Eggs	0.4	318.2	5.0	Marcus et al. 1997
Crustacea	Decapoda	<i>C. crangon</i>	Adult	1.9		1.9	Diaz and Rosenberg 1995
Crustacea	Isopoda	<i>I. balthica</i>	Adult	6.0		6.0	Diaz and Rosenberg 1995
Crustacea	Amphipoda	<i>G. oceanicus</i>	Adult	7.7		14.2	Diaz and Rosenberg 1995
Crustacea	Decapoda	<i>C. maenas</i>	Adult	31.1		50.2	Diaz and Rosenberg 1995
Echinodermata	Asteroidea	<i>Asterias rubens</i>	Adult	67	196.2	84	Theede et al. 1969
Echinodermata	Asteroidea	<i>Ctenodiscus crispatus</i>	Adult	236	196.2	248	Shick 1976
Echinodermata	Asteroidea	<i>A. rubens</i>	Adult	64.1		85.8	Diaz and Rosenberg 1995
Echinodermata	Ophiuroidea	<i>Ophiura albida</i>	Adult	30	196.2	32	Theede et al. 1969
Echinodermata	Ophiuroidea	<i>O. albida</i>	Adult	28.9		73.5	Diaz and Rosenberg 1995

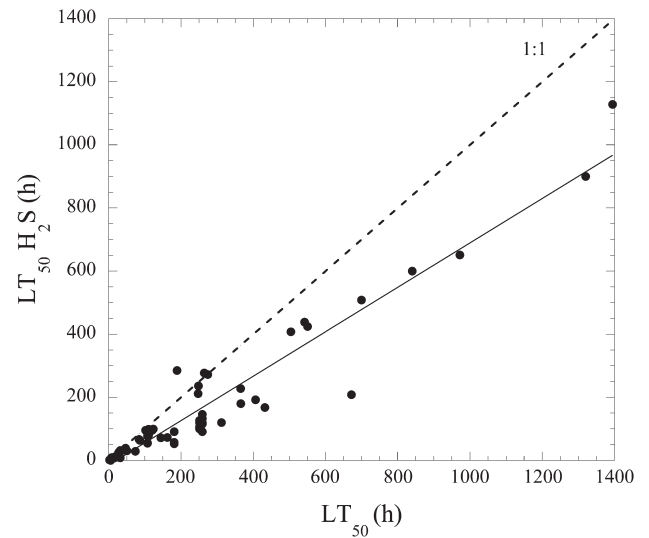


Fig. 1. The relationship between the median lethal time in the presence of sulfide (LT₅₀ H₂S) and the median lethal time without sulfide (LT₅₀). The solid line represents the fitted regression equation, LT₅₀ H₂S (h) = -8.45 (± 13.74) + 0.70 (± 0.04) × LT₅₀ (h) (R² = 0.84, p < 0.0001, n = 68). The dotted line represents the line 1 : 1. See Table 1 for data sources.

statistically derived time interval at which 50% of a given population dies after exposure to low O₂ levels, involving 30 different species of marine benthos. The vast majority (98.5%) of the experiments used very low oxygen concentrations to assess median lethal time in presence of sulfide (mean ± SE = 0.2 ± 0.1 mg O₂ L⁻¹), and one experiment used 5.75 mg O₂ L⁻¹ to compare survival under normoxia in the presence and the absence of sulfide. The full data set is shown in Table 1.

A general linear nested model was used to assess changes in median survival time under hypoxia of animals exposed to sulfide as a function of hydrogen sulfide concentration and the median lethal time in the absence of sulfide, and analysis of covariance (ANCOVA) was used to test for differences in response of changes in LT₅₀ in the presence and absence of sulfide among taxonomic groups.

Results

We found a total of 68 published experiments involving 30 species of benthic macrofauna in which the median lethal time (LT₅₀) was assessed in the presence and absence of sulfide. In all of these experiments the median lethal time in the presence of sulfide was lower than when the organisms were exposed to hypoxia alone (Fig. 1). The median lethal time under hypoxic conditions was reduced by, on average (±SE), 30% ± 2% in the presence of sulfide relative to that under hypoxia alone (Fig. 1).

A nested general linear model showed that the median lethal time in presence of sulfide (LT₅₀ H₂S, h) decreased with increasing sulfide concentration (Log₁₀ H₂S, μmol L⁻¹) and the interaction between the two variables,

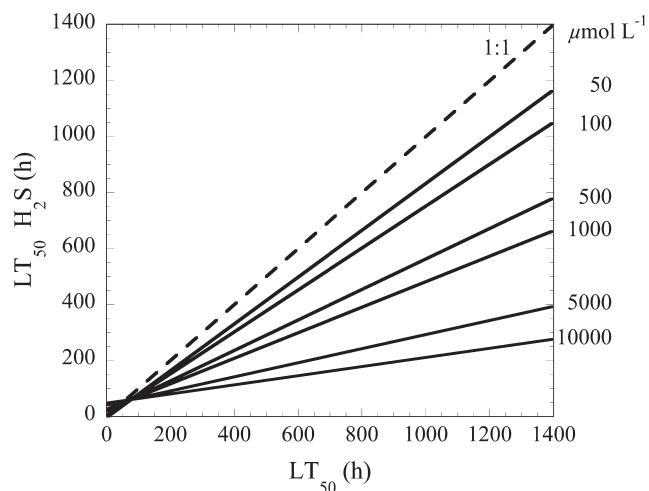


Fig. 2. The decrease in median lethal time in presence of hydrogen sulfide ($LT_{50} H_2S$) at different sulfide concentrations (50, 100, 1000, 5000, and 10,000 $\mu\text{mol L}^{-1}$) predicted by the model described the effect of sulfide concentrations on survival fitted here (Eq. 1).

as shown by the fitted regression equation

$$LT_{50} H_2S = 135.6 - 39.7 \times \text{Log}_{10} H_2S + 0.5 \times LT_{50} - 0.3 \times [(LT_{50} - 212.9) \times \text{Log}_{10} H_2S - 2.9] \quad (1)$$

$(R^2 = 0.92, p < 0.0001).$

The model indicates no significant negative effect of sulfide concentration on marine benthic organisms at sulfide concentrations below 14 $\mu\text{mol L}^{-1}$ (Fig. 2). The model shows that millimolar sulfide concentrations reduce the median lethal time to half of that in the absence of

sulfide (Fig. 2). Even greater reductions in survival time, to 15% of those in the absence of sulfide, result from exposure of benthic animals to exceptionally high concentrations in the order of 10 mmol L^{-1} (Fig. 2). Such exceptionally high sulfide concentrations have indeed been reported in nature, with a value of 15,000 $\mu\text{mol L}^{-1}$ H_2S reported from sediment pore waters of the Gulf of California (Goldhaber and Kaplan 1974; Table 2), which, according to our model, would reduce survival time of the associated fauna by 89% relative to that in the absence of sulfide. Indeed, the reduction in survival time can be quite high for various benthic environments (Table 2), showing that sulfide can indeed substantially shorten the life span of organisms under hypoxia in nature.

ANCOVA did not reveal any significant differences between taxonomic groups, in either the slope or the intercept (t -test, $t = -1.08$, $df = 64$, $p = 0.29$), in the regression equation relating LT_{50} in the presence or absence of sulfide under hypoxia. We found, however, ontogenic differences in the ratio of LT_{50} in the absence of sulfide and that in the presence of sulfide (analysis of variance, $F_2 = 8.20$, $p = 0.0006$), with eggs having the shortest survival in the presence of sulfide relative to hypoxia alone, followed by adults and then by juveniles (Fig. 3). Juveniles did not show significant differences in this ratio from adults or eggs, whereas eggs and adults showed significant differences from each other in the extent of response to sulfide presence (Tukey post hoc test, $p < 0.05$).

Discussion

The results presented here confirm the existence of a synergistic effect of hypoxia and the presence of sulfide in accelerating mortality of benthic macrofauna. The survival

Table 2. Sulfide concentrations reported in different habitats, the species inhabiting these habitats, and the reduction in survival time expected for the reported sulfide concentration as described by the lineal model reported here (Eq. 1).

Organism	Habitat	Reference	Sulfide concentration ($\mu\text{mol L}^{-1}$)	Reduction in LT_{50} (%)
<i>Urechis caupo</i>	Burrows, northern California	Arp et al. 1992	66	20
<i>Arenicola marina</i>	Burrows in summer, intertidal flat, France	Völkel and Grieshaber 1992	3.2–336	0–41
<i>A. marina</i>	Burrows in spring, intertidal flat, France	Völkel and Grieshaber 1992	1.7–138	0–30
<i>Tubificoides benedii</i>	Top 5 cm sediment, sand flat, North Sea	Dubilier et al. 1994	20–50	5–16
Mud shrimps (Thalassinidea)	Burrows, Scotland	Johns et al. 1997	260	37
Burrowing animals	Burrows, mudflat, France	Völkel et al. 1995	36	12
<i>Streblospio benedicti</i>	Top 1 cm sediment, subtidal mud habitat, Virginia	Llansó 1991	100	25
<i>Macoma balthica</i>	5 cm sediment depth, North Sea and Baltic Sea	Jahn and Theede 1997	1–432	0–44
<i>Branchioasychis americana</i>	Intertidal mudflat, Cedar Key, Florida	Wohlgemuth et al. 2007	10–170	0–32
	Sediment in Western Baltic	Oeschger and Vetter 1992	665	49
	Water column, Port Angeles harbor, Washington	Ziebell et al. 1970	3	n.e.
	Sediment pore water, Gulf of California	Goldhaber and Kaplan 1974	15,000	89
	Soft bottoms of North Sea mud flats	Thamdrup 1935	180	33

n.e., no effect

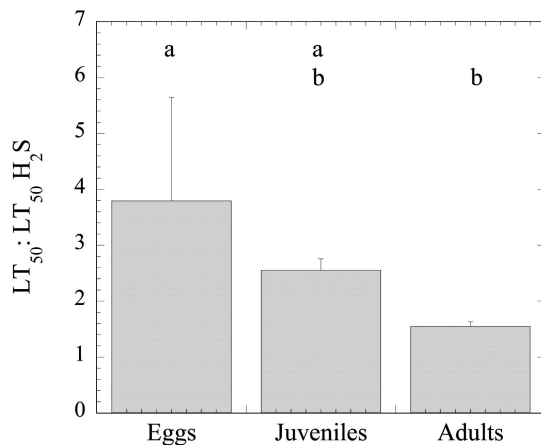


Fig. 3. Ratio of LT_{50} in the absence of sulfide to LT_{50} in the presence of sulfide for different life stages. The letters indicate the results of the Tukey-Kramer honestly significant differences (HSD) test, whereby the ratio did not differ significantly for life stages with the same letter.

of benthic organisms under hypoxia is reduced by 30% in the presence of sulfide and is reduced even more for egg stages. Sulfide is quite toxic to aerobic aquatic animals (Theede et al. 1969) and plants (Terrados et al. 1999) at micromolar concentrations; $10 \mu\text{mol L}^{-1}$ sulfide causes *Posidonia oceanica* meadows to decline (Calleja et al. 2007). The reason why the survival of benthic macrofauna under hypoxia is reduced in the presence of sulfide is that the toxicity of sulfide, once its oxidation to thiosulfate is not possible because of the high sulfide content or the lack of oxygen necessary to oxidize it, operates through interferences with cytochrome *c* oxidase activity (Nicholls 1975b; Petersen 1977; Nicholls and Kim 1982). These interferences affect respiratory processes directly and indirectly through the depletion of organic substrates and oxygen in sulfide oxidation by mitochondria, when animals are already compromised by low oxygen concentration. Nanomolar to low micromolar sulfide concentrations inhibit the cytochrome *c* oxidase of various organisms (Bagarinao 1992), with such toxic effects evident at sulfide concentrations ranging from $0.002 \mu\text{mol L}^{-1} \text{H}_2\text{S}$ for the bivalve *Mercenaria mercenaria* (Hand and Somero 1983) to $14 \mu\text{mol L}^{-1}$ for the annelid *Tubifex* sp. (Degn and Kristensen 1981). Mitochondrial respiration has been reported to be inhibited at $2 \mu\text{mol L}^{-1} \text{H}_2\text{S}$ for the annelid *Tubifex* sp. (Degn and Kristensen 1981) and at $38 \mu\text{mol L}^{-1} \text{H}_2\text{S}$ for the soil amoeba *Acanthamoeba castellanii* (Lloyd et al. 1981).

Under normoxic conditions, sulfide is present at some depth in the sediments. The depth of the sulfide front is set dynamically by the balance between the sulfide production through sulfate reduction in the anaerobic zone of the sediment and the diffusive and advective (though bioturbation) penetration of oxygen into the sediments. In particular, the depth of the sulfide horizon is set by the depth to which sufficient oxygen reaches to satisfy the respiratory requirements of the aerobic community and oxidize the sulfide diffusing upwards from the anoxic layers

of the sediment. Depletion of water-column oxygen during hypoxic events leads to upward displacement of sulfide within the sediments, which may be accelerated by the loss of bioturbating fauna. Sulfide may even invade the water column, resulting in sulfide events that may be more common in hypoxic waters than reported because sulfides may be oxidized before they reach the air–sea interface. The extent of sulfide oxidation will depend on the balance between sulfide production and oxygen penetration, but sulfides can also be removed by chemolithotrophs. Indeed, Lavik et al. (2009) reported, for the first time, an event of large-scale detoxification of sulfidic shelf waters by a bloom of chemolithotrophs, and postulate, on the basis of their results, that many sulfidic events in coastal waters may remain unnoticed because bacteria consume sulfide before it reaches the air–sea interface. Consequently, sulfide episodes in bottom waters on continental shelves may be more common than hitherto believed and could have an important but as yet neglected effect on benthic communities. Sulfide typically appears in the water column with a time lag, hours to days following the onset of hypoxia (Riedel et al. 2008). Hence, as hypoxia develops, the likelihood of mass mortality increases faster than anticipated, as the appearance of sulfide will shorten survival times.

There are multiple strategies that benthic organisms can adopt to survive in the presence of hydrogen sulfide. Escape may help mobile organisms avoid areas with high sulfide content. Whenever escape is not possible, organisms may try to isolate themselves from sulfide. For instance, bivalves can close their valves, but such responses are effective only over limited time spans (Hagerman 1998). Some organisms detoxify after sulfide intrusion by oxidizing the sulfide to thiosulfate, a capacity reported for the ostracod *Cyprideis torosa* (Jahn et al. 1996), the Baltic clam *Macoma balthica* (Jahn and Theede 1997), the priapulid worm *Halicryptus spinulosus* (Oeschger and Vetter 1992), the polychaetes *Heteromastus filiformis* (Oeschger and Vismann 1994) and *Arenicola marina* (Völkel and Grieshaber 1994), and the crustacean *Saduria entomon* (Vismann 1991), among others.

Sulfide oxidation can also be used as an energy source in some species, such as the ribbed mussel *Geukensia demissa* (Kraus and Doeller 2004), the polychaete *H. filiformis* (Oeschger and Vismann 1994), and the lugworm *A. marina* (Völkel and Grieshaber 1997). The mitochondria of these three species use sulfide as a respiratory substrate for adenosine triphosphate production.

Two main strategies appear to be involved in sulfide detoxification through sulfide oxidation: mitochondrial and blood-based sulfide oxidation (Grieshaber and Völkel 1998). Some organisms can oxidize sulfide in the hepatopancreas, such as the crabs *Bythograea thermydron* (Vetter et al. 1987) and *S. entomon* (Vismann 1991). In natural environments, where the presence of sulfide is normally associated with oxygen deficiency, mitochondrial sulfide oxidation is effective only at micromolar ambient sulfide concentrations for most metazoans and requires the presence of oxygen to oxidize the sulfide to thiosulfate, sulfite, or sulfate. Although the inhibition of mitochondrial

respiration occurs at concentrations ranging from 2 to 38 $\mu\text{mol L}^{-1}$ H_2S , sulfide acts as a mitochondrial substrate and stimulates oxygen consumption at slightly lower concentrations, reported at 5 to 15 $\mu\text{mol L}^{-1}$ H_2S (Bagarinao 1992). Sulfide detoxification by mitochondrial oxidation involves a delicate dynamic balance wherein sulfide must be promptly oxidized before it reaches internal concentrations inhibitory to cytochrome *c* oxidase (Bagarinao and Vetter 1990). Mitochondrial sulfide oxidation is a detoxification that competes with organic molecules for oxygen, reducing oxygen available for aerobic respiration. The detoxification of sulfide by oxidation to thiosulfate is the most favorable energetically, as 1 mol of sulfide is removed for every 1.5 mol of oxygen, whereas the oxidation to sulfite and sulfate requires 3 and 4 mol of oxygen per mol of sulfide, respectively (Johns et al. 1997).

A last strategy is to switch to anaerobic metabolism. During hypoxia, sulfide will compete with the electron transport chain for oxygen and force animals to change to anaerobic metabolism at a higher oxygen content than that at which this process would be triggered in the absence of sulfide (Grieshaber and Völkel 1998). A complete inhibition of the respiratory chain occurs at hydrogen sulfide concentrations exceeding 2 to 38 $\mu\text{mol L}^{-1}$ sulfide (Bagarinao 1992), and anaerobic metabolism is triggered even if the oxygen concentration is high enough to allow aerobic metabolism. Anaerobic pathways must proceed with the lowest possible consumption of energy reserves, both as a protection and to extend survival time (Hagerman 1998). That benthic animals may remain alive after exposure to anoxia or sulfide does not necessarily mean long-term survival, as the animals may be compromised and die subsequently. Processes occurring during recovery following exposure to sulfide are, therefore, important in determining the fates of the animals. Animals that turn to anaerobic metabolism and accumulate metabolites must restore their energy resources and reoxidize at least part of the metabolites when aerobic metabolism is resumed (Hagerman 1998). Some organisms can eliminate the thiosulfate produced for sulfide oxidation through passive diffusion through the hindgut, as the echiuran worm *Urechis caupo* does (Julian et al. 1999), or through the body wall, as the lugworm *A. marina* does (Hauschild et al. 1999).

After sulfide exposure, some organelles can be damaged by sulfide. Wohlgemuth et al. (2007) proposed that electron-dense organelles (EDOs) represent transient organelles that sequester and degrade mitochondria or other cellular constituents damaged by sulfide. These EDOs are intracellular, membrane-bounded structures that appear electron-dense by transmission electron microscopy. EDOs are characteristic features of epithelial cells from marine annelids evolutionarily adapted to sulfide exposure and have been reported in all sulfide-adapted annelids that have been investigated using transmission electron microscopy (Wohlgemuth et al. 2007). An alternative pathway of sulfide detoxification is the precipitation of free sulfide by metals (commonly copper), as demonstrated in the Baltic clam *M. balthica* by Windoffer et al. (1999). This mechanism is

temporary and only works at low sulfide conditions (10 $\mu\text{mol L}^{-1}$).

Although many marine benthic invertebrates have developed strategies to cope with sulfide exposure, we found no differences in the negative effects of sulfide exposure among broad taxonomic groups. Egg stages, however, showed more negative response to the presence of sulfide than adult and juvenile stages, indicating that a sulfide event can result in catastrophic consequences for hatching success and therefore population dynamics.

The meta-analysis conducted here shows that the presence of hydrogen sulfide will decrease survival times under hypoxia by an average of 30% in marine benthic communities. This reduction is concentration-dependent and varies with the sulfide levels that animals experience in their natural environments, and can reach a reduction in survival time of up to 90% at $> 10 \text{ mmol L}^{-1}$ sulfide concentrations, the highest levels reported in nature (Table 2). In contrast, other organisms such as *Tubificoides benedii* that inhabit the upper 5 cm of the sediment can encounter moderate sulfide concentrations, ranging between 20 and 50 $\mu\text{mol L}^{-1}$ (Dubilier et al. 1994), which, using our fitted model, would lead to modest reductions in survival time under hypoxia ranging from 5% to 16% (Table 2). Current frameworks defining hypoxia consider oxygen concentrations alone and do not include the possible synergistic effect of hypoxia and other stresses, such as sulfide toxicity. Results presented here suggest that the survival of benthic invertebrates may be shorter than anticipated as sulfide appears, possibly accelerating mortality events. Other stresses, including pollutants and ammonia, may further reduce their survival. Aggravation of the negative effects of spreading hypoxia by the presence of sulfide suggests that the threats derived from hypoxia to marine biodiversity are greater than anticipated.

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