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Survival of the copepod *Acartia tonsa* following egg exposure to near anoxia and to sulfide at different pH values

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ABSTRACT: The impact of near anoxia (1.78×10^{-3} to 3.56×10^{-3} mmol O_2 l^{-1}) and anoxia/sulfide (~1 mmol l^{-1}) on hatching and viability of eggs of the planktonic copepod *Acartia tonsa* was evaluated. Since the equilibrium concentration of the sulfide species is influenced by pH and the different species have different capacities to enter cells, the impact of sulfide was analyzed at pH 8.2 and 6.5. The consequences of egg exposure for growth and survival of the hatched organisms were also studied. Subitaneous eggs, spawned by laboratory-reared organisms, were incubated in near anoxia or anoxia/sulfide for different periods (1, 4, 15 and 32 d) and then transferred to normoxic conditions. Short exposure to near anoxia or anoxia/sulfide did not affect egg viability and subsequent growth and survival. Exposure times ≥15 d caused significant declines in hatching and strong reductions in life expectancy. No significant differences between the effects of near anoxia and anoxia/sulfide (at both pHs) were observed following incubation for 15 d. After 32 d incubation, the hatching success of eggs exposed to anoxia/sulfide at pH 8.2 was significantly higher than that of eggs exposed to near anoxia or to anoxia/sulfide at pH 6.5, and life expectancy was also less reduced. The results indicate that long exposure of eggs to anoxia/sulfide is less detrimental to *A. tonsa* than near anoxia alone when the pH is in the range of natural seawater (7.9 to 8.3). It seems to be more detrimental when pH is as low as that reached in pore waters (6.0 to 6.5).

KEY WORDS: Near anoxia · Sulfide · pH · *Acartia tonsa*

INTRODUCTION

During the last decades nutrient over-enrichment has affected many shallow coastal and estuarine areas around the world, bringing large-scale consequences such as an increasing and spreading occurrence of hypoxic and anoxic waters (Diaz & Rosenberg 1995, Nixon 1995, Paerl 1997, Diaz 2001, Rabalais & Turner 2001). Oxygen depletion is frequently coupled with the production of hydrogen sulfide, which is toxic to most aerobic organisms. In aqueous solution, the equilibrium concentration of the different sulfide species varies with pH. In natural seawater, where pH ranges from 7.9 to 8.3, the predominant species is the hydrosulfide anion (HS^-). In pore water, where pH can be as low as 6.0 to 6.5, the neutral molecular H_2S may prevail (Vismann 1996, Wang & Chapman 1999). While H_2S freely crosses membranes, the HS^- anion may be electrically excluded (Bagarinao 1992, Wang & Chapman 1999). It has been shown that total sulfide toxicity is modulated by pH and increases with decreasing pH (Powell & Somero 1986, Vismann 1996). According to Bagarinao (1992), however, the HS^- anion also seems to contribute to toxicity at high sulfide concentrations.

Hypoxic (<0.089 mmol l^{-1} dissolved oxygen, according to Diaz & Rosenberg 1995), anoxic, and sulfidic conditions occur more frequently in sediments and bottom waters. Numerous studies of the impact of these stressors on biota have therefore focused on benthic fauna (Pihl et al. 1991, Nilsson & Rosenberg 1994, Gamenick et al. 1996, Vistisen & Vismann 1997, Vopel et al. 1998). Many planktonic species release

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subitaneous or diapause eggs freely into the water column. These eggs tend to sink and so can spend hours to years in the seabed before hatching (Marcus & Boero 1998); they can, hence, be exposed to hypoxia, anoxia and/or sulfide. Thus, these stress conditions, even if occurring at the water/sediment interface, may affect population dynamics of planktonic species. Many of the studies carried out on planktonic species (Uye & Fleminger 1976, Ambler 1985, Lutz et al. 1992, 1994, Roman et al. 1993, Marcus & Lutz 1994, Stalder & Marcus 1997) have dealt with *Acartia tonsa*, a calanoid copepod very common in many lagoons and estuarine areas. Lutz et al. (1994) reported that the eggs of this species stop hatching at oxygen concentrations lower than 3.12×10^{-3} mmol l^{-1}, but are not killed since they resume hatching when transferred to normoxic seawater. The results obtained by various authors (Uye & Fleminger 1976, Ambler 1985, Lutz et al. 1992, 1994, Roman et al. 1993, Marcus & Lutz 1994) differ slightly in regard to the concentration of oxygen that induces quiescence and the maximum survival times for eggs exposed to low oxygen concentrations. The impact of anoxia and sulfide (at pH 8.2) on egg viability of planktonic copepods under laboratory conditions has been studied only by Marcus et al. (1997). The authors exposed the eggs of the calanoids *A. tonsa*, *Labidocera aestiva* and *Centropages hamatus* to anoxia and anoxia/sulfide and concluded that there is no significant difference in viability between eggs exposed to anoxia/sulfide and those exposed to anoxia alone. All these studies only took hatching success into account, and no data are available on the development and life expectancy of the individuals hatched from eggs exposed to these stress conditions. But, according to Hagerman (1998), 'to be alive after exposure to hypoxia or sulfide is not the same as surviving', since processes acting during the recovery phase to restore the fuel resources and reoxidise metabolites (oxygen debt) are also important. As a consequence, the cost of the recovery borne during the embryonic stage might affect growth and survival of subsequent developmental stages.

The aim of the present study was to assess hatching and viability of *Acartia tonsa* eggs exposed to near anoxia and sulfide for different times, and to evaluate the consequences of egg exposure on growth and survival at the subsequent developmental stages. The experiments were performed at high concentrations of dissolved sulfide (~1 mmol l^{-1}) to evaluate the effects of extreme conditions. This choice was made on the basis of the highest sulfide concentrations reported in the literature for highly eutrophic environments (Bagarinao 1992, Bartoli et al. 1996, Giordani et al. 1996) and detected in water column and at sediment superficial layers. Considering that the equilibrium concentration of the sulfide species is influenced by pH, and that the different chemical species may have different capacities to enter cells, the experiments were carried out at 2 different pHs: 8.2 and 6.5.

MATERIALS AND METHODS

Sampling and maintenance cultures. *Acartia tonsa* eggs used in the experiments came from laboratory cultures. To set up cultures, zooplankton samples were originally collected in the Venice lagoon (North Adriatic Sea, Italy) using a 50 µm mesh plankton net. The samples were diluted in a 5 l tank immediately after collection and transported to the laboratory within 4 h.

Adults (females and males) of *Acartia tonsa* were sorted under a dissecting microscope and transferred into aquaria ($21 \times 20 \times 25$ [height] cm) containing natural seawater (S = 30 to 32‰; pH = 8.2 ± 0.1) filtered through a 0.45 µm mixed cellulose ester filter. The aquaria were maintained in a room with constant temperature at 20 ± 1°C and a 14 h light:10 h dark cycle. The cultures were fed a mixture of 3 algal species: *Isochrysis galbana* (CCAP 927/1), *Tetraselmis suecica* (CCAP 66/22A) and *Rhinomonas reticulata* (CCAP 995/2).

Algae were cultured in the laboratory in artificial medium according to APHA, AWWA, WPCF (1989). The original recipe was modified: $CoCl_2$ (0.01 mg l^{-1}) was added and the amounts of $NaNO_3$ and K_2HPO_4 were increased to obtain final concentrations of P and N more appropriate for the algae we were cultivating, i.e. 12.6 mg N l^{-1}, 1.12 mg P l^{-1}, N:P = 11:3. The cultures were maintained in a room with constant temperature at 20 ± 1°C. Cool, white, fluorescent lights provided 3000 lux illumination on a 12 h light:12 h dark cycle.

Experimental procedures. To guarantee the constant composition of the medium, all the experiments were carried out in artificial seawater (APHA, AWWA, WPCF 1989, modified in the content of NaCl to obtain a salinity of 30‰).

The water was deoxygenated by bubbling it for about 2 h with nitrogen gas previously washed using Dreschel bottles containing a mixture of 1 part pyrogallol solution (25%) and 5 parts caustic liquid potassium (1.52 to 1.54 density). The pH was then measured and adjusted to 8.2 with diluted HCl. Screw-capped vials (25 ml) were carefully filled with the water to avoid trapping any gas bubbles. A suitable number of vials (from 4 to 10 for each exposure time, depending on the number of spawned eggs) was used for the incubation of the eggs. Initial and final (at the end of the incubation period) oxygen concentrations were determined by the micro-Winkler

method (APHA, AWWA, WPCF 1989). At the low values obtained (dissolved oxygen < 3.56×10^{-3} mmol l^{-1}, from now on referred to as 'near anoxia'), oxygen determination required the use of the entire volume of water in a single vial, causing the loss of the incubated eggs. As preliminary tests (data not reported) indicated that even after 32 d of incubation there were no significant differences in the oxygen concentrations between vials with and without eggs, oxygen determination in the experiments was made on vials without eggs (6 vials per experiment).

Sulfide stock solution (~0.1 M) was prepared as follows: 2.1 g anhydrous Na_2CO_3 were added to 100 ml bidistilled water, the solution was boiled for 10 min to remove oxygen, the hot solution was added to about 2.4 g $Na_2S \times 7\text{-}9H_2O$ (only white crystals were used) and mixed well. Stock solution was dispensed into screw-capped glass vials with minimum headspace. Sulfide concentration of the stock solution was determined according to the method of Cline (1969). The vials were stored at room temperature in the dark.

To obtain the required sulfidic conditions (~1 mmol l^{-1} of total sulfide), a suitable aliquot of sulfide stock solution was added to artificial water deoxygenated as described above. The pH was then readjusted to 8.2 or 6.5 with diluted HCl, and screw-capped vials (25 ml) were filled for the treatment of the eggs. Before and after filling the vials, 2 subsamples of water were taken to determine the initial sulfide concentration (Cline 1969). At the end of the incubation period, sulfide concentration was determined on aliquots taken from the vials used for the egg incubation.

The preparation of the near-anoxic and sulfidic water and the filling of the vials were done inside a modified atmosphere (~100 % N_2) chamber. Before and during the incubation, the vials filled with near-anoxic water were kept in an Anaerojar (HP11, Oxoid) filled with nitrogen gas, to avoid increases in oxygen concentration.

In all experiments the vials were prepared 24 h before the introduction of the eggs. The addition of high sulfide amounts to near-anoxic water brought the system (vial) to anoxia (as determined according to the method of Mor & Beccaria 1971) within the 24 h. From now on, the term 'sulfide' will be used to indicate this treatment.

Five days before the beginning of the experiments, about 20 females and 20 males were isolated from 15 or 21 d old cohorts, transferred into a crystallizing dish containing 300 ml natural seawater (NSW) and 300 ml artificial seawater (ASW) and fed a mixture of the 3 algal species. About 15 h before the beginning of the experiments the organisms were distributed into 4 smaller crystallizing dishes containing 200 ml ASW and fed. This procedure was used to avoid a drastic transfer of the adults from natural into artificial seawater, and to allow the spawning of the eggs in artificial seawater. The eggs spawned during the subsequent 15 h were collected under a dissecting microscope and transferred into 24-well plates, 1 egg per well, containing 2.5 ml normoxic ASW (controls) at pH 8.2 or 6.5, or into the vials (8 to 10 eggs per vial) containing near-anoxic or sulfidic seawater at pH 8.2 or 6.5. The vials were incubated for 1, 4, 15 or 32 d at a temperature of 20 ± 1°C and a 14 h light:10 h dark cycle. At the end of the incubation period the eggs were transferred to 24-well plates containing normoxic ASW at pH 8.2.

The eggs were checked daily until hatching or degeneration. Since the nauplii were not able to reach the copepodid stage in the multi-well plates (data not reported), they were transferred into small crystallizing dishes containing 45 ml ASW (3 nauplii per dish) just after hatching. The hatched individuals were fed a mixture of the algae *Isochrysis galbana* (5×10^4 cells ml^{-1}) and *Tetraselmis suecica* (1×10^4 cells ml^{-1}) and maintained at the same conditions of temperature and photoperiod as the bulk cultures. Water and algae were renewed once a week. Survivorship and the number of organisms at the different developmental stages (nauplius, copepodite and adult) were recorded daily till the cohorts died out. Females and males were placed together to allow egg fertilisation and spawning.

The experiments were repeated once or twice.

Data analysis. Each vial was considered as a replicate for hatch data analysis. Since the data were counts with a binary outcome (live/dead), a series of nonparametric tests was used to evaluate and compare the influence of the different conditions (normoxia, near anoxia, sulfide at pH 8.2 and sulfide at pH 6.5) and exposure times on hatching success. The Jonckheere-Terpstra trend test for ordered differences among classes (exposure time) and Kruskal-Wallis 1-way analysis of variance by ranks, followed by the comparison procedure described by Dunn (1964), were applied.

To evaluate and compare the influence on growth and survival, data from replicated experiments were pooled. Two kinds of survival curves were constructed: mortality pattern of hatched organisms over time and survivorship over the course of the entire life cycle, dividing development into 4 stages (egg, larval, i.e. nauplius, juvenile, i.e. copepodite and adult). The Kolmogorov-Smirnov nonparametric test was applied to compare survival curves over time and Survival-Life Table analysis by Wilcoxon (Gehan) nonparametric test was applied to compare survivorship during development (life expectancy).

RESULTS

In near-anoxic vials, final oxygen concentrations ranged from 1.78×10^{-3} to 3.56×10^{-3} mmol l^{-1}. Only during 32 d experiments were dissolved oxygen values slightly increased to between 4.02×10^{-3} and 4.46×10^{-3} mmol l^{-1}. Initial sulfide concentrations ranged from 0.78 to 1.12 mmol l^{-1}. The final concentrations showed a slight decrease with increasing incubation time. The lowest final value (0.66 mmol l^{-1}) was observed after 32 d exposure. pH did not change in all experimental vials.

Vials containing a gas bubble at the end of the incubation period were not considered and some eggs were lost during the transfer to or from the vials. This caused variation in the vial and egg numbers among the experiments.

Hatching success

In normoxic conditions (control), hatching success ranged from 88 to 100%, with the exception of 2 multiwell plates at pH 6.5, where it was 75% (Fig. 1). No hatching occurred during either near anoxia or sulfide treatment, irrespective of the exposure time and pH: the eggs resumed hatching only after transfer to normoxic seawater.

Eggs exposed to near anoxia resumed hatching within 48 h. Incubation in near-anoxic conditions for 1 or 4 d did not significantly reduce the final hatching success (Fig. 1), which almost always reached 100% after 1 d exposure and ranged from 67 to 100% after 4 d. Increasing the incubation time reduced hatching (Jonckheere-Terpstra trend test; $p < 0.001$). The hatching success of the eggs incubated for 15 d showed the highest variability, ranging from 0 to 100%, and was significantly ($p < 0.05$) lower than that of control eggs. Hatching of the eggs incubated for 32 d ranged from 0 to only 14% (with the exception of 1 replicate, where it was 75%), a significant reduction with respect to control eggs and eggs incubated for 1 and 4 d.

Most of the eggs exposed to sulfide resumed hatching within 48 h of the transfer to normoxic seawater, but hatching was observed even after 10 d. Incubation in sulfidic conditions for 1 or 4 d at both pHs did not significantly reduce the final hatching success (Fig. 1), which ranged from 75 to 100% after 1 d exposure and from 63 to 100% after 4 d. Sulfide-treated eggs also underwent a hatching decline with increase of the incubation time (Jonckheere-Terpstra trend test; $p < 0.001$). The hatching success of the eggs exposed to sulfide for 15 d at pH 8.2 ranged from 20 to 88%, and was significantly ($p < 0.05$) reduced with respect to control eggs and eggs treated for 1 and 4 d. At pH 6.5, hatching success ranged from 0 to 88% and was significantly ($p < 0.05$) reduced only with respect to control eggs. When the exposure time was prolonged to 32 d, hatching ranged from 20 to 80% in the eggs incubated at pH 8.2, while it was almost completely suppressed in the eggs incubated at pH 6.5. In both cases the reduction was significant with respect to control eggs and eggs treated for 1 and 4 d.

Comparison of the different conditions (near anoxia, sulfide at pH 8.2, sulfide at pH 6.5) showed that hatching success differed only among the eggs that had been incubated for 32 d, the hatching of the eggs

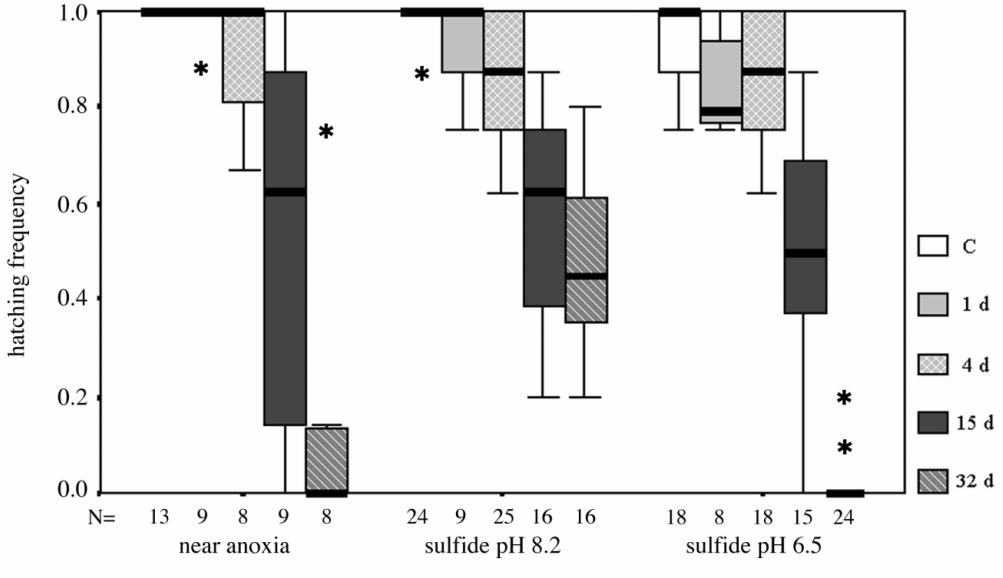

Fig. 1. *Acartia tonsa*. Box-and-whisker plots of hatching success (frequency) of eggs exposed to the different conditions. The line across each box indicates the median of the data, the top and bottom of each box are the 25th and 75th percentiles (quartiles), the vertical lines extending from the boxes indicate the data remaining outside. C = control (normoxic condition), N = number of replicates (vials), * = outliers

exposed to sulfide at pH 8.2 being significantly ($p < 0.05$) higher than that of the eggs exposed to the other conditions. No significant difference was found between near anoxia and sulfide at pH 6.5.

Growth and survival

In all the cohorts, the first nauplii generally developed to copepodites within 5 to 7 d after hatching and to adults within 10 to 12 d, irrespective of egg treatment, exposure time and pH. Longevity of adults ranged from 10 to 50 d, being higher for females. Even though fecundity was not quantified, in all cohorts where individuals reached adulthood, eggs that hatched into nauplii were observed.

High variability in the percentages of nauplii that developed to copepodites and adults was observed in the control cohorts: 66 to 76% of the nauplii reached the copepodite stage and 39 to 56% reached adulthood. Consequently, when the impacts of the different treatments on survivorship and life expectancy were compared, only highly significant differences ($p < 0.001$) were accepted.

Survivorship of the copepods hatched from near-anoxia exposed eggs showed the same pattern as the control (Fig. 2). The percentages of nauplii that reached juvenile (from 50%, after 32 d exposure, to 74%, after 1 d exposure) and adult stages (from 32%, after 15 d exposure, to 52%, after 1 d exposure) were as high as those observed in control cohorts.

Incubation of eggs in sulfidic water for 1 or 4 d at both pHs did not affect subsequent survival, as shown by survivorship curves that were similar to those of the control cohort (Fig. 2). The percentages of nauplii that reached the copepodite stage ranged from 53% (after 4 d exposure at pH 6.5) to 69% (after 4 d exposure at pH 8.2). The percentages of nauplii that developed to adults varied from 36% (after 4 d exposure at pH 6.5) to 59% (after 1 d exposure at pH 6.5). Exposure to sulfide for 15 d at pH 8.2 caused a decline in survival of the hatched organisms: survival was significantly ($p < 0.001$) reduced with respect not only to the control but also to the cohorts hatched from 1 and 4 d exposed eggs. Only 32% of the nauplii developed to copepodites and 18% became adults. Incubation of eggs at pH 6.5, in contrast, did not affect survivorship. Survival showed the same pattern as the control and the percentage of nauplii that reached subsequent developmental stages was high: 46% of them became copepodites and 39% developed to the adult stage. Exposure to sulfidic conditions for 32 d, at both pHs, caused a significant ($p < 0.001$) reduction in survival of the hatched organisms. At pH 8.2, only 16% of the nauplii reached the copepodite and 12% the adult

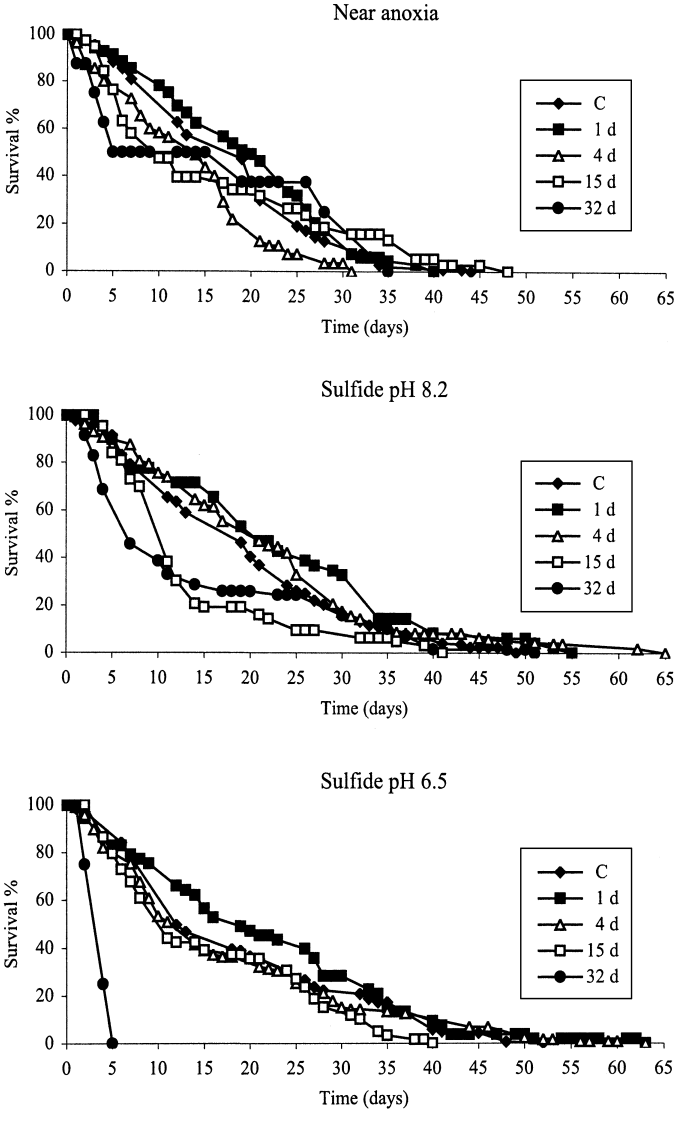

Fig. 2. *Acartia tonsa*. Survivorship pattern of the copepods hatched from eggs exposed to the different conditions. C = control (normoxic condition)

stage. When the eggs were incubated at pH 6.5, the few hatched nauplii died within 4 d of the restoration of normoxic conditions and none reached the copepodite stage.

To assess the consequences of egg exposure to near-anoxic and sulfidic conditions for the entire life cycle of *Acartia tonsa*, data on hatching and on subsequent development and survival were compiled (Fig. 3) and life expectancies were compared (Table 1). Exposure of eggs to near anoxia or to sulfide at both pHs for 1 or 4 d did not reduce life expectancy: the survival curves over the course of the entire life cycle showed the same pattern as the control curves. Exposure of eggs to the different conditions for 15 d caused a significant ($p <$

0.001) reduction in life expectancy with respect to control and to 1 and 4 d exposed cohorts. Life expectancy after exposure to sulfide at pH 8.2 did not suffer a further reduction when exposure time was increased from 15 to 32 d, whereas it was significantly reduced after exposure to sulfide at pH 6.5 or to near anoxia. Comparison of the different conditions (near anoxia, sulfide at pH 8.2, sulfide at pH 6.5) (Table 2) showed significant differences in life expectancy only after incubation for 32 d. Specimens exposed to sulfide at pH 6.5 were the most heavily impacted.

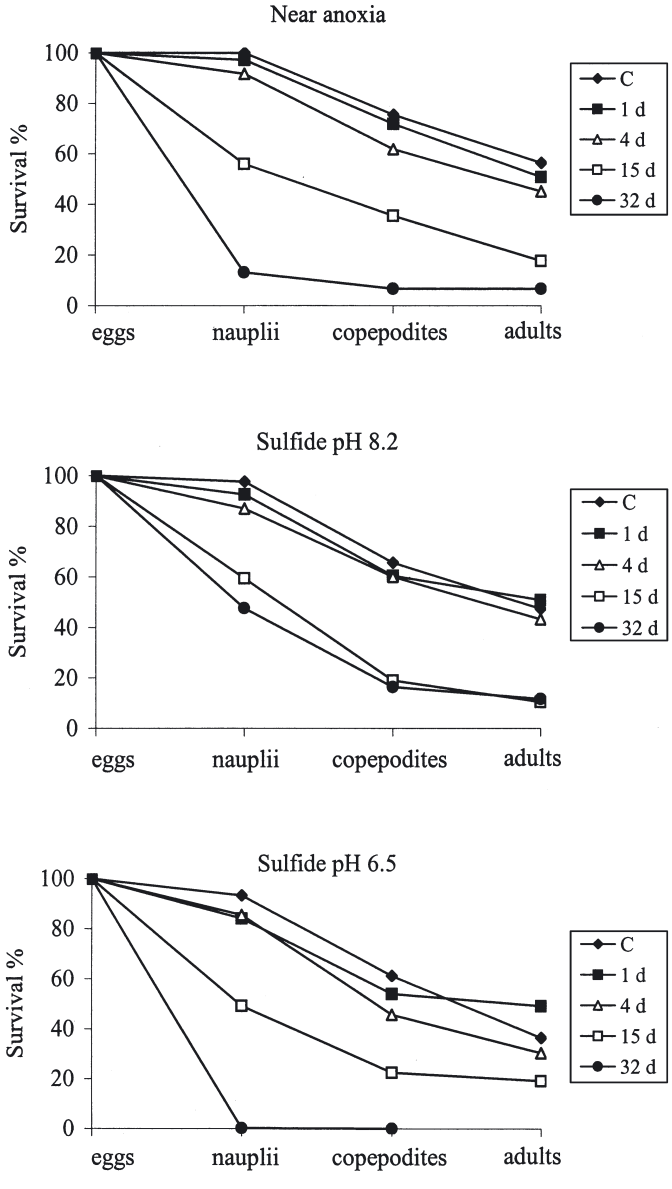

Fig. 3. *Acartia tonsa*. Survivorship at the different developmental stages (egg, larval, i.e. nauplius, juvenile, i.e. copepodite, and adult) after egg exposure to the different conditions. All the eggs were considered viable before treatments (100% survival). C = control (normoxic condition)

DISCUSSION

Exposure to near anoxia (oxygen concentrations 1.78×10^{-3} to 3.56×10^{-3} mmol l^{-1}) and sulfide at pH 8.2 and pH 6.5 induced quiescence in the eggs of *Acartia tonsa*: the eggs were able to resume hatching after restoration of normoxic conditions. However, in all the

Table 1. Wilcoxon (Gehan) nonparametric test: p-values obtained by comparing life expectancy after egg exposure to each condition for different times. C = control

	C	1 d	4 d	15 d
Near anoxia				
C				
1 d	0.412			
4 d	0.050	0.271		
15 d	<0.001	<0.001	<0.001	
32 d	<0.001	<0.001	<0.001	<0.001
Sulfide pH 8.2				
C				
1 d	0.805			
4 d	0.094	0.411		
15 d	<0.001	<0.001	<0.001	
32 d	<0.001	<0.001	<0.001	0.090
Sulfide pH 6.5				
C				
1 d	0.959			
4 d	0.014	0.137		
15 d	<0.001	<0.001	<0.001	
32 d	<0.001	<0.001	<0.001	<0.001

Table 2. Wilcoxon (Gehan) nonparametric test: p-values obtained by comparing life expectancy after egg exposure to the different conditions for different times: (a) near anoxia versus sulfide at pH 8.2 and sulfide at pH 6.5; (b) sulfide at pH 8.2 versus sulfide at pH 6.5

	(a) Near anoxia			
	1 d	4 d	15 d	32 d
Sulfide pH 8.2				
1 d	0.502			
4 d		0.653		
15 d			0.440	
32 d				<0.001
Sulfide pH 6.5				
1 d	0.158			
4 d		0.027		
15 d			0.287	
32 d				<0.001
	(b) Sulfide pH 8.2			
	1 d	4 d	15 d	32 d
Sulfide pH 6.5				
1 d	0.480			
4 d		0.019		
15 d			0.495	
32 d				<0.001

experimental conditions, egg viability decreased with increasing exposure time and, after 15 and 32 d incubation periods, significant declines in hatching were observed. The maximum survival times for the eggs exposed either to near anoxia or sulfide were not detectable, since after 32 d exposure some eggs were still viable in all the experimental conditions. Marcus & Lutz (1994) and Marcus et al. (1997) found that the hatching success of *A. tonsa* eggs exposed to anoxia or anoxia/sulfide declined with increasing exposure time. Maximum survival times reported by Marcus & Lutz (1994) for eggs exposed to anoxia were 28 d for 'newly spawned' eggs (0 to 3.5 h post-spawning) and 20 d for fully developed eggs (10 to 13.5 h post-spawning). Marcus et al. (1997) exposed newly spawned eggs to anoxia and anoxia/sulfide (at concentrations ranging from 0.283 to 0.352 mmol l^{-1} and pH 8.11 to 8.67) and observed viable eggs after a 32 d incubation period for both conditions. The differences, according to Marcus & Lutz (1994), were related to variations in the developmental stages of the eggs used in the experiments or, as reported by Marcus et al. (1997), 'may have been due to differences in the genetic and/or environmental histories of the females used to obtain eggs'. Both studies used females collected in the field (Gulf of Mexico) during different periods of the year and eggs at 2 stages of embryological development. Although we used females bred under standard conditions, so that environmental differences were minor and genetic variability would be presumably lower than in the field, and treated eggs ranging from 0 to 15 h old, our results are in the range of values reported by the cited studies. The differences in hatching success observed within each treatment appear consistent with the variability reported by Marcus & Lutz (1994) and Marcus et al. (1997).

Comparison of the impacts of near anoxia and sulfide on viability of the eggs did not show significant differences when the exposure time was ≤15 d. But when the incubation was prolonged to 32 d, the hatching success of the eggs exposed to sulfide at pH 8.2 was significantly higher than that of the eggs exposed to near anoxia. Lutz et al. (1994) suggest that 'perhaps prolonged exposure to low oxygen concentrations, as opposed to zero oxygen, is more detrimental to the eggs because, in the absence of oxygen, metabolism is shut down completely, whereas under low oxygen conditions some metabolic functions proceed'. Survival under anoxia depends on an overall reduction in metabolic rate to conserve substrates and reduce the accumulation of toxic end products, a phenomenon commonly referred to as 'metabolic rate depression' (Clegg 1997). Our results seem consistent with this. In our experiments, the addition of sulfide to near-anoxic water caused anoxia in the incubation vials. Under these conditions the metabolic rate depression is probably deeper than in near anoxia and cell poisoning is slowed down so that eggs can overcome longer exposure to stress conditions. It seems, therefore, that the hatching reduction observed after 32 d exposure was only due to the lack of oxygen, and that the exposure to sulfide at pH 8.2 did not have additional negative effects on hatching success. These results appear to be consistent with the suggestions of Marcus et al. (1997): they reported no significant differences in viability for eggs from 3 calanoid copepod species (*Acartia tonsa*, *Labidocera aestiva* and *Centropages hamatus*) exposed to anoxia/sulfide (at pH values ranging from 8.11 to 8.67) and to anoxia alone, and suggested that the eggs which survived sulfide or prolonged exposure to anoxia had switched to anaerobic metabolism.

At pH 6.5, sulfide appeared to affect egg viability. Hatching was almost completely inhibited after 32 d exposure, showing significant differences with respect to pH 8.2 and a reduction close to that observed in the eggs exposed to near anoxia alone. Metabolic rate depression and sulfide speciation may explain these different responses. The presence of sulfide brings the system (vial) to anoxia so that, according to Marcus et al. (1997) and Hagerman (1998), the eggs have to turn to anaerobic metabolism. The metabolic rate depression due to zero oxygen should presumably be the same at both pHs. All sulfide species can be considered toxic due to the equilibrium $H_2S \leftrightarrow HS^- \leftrightarrow S^{2-}$ (Vismann 1996). However, it has been shown that total sulfide toxicity depends on pH (Broderius et al. 1977, Powell & Somero 1986, Vismann 1996): at pH 6.5 the molecular H_2S, which can penetrate into cells more readily than the anion HS^-, represents more than 70% of total sulfide, whereas at pH 8.2 the dominant species, present at more than 90%, is HS^-.

Studies of benthic invertebrates (Vismann 1990, De Zwaan et al. 1993, Cattani et al. 1996, Visman & Hagerman 1996, Hagerman 1998) indicate that exposure to hypoxia/anoxia plus sulfide (at pH 8.0 to 8.5) is more detrimental to the organisms than hypoxia or anoxia alone. Vismann & Hagerman (1996) and Hagerman (1998) suggested that, when sulfide diffuses in biological systems, the metabolic rate cannot be drastically reduced since mechanisms of sulfide detoxification are probably needed even during anaerobiosis. Our results with copepod eggs contradict these studies. Sulfide exposure for ≤15 d, even at the high concentrations we used, did not appear to be toxic to the *Acartia tonsa* eggs, irrespective of pH. Sulfide toxicity became evident only after 32 d exposure at pH 6.5, but even in this case the reduction in hatching success is not significantly different with respect to near anoxia alone. Bagarinao (1992) reported that there is no evidence that marine animals can exclude sulfide at the

body wall. Due to the tissue diffusion coefficient of H_2S, similar to that of O_2 (Powell 1989), Vopel et al. (1998) suggested that the equilibrium between external and internal sulfide concentration in small-size organisms should be reached within a few hours. On the basis of these considerations, the different response to sulfide at pH 8.2 and 6.5, detectable only after 32 d exposure, is not easy to explain. In this study we considered eggs instead of adult organisms: the presence of an egg wall able to block the entry of sulfide might explain the high tolerance of *A. tonsa* eggs. In this case, internal sulfide concentration should increase slowly, as a function of the exposure time and external molecular H_2S concentration; only when the eggs are exposed for a long time to high concentrations of H_2S, can the internal sulfide reach such a level where toxic effects are observed.

Growth and survival of hatched organisms were not affected when eggs were exposed to the different experimental conditions for short times (1 and 4 d). As for hatching, the most considerable differences in the effects of near anoxia and sulfide at different pH were observed after 32 d exposure: incubation in near anoxia did not affect the survival of the organisms hatched from the exposed eggs, exposure to sulfide at pH 8.2 caused a significant decline with respect to the control, and exposure to sulfide at pH 6.5 brought about the death at the naupliar stage of the few hatched organisms. Differences in the accumulation of toxic end products (e.g. lactate) during quiescence or sublethal toxic effects of sulfide may explain these responses. It may be that in near-anoxic conditions selection occurs at the egg stage: the few eggs that survive probably have the highest tolerance and are able to develop to adult stage. In sulfidic conditions, toxic end product accumulation is presumably lower and reaches a sublethal level. Consequently, hatching success is higher than in near anoxia but many nauplii may be not able to grow and develop. Besides, a toxic effect of sulfide cannot be excluded: after 32 d exposure at pH 8.2 the internal sulfide concentration may reach a sublethal level affecting the growth and survival of nauplii. At pH 6.5, internal sulfide probably reaches a level where hatching success is near zero.

The highest variability in hatching data was observed after 15 d exposure. In all conditions the hatching median values were near 50%, a value around which quantal data generally have the highest variability. Moreover, exposure of the eggs to near anoxia and to sulfide at pH 6.5 for 15 d did not seem to affect growth and survival of the nauplii, whereas incubation in sulfidic conditions at pH 8.2 caused a decline with respect to the control and to 1 and 4 d (short-time) exposure. However, comparison of the various conditions did not show significant differences among the survival curves of the copepods hatched from eggs exposed for 15 d. These results cannot be easily explained. It could be that differences in responses to the various experimental conditions become noticeable only when the ability to withstand the cost of quiescence is severely reduced. The 15 d period might be a threshold time, during which adaptation mechanisms to overcome exposure to near anoxia and sulfide become close to exhaustion. At this exposure time, even minor variations in experimental conditions (i.e. oxygen concentration, genetic variability, age of the eggs) could bring about a high variability in the responses. Furthermore, the small sample size could have had an effect.

When hatching success and subsequent growth and survival are considered together, the consequences of egg exposure to near anoxia or sulfide for the entire life cycle and, hence, possible effects on the population dynamics of *Acartia tonsa* are more appreciable. Exposure times ≥15 d caused a strong reduction in life expectancy. Thus, when the eggs of *A. tonsa* experience near-anoxic or sulfidic conditions for a long time, the population dynamics of this species will be adversely affected. When exposure time was increased from 15 to 32 d, the effects on life expectancy differed depending on the different conditions experienced by the eggs. The eggs exposed to sulfide at pH 8.2 did not suffer a further decrease in life expectancy, whereas the eggs exposed to near anoxia or sulfide at pH 6.5 underwent a severe reduction. In particular, exposure at pH 6.5 completely hindered survival.

Our study indicates that long-time exposure of eggs to near anoxia is more detrimental than exposure to high sulfide concentrations, when pH is in the range of natural seawater (7.9 to 8.3) and the dominant sulfide species is HS^-. On the contrary, when exposure to sulfide is at a pH as low as the values that can be reached in pore waters (6.0 to 6.5) and the species H_2S prevails, the effects on organisms are more detrimental than in near anoxia alone.

Acartia tonsa is a species typical of eutrophic coastal areas (Brylinsky 1981). It was recorded for the first time in the Mediterranean Sea by Gaudy & Viñas (1985) and in the Adriatic Sea by Farabegoli et al. (1989). In recent years it has become the dominant species of the planktonic copepod component in the North Adriatic lagoons. The greater numerical importance of *A. tonsa* led to the progressive disappearance of the congeneric species *A. margalefi* and *A. latisetosa*, which were typical of the most confined areas of these lagoons (Sei et al. 1996). The high tolerance to both near anoxia and sulfide shown by the eggs of *A. tonsa* could favor its strong development in the lagoons to the detriment of congeneric species. Differences in tolerance to anoxia between *A. tonsa* and *A. clausi* eggs has been sug-

gested by Gaudy et al. (2000) as a possible factor explaining the absence of an *A. clausi* population in the Berre lagoon (French Mediterranean coast), where *A. tonsa* is the dominant species. The comparative evaluation of the tolerance of the *Acartia* species eggs to anoxia and anoxia/sulfide could allow better understanding of the role played by these stress factors in the distribution of these species in coastal environments.

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