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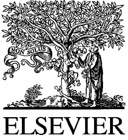

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Survivorship of juvenile surf clams *Donax serra* (Bivalvia, Donacidae) exposed to severe hypoxia and hydrogen sulphide

J. Laudien [a,*], D. Schiedek [b], T. Brey [a], H.-O. Pörtner [a], W.E. Arntz [a]

[a] *Alfred Wegener Institute for Polar and Marine Research, P.O. Box 120161, 27515 Bremerhaven, Germany*
[b] *Baltic Sea Research Institute, Warnemünde, Germany*

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Abstract

Toxic "sulphide eruptions" sporadically occur in the highly productive inshore regions of the central Namibian Benguela upwelling system. The surf clam *Donax serra* (Röding, 1798) dominates the intertidal and upper subtidal of large exposed sandy beaches of southern Africa and its recruitment seems to be affected by sulphide events. The reaction of juvenile surf clams to low oxygen concentrations and sulphide occurrence (0.1 mmol l^{-1}) was examined by in vitro exposure experiments in a gas-tight continuous flow system. After 2 h of hypoxic- and hypoxic/sulphidic conditions, clams moved to the sediment surface, aiding their passive transport to areas with more favourable conditions. The clams showed a high sulphide detoxification capacity by oxidising the penetrating hydrogen sulphide to non-toxic thiosulphate. Moreover, juvenile *D. serra* switched to anaerobic energy production, indicated by the significant accumulation of succinate and, to some extent, alanine. Test animals were not able to reduce their energy requirements enough to withstand long periods of exposure, leading to a median survival time (LT_{50}) of 80 h under hypoxic sulphide incubation. In conclusion, natural "sulphide eruptions", especially those with a large spatial and temporal extension, have to be considered as an important factor for *D. serra* recruitment failures. Hydrogen sulphide is assumed to be a potential community structuring factor. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Benguela upwelling system; *Donax serra*; Hydrogen sulphide; Hypoxia; Succinate; Sulphide oxidation; Thiosulphate

* Corresponding author. Tel.: +49-471-4831-1643; fax: +49-471-4831-1149.
E-mail address: jlaudien@awi-bremerhaven.de (J. Laudien).

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1. Introduction

Coastal upwelling regions are frequently exposed to hypoxic conditions (Rosenberg et al., 1983; Arntz et al., 1991; Tarazona et al., 1991; Diaz and Rosenberg, 1995; Gallardo et al., 1998) owing to extremely high primary production and subsequent oxidative degeneration of organic matter (Van der Plas, 1999; Fossing et al., 2000), Advancing hypoxic water has the potential to cause mass mortalities of benthos and fish (Brongersma-Sanders, 1957; Hart and Currie, 1960; Arntz, 1981). Further anaerobic degradation of organic matter due to sulphate reducing bacteria results in the production of hydrogen sulphide (e.g., Huxtable, 1986; Widdel, 1988; Jørgensen, 1990). For the Benguela upwelling system permanent hypoxic conditions are recorded in the inshore areas downstream of the major upwelling centres (Bailey, 1991, 1999; Fossing et al., 2000). Especially in summer, a combination of physical and biochemical processes causes sulphidic water to rise sporadically to the surface during "sulphide eruptions" (Bailey, 1991). As a consequence, extremely low oxygen concentrations and high hydrogen sulphide concentrations are occasionally recorded in the intertidal and upper subtidal (Currie, 1999). The impact is perpetual as hydrogen sulphide has a half-life of a few hours even in oxygen-saturated seawater (Morse et al., 1987).

Many benthic organisms live in environments with regular occurrence of hypoxia and hydrogen sulphide. Hydrogen sulphide is highly toxic in nanomolar concentrations to aerobic eukaryotic organisms (Evans, 1967; Nicholls, 1975; National Research Council, 1979). Most intertidal bivalves are capable of sustaining their energy production by the use of anaerobic pathways (reviews: De Zwaan, 1977; Storey and Storey, 1990; Grieshaber et al., 1994). Furthermore, they have evolved a detoxification strategy by oxidising the poisonous hydrogen sulphide to non-toxic compounds, mainly thiosulphate (Jahn and Theede, 1997; Jahn et al., 1997). However, there is extensive evidence for stressful and harmful effects of hypoxia and anoxia from both in situ and laboratory experiments (review: Diaz and Rosenberg, 1995). These effects are even more pronounced in the presence of hydrogen sulphide (National Research Council, 1979; Shumway et al., 1983; Diaz and Rosenberg, 1995; Jahn and Theede, 1997).

Ecophysiological studies concerning hypoxia and occurrence of hydrogen sulphide are almost entirely devoted to species from hydrothermal vents or from eutrophicated coastal areas of the Northern Hemisphere. At present, not much is known about the metabolic effects of naturally occurring hydrogen sulphide on macrobenthic species in a coastal upwelling ecosystem such as the Benguela. The surf clam *Donax serra* (Röding, 1798) dominates the intertidal and upper subtidal of large exposed sandy beaches of southern Africa and was affected by sulphide events (Bailey, 1999). Laudien et al. (in press) hypothesised that an observed *D. serra* recruitment failure could have resulted from the occurrence of hypoxia and hydrogen sulphide affecting meroplanktic larvae and early juvenile survivorship. Therefore, the objectives of the present study were: (i) to investigate how juvenile *D. serra* deal with severe hypoxia and sulphide exposure (100 $\mu mol\ l^{-1}$) and (ii) to investigate as to what extent juvenile *D. serra* are able to detoxify this poisonous compound.

2. Material and methods

2.1. Study area and sampling

D. serra was collected during low tide in the intertidal of an exposed Namibian sandy beach (Langstrand: 22°47′ S, 14°33′ E), which is impacted by continuous wave action. The area is subjected to subequal semi-diurnal micro- to macrotides with a maximum range of about 2 m (McLachlan, 1986). The study site is described by Laudien et al. (in press) and main features of the beach are summarised in McLachlan (1985).

During a recruitment event in November and December 1999, post-settled *D. serra* of the smallest cohort (2–6 mm anterior–posterior length, approx. 5.5 mg) were collected by gently sieving the sediment (1 mm mesh). Within 1 h after sampling, animals were transported to the laboratory (burrowed in wet sand) and transferred into the experimental chambers. Experiments were started after 3 h.

2.2. Experiments

2.2.1. Tolerance of severe hypoxia and sulphide

Survivorship of juvenile *D. serra* was investigated in vitro under normoxic and hypoxic conditions in (i) the absence and (ii) the presence of hydrogen sulphide using a gas-tight system with continuous flow of seawater from a refillable reservoir (Fig. 1). The water reservoir (R1) contained 70 l of seawater (S = 35) filtered twice (National aquarium filter system and paper filter Whatman 2V). For deoxygenation of the water to a minimum oxygen content, it was warmed to 30–35 °C with an external red light, bubbled with pure nitrogen gas for 4 h and then cooled to room temperature. Deoxygenation was confirmed titrometrically (Grasshoff et al., 1983). Six single 250-ml filter flasks (F) containing a sand layer (100 ml of sand from the natural habitat, sterilized at 700 °C then cooled) were connected to the reservoir with oxygen impermeable Viton tubes. One juvenile *D. serra* was introduced into each filter flask before it was closed gas-tight with a rubber plug. This experiment was repeated four times using always a new individual.

At the beginning of each incubation period, all filter flasks were filled completely and kept at 16 ± 0.5 °C. Flow rate through each filter flask was set to 250 ml h^{-1} with variable clamp valves (CV). Due to varying water level in R1 causing changes in hydrostatic pressure, the flow rate was controlled and readjusted when necessary every 2 h. The volume of the effluent water of R1 was replaced continuously by overlaying pure nitrogen, and excessive gas could exhaust through a valve (V). Viton tubes (VT) were connected to glass tubes (GT), which were plunged through gas-tight plugs (P) of the filter flasks. The openings of the glass tubes were placed in the sand approx. 5 mm above the flask bottom. Inflowing water replaced the water in the filter flasks, which drained out through glass capillaries (C). A similar reservoir (R2) placed above the first one was set-up in order to be able to prepare new hypoxic water and refill R1. Again, the outflowing volume was replaced by pure nitrogen gas. In addition hypoxic and aerated normoxic controls were set up. In total, 72 clams were tested, 24 under

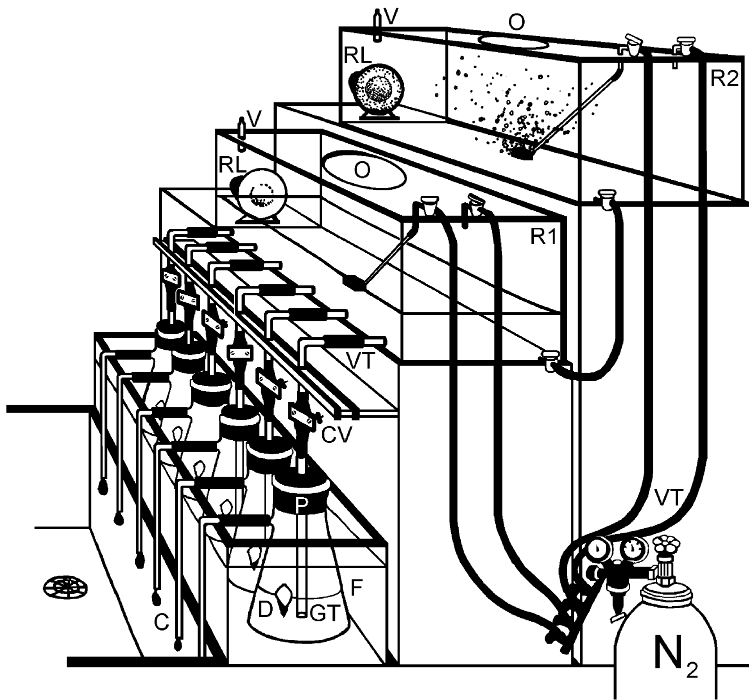


Fig. 1. Gas-tight system flow-through system C = glass capillaries, CV = clamp valve, F = filter flask, GT = glass tube, N = nitrogen gas, O = closable opening, P = gas-tight plugs, R1 = water reservoir 1, R2 = water reservoir 2, RL = redlight, V = valve, VT = Viton tube.

normoxia, 24 under hypoxic conditions and 24 under hypoxic/sulphidic conditions, respectively.

Stock solution of hydrogen sulphide (approx. 10 mmol) was prepared by dissolving (aqua bidest-washed) $Na_2S \times H_2O$ crystals ($\chi = 7-9$) in N_2-saturated sea-water taken from R1. Prior to the experiments, the concentration of the stock was determined by iodometric titration (Poethke, 1973) and sulphide was added to R1 through a closeable opening (O) to a final sulphide concentration of 0.1 mmol l^{-1}. To monitor the sulphide concentration during the course of the incubation, a 50-ml water sample was collected once every 2 h at the outflow of one glass capillary. Sulphide concentrations were determined spectrophotometrically at 660 nm according to the methylene blue method (Fonselius, 1976). Whenever R1 was refilled, at the start as well as at the end of the experiment, oxygen concentration in the water was checked titrometrically (Grasshoff et al., 1983). Sulphide did not change the pH of the incubation medium significantly as confirmed by pH controls (see also Hahlbeck et al., 2000).

Mortality assessment of test animals was based on failure of the valve closure reflex (Jahn and Theede, 1997) when the gaping surf clams were touched with the glass pipe of the water inlet. The number of surviving bivalves was monitored once every 2 h over a period of 7 days and mortality is given as median survival time

(LT_{50}) using the probability relation between percent mortality and time (Litchfield, 1949).

2.2.2. Short-term incubations in the presence of sulphide (0.1 mmol l^{-1})

Three replicated groups, with six juvenile *D. serra* each, were introduced into the above-described set-up for seven different time periods. In total, 21 groups of test animals (3 replicates × 7 time periods) were investigated. Incubation was terminated after 0, 1, 3, 6, 12, 24 and 48 h of exposure. Thereafter the tissues of the test animals were rapidly dissected, blotted dry and the pooled sample of each replicate immediately stored in liquid nitrogen until biochemical analysis.

2.2.3. Biochemical analyses

Sulphide oxidation products, e.g. sulphite and thiosulphate were determined in the juvenile surf clams as described in Schiedek et al. (1997): The pooled sample of six shell free juvenile *D. serra* (one replicate; approx. 0.15 g wet mass) were ground to fine powder in a mortar precooled by liquid nitrogen. Concentrations of sulphite (SO_3^{2-}), sulphide (H_2S), cystein ($C_3H_7NO_2HS$), thiosulphate ($S_2O_3^{2-}$) and glutathione ($C_{20}H_{32}N_6O_{12}S_2$) were quantified in the tissue by High-Performance Liquid Chromatography (HPLC) after derivatisation with monobromobimane (e.g., Völkel and Grieshaber, 1994; Jahn et al., 1996; Jahn 1997). The content of succinate and of the free amino acids alanine, aspartate and glutamate were measured as indicators for the onset of anaerobiosis. The free amino acids were analysed from perchloric acid (PCA) extracts. A 50-mg portion of the frozen tissue powder was transferred to 0.25 ml 3 mol l^{-1} PCA and homogenised with an Ultra-Turrax for 20 s. After centrifugation (12 min, 12,000 × *g*), the pH of the supernatant was neutralised by adding 5 mol l^{-1} K_2CO_3, controlled by the colour change of methylorange (1 drop). The precipitate was removed by centrifugation (20 min, 17,000 × *g*). Amino acids were separated and determined via HPLC (5 μm, Superspher 60, 125-4, RP-8, MERCK) according to Schiedek (1997). Succinate was analysed via capillary electrophoresis according to a modified method after "Agilent" (Organic Acid Analysis Kit P/N 5063-6510) (T. Hirse and H.-O. Pörtner, unpublished). Trichloric acetic acid (TCA) extracts were prepared using TCA (15%) and 0.12 g l^{-1} tatrate as an internal standard. Frozen tissue powder (100 mg) was suspended in the 3.5-fold volume of cold TCA and homogenised with an Ultra-Turrax for 10 s. After centrifugation (3 min, 16,000 × *g*, 0 °C) the supernatant was neutralised by the 3-fold volume of 1:4 *n*-Octylamine: 1,1,2-Trichlortrifluorethan (Freon), mixed and centrifuged (1 min, 16,000 × *g*, 0 °C). The upper phase was stored at − 80 °C and diluted 1:4 and filtered through a 0.2-μm injection filter prior to analyses. Succinate was separated with a "eCAP Capillary Tubing" (75 μm, 120 cm, Beckman 338473) at 27 kV and 15 °C and detected (PDA) at 214 nm. The separating buffer consisted of 1/5/20 Brij 35 (Fluka)/Acetonitril (Riedel, chromasolv)/Organic Acid Buffer (Agilent).

2.3. Statistical analyses

In order to test the reduction of survival time a log-rank test was performed. Thiosulphate, succinate and amino acid concentrations were tested for statistical signifi-

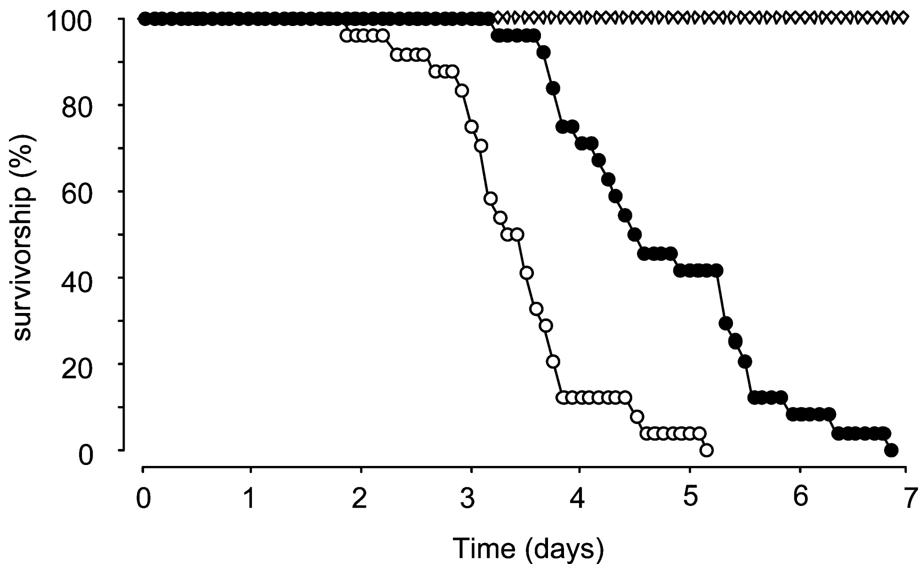


Fig. 2. Percentage of surviving post-settled *D. serra* during severe hypoxia in the absence (solid circles) or presence (open circles) of sulphide. Normoxic controls are shown as rhombi. $N = 24$ per treatment.

cance using ANOVA at the 5% level. Prior to the test, data of thiosulphate were square-root transformed, data of succinate were logarithmical transformed in order to achieve normality and induce homogeneity of variances.

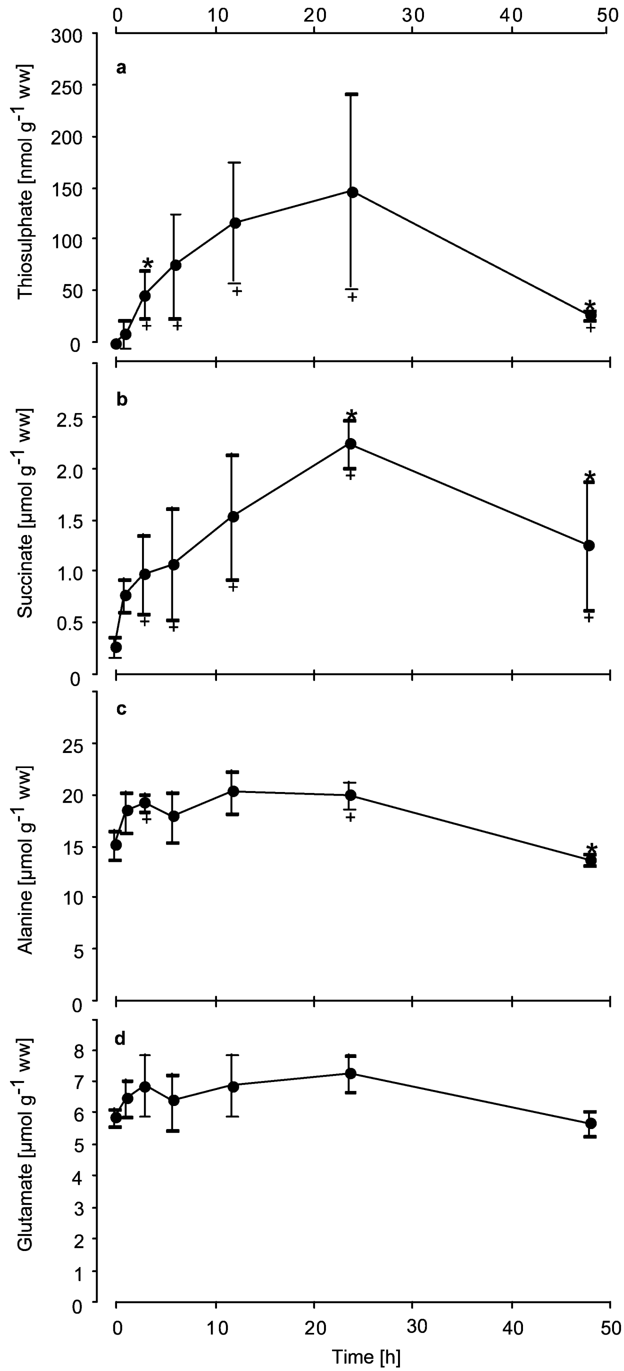
3. Results

3.1. Experimental hypoxia

The average oxygen concentration of the incubation water was 0.337 ml l^{-1} with very little variation between the different experimental runs and during each run ($\pm$ 0.002 ml l^{-1}, $n = 17$). Sulphide levels were in the range of 100 $\pm$ 25 μmol l^{-1}. Normoxic control flasks had a constant average oxygen concentration of 10.491 $\pm$ 0.153 ml l^{-1}.

Under normoxic conditions, post-settled *D. serra* were buried in the sediment and the tips of the siphons were visible at the sediment surface. In contrast, under hypoxic conditions, test clams remained buried and the siphons extruded only for the first 2 h regardless of the presence or absence of sulphide. Thereafter, the clams were found lying on the sediment surface and extended their siphons into the water column. Finally, siphons collapsed and the valves gaped. When exposed to hypoxia or hydrogen sulphide, survivorship was affected. Under severe hypoxia the first animals were recorded dead

Fig. 3. Juvenile *D. serra*: content of thiosulphate (a), succinate (b), alanine (c) and glutamate (d) after hypoxic incubation (0–48 h) in the presence of hydrogen sulphide (100 μmol l). Mean $\pm$ S.E., $n = 3$, *: significantly different from previous value; +: significantly different from control.



Thiosulphate [nmol g-1 ww]
Succinate [µmol g-1 ww]
Alanine [µmol g-1 ww]
Glutamate [µmol g-1 ww]
Time [h]
a
b
c
d

after 78 h with 110 h as a median survival time (LT_{50}) (Fig. 2). The presence of sulphide (100 μmol l^{-1}) significantly reduced the survival time even further (log-rank test, $z = -4.07$, $p < 0.01$). The first animal died after 46 h and an LT_{50} of 80 h was calculated. All surf clams kept under normoxic conditions were still alive when the experiment was terminated after 200 h.

3.2. Sulphur compounds in the tissue

The time course of sulphide uptake in the soft tissue was followed during 48 h of sulphide exposure (100 μmol l^{-1}). Sulphide itself was not detectable in the soft tissue and sulphite was only found in low concentrations (0.015 μmol g^{-1} wet mass) in some specimens. Thiosulphate was the only sulphur compound that clearly accumulated, reaching a maximum of 0.148 μmol g^{-1} wet mass after 24 h (Fig. 3a), while cystein was recorded at constant concentrations (0.25 μmol g^{-1} wet mass).

3.3. Anaerobic energy production under sulphide exposure

The concentrations of metabolites resulting from anaerobic energy production were followed over the same time course (48 h). Succinate concentration increased 9-fold from the onset of exposure (0.25 μmol g^{-1} wet mass) to 2.23 μmol g^{-1} wet mass after 24 h (Fig. 3b). The concentration of alanine (17.5 ± 2.5 μmol g^{-1} wm) was significantly elevated after 3 and 24 h (ANOVA, $p < 0.05$), while it decreased significantly between 24 and 48 h (ANOVA, $p < 0.05$). Glutamate levels (6.5 ± 0.8 μmol g^{-1} wm) did not change significantly during the experiment (ANOVA, $p > 0.05$; Fig. 3d) and aspartate was not detectable.

4. Discussion

Marine invertebrates inhabiting coastal zones with sporadical hydrogen sulphide occurrence such as the central Namibian coastal upwelling system use a variety of behavioural, biochemical and physiological traits to either avoid the contact or to withstand this poisonous substance. The epibenthic blue mussel, *Mytilus edulis*, for instance closes the shells as a passive protection against the poisonous effects of hydrogen sulphide (Jørgensen, 1980). A similar behaviour has been observed for *Perna perna* (Schiedek and Currie, unpublished data). The studied juvenile *D. serra*, however, were observed lying on the sediment surface with siphons extended into the water column when exposed to hypoxia or sulphidic hypoxia. This is in agreement with observations from other infauna bivalves during experimental (*Mulinia lateralis*: Shumway et al., 1983; *Abra alba*: Rosenberg et al., 1991; *Scrobicularia plana*: Oeschger and Pedersen, 1994) and field studies (*Mya arenaria*, *Cerastoderma edule* and *A. alba*: Jørgensen, 1980). Deposit feeding bivalves, such as *D. serra*, use their siphons to take up organic particles by filtering the water. The observed permanent siphon extrusion in the presence of sulphide may reflect a strategy to monitor the water for better conditions and might not be coupled with high ventilation activity. The clam *S. plana*, for instance, reduced ventilatory water

flow under exposure to keep the sulphide concentration low in their extrapallial fluid. In addition, a diffusive barrier against sulphide might exist in the siphons (Oeschger and Pedersen, 1994).

The observed migration of *D. serra* to the sediment surface during hypoxic sulphide exposure appears to be an adaptation to the specific conditions prevailing in such kind of coastal systems. Natural "sulphide eruptions" within the Benguela upwelling system often affect restricted surface areas of some 100 m^2 only (Currie, 1999; personal observation). Therefore, moving to the sediment surface favours the transport with currents to other areas where the mobile clams might find better conditions. On the other hand the clams have to cope with dangers like (i) a possible wash up followed by mortality due to overheating in the sun or predation by seagulls (McLachlan et al., 1980; J. Laudien, unpublished data); and/or (ii) a constant exposure to pelagic and epibenthic predators, able to enter the toxic water body while feeding (Phil et al., 1992). The advantages of being transported to more favourable conditions seem to compensate for these disadvantages.

As mentioned above, marine invertebrates regularly exposed to hydrogen sulphide have acquired a variety of biochemical adaptations to eliminate this poisonous compound (reviews: Vismann, 1991b; Bagarinao, 1992; Völkel and Grieshaber, 1995; Grieshaber and Völkel, 1998). The detoxification of sulphur compounds to less or non-toxic oxidation products is known to be a common strategy in marine bivalves (Cary et al., 1989; O'Brien and Vetter, 1990) and other invertebrates (Vismann, 1991a; Völkel and Grieshaber, 1995). In *D. serra*, thiosulphate appears to be the main product of mitochondrial sulphide oxidation, as it was the only detoxification product found at elevated amounts after experimental sulphide exposure. Thiosulphate accumulated from the onset of exposure to a maximum of 0.148 μmol g^{-1} wet mass (wm) after 24 h. This demonstrates that oxygen stored in body fluids and/or oxygen remains in the incubation water (<0.3 ml l^{-1}, due to the remaining oxygen in the nitrogen gas corresponding to the purity grade noted in the techsheet) seems to be still available for oxidative processes. Thiosulphate is almost non-toxic (Voegtlin et al., 1924; Sörbo, 1972), not inhibiting oxidative metabolism (Vetter et al., 1989) and highly soluble. The detected value is in the same range as found in the Baltic clam *Macoma balthica* (0.177 μmol g^{-1} wm, Jahn and Theede, 1997) but lower than concentrations seen in other marine invertebrates (*Marenzelleria* cf. *wireni*: 1 μmol g^{-1} wm, Schiedek et al., 1997; *Arenicola marina* 30 μmol g^{-1} wm, Grieshaber et al., 1995; *Hediste diversicolor*: 100 μmol g^{-1} dm, Hahlbeck et al., 2000).

The observed decrease of the thiosulphate concentration in the soft tissue of *D. serra* after 24 h may be explained with a decreasing oxygen tension in the tissue. As thiosulphate is highly soluble it can, therefore, not be accumulated to infinite concentrations within tissues. Grieshaber and Völkel (1998) concluded that it is generally not further metabolised but eliminated by outward diffusion. Accordingly, no thiosulphate transport system could be identified in invertebrates (Hauschild et al., 1999).

It is remarkable, that no sulphide could be detected in the clams' soft tissue after exposure to sulphide. This leads to the assumption that sulphide detoxification is very efficient allowing the clams to maintain a very low sulphide level for a period of about 24 h when exposed to 0.1 mmol l^{-1} hydrogen sulphide. This level represents concentrations measured in the coastal area off Namibia during sulphide eruption events (A. van der Plas, personal communication). These findings further support that juvenile *D. serra* are able to

deal with hydrogen sulphide for a short period, giving them the opportunity to move to a more suitable location as described above.

Many euryoxic marine bivalves and other invertebrates use alternative pathways of anaerobic energy production for long-term hypoxic survival (e.g., Storey and Storey, 1990; De Zwaan, 1991; Grieshaber et al., 1994). One predominant anaerobic product is succinate, which accumulates in the tissue (e.g., Kluytmans et al., 1977; Widdows et al., 1979; Kluytmans and Zandee, 1983; Demers and Guderley, 1994; Sukhotin and Pörtner, 1999). The present results support these findings: when exposed to sulphidic hypoxia, post-settled *D. serra* switched to anaerobiosis—which resulted in a 9-fold increase of succinate levels. Starting from relatively high levels, alanine accumulated to an even higher extent. For intertidal species, this might be a regular event, since they must switch to anaerobiosis during low tide when the substratum is exposed to air and the penetration of oxygen into the sediment is not sufficient to support fully aerobic energy production (reviews: Schöttler and Bennet, 1991; Grieshaber et al., 1994). Cockcroft (1990) stresses that anaerobic metabolism includes a significant energy saving strategy and may even explain the obvious success of *D. serra* on southern African coasts.

Relying on anaerobic metabolism, however, does not provide complete protection against "sulphide eruptions". Under severe hypoxia and in the absence of sulphide, all of the juvenile *D. serra* survived for more than 4 days (LT_{50} 110 h, Fig. 2). Under hypoxic–sulphidic conditions, survivorship was significantly reduced (LT_{50} 80 h). Non-dissociated hydrogen sulphide diffuses easily through biochemical membranes (Powell, 1989; Julian and Arp, 1992) and reversibly inhibits the last enzymatic reaction of the respiratory chain by forming a stable complex with cytochrome *c* oxidase. In the presence of sulphide, aerobic respiration is therefore impossible (Nicholls, 1975; Nicholls and Kim, 1981, 1982). In the juvenile surf clams, succinate was accumulated almost directly after the onset of hypoxic–sulphidic conditions. At the same time, thiosulphate started to increase. As pointed out earlier, this suggests that the animals used much of the remaining oxygen to detoxify the penetrating hydrogen sulphide and as a consequence switched to anaerobiosis immediately. A similar reduction in survival and a more pronounced anaerobic metabolism in the presence of hydrogen sulphide has been reported for several other marine invertebrates, e.g. the polycheate worms *Arenicola marina*, *Nephtys homgergü* or *Marenzelleria* cf. *wireni* (Völkel and Grieshaber, 1992; Arndt and Schiedek, 1997; Schiedek et al., 1997). This leads to a faster breakdown of glycogen, which is known to be the major energy resource during long-term anaerobiosis (Schöttler and Bennet, 1991) and may limit survival upon its depletion. Glycogen content was not measured in the juvenile *D. serra* because of limited amount of tissue, but their stores are likely to be lower than in the adults. A size and age related increase in glycogen content has been shown for instance for juvenile lugworms (Schiedek and Schöttler, 1990).

In terms of survival periods, juvenile *D. serra* are 3-fold more sensitive to hydrogen sulphide than adult Baltic clams *Macoma balthica* (100 $\mu mol\ l^{-1}$, 10 °C) (Jahn and Theede, 1997). However, some of these differences are probably due to the higher temperatures used in our experiments (15 °C). Furthermore, the susceptibility might not only be species related, but seems also to decrease with increasing age and size (e.g., *Theora lubrica*: Imabayashi, 1986, *Mytilus edulis*: Wang and Widdows, 1991; C. Bittkau unpublished data). Jahn et al. (1997) argued that in contrast to larger animals, small

individuals with a higher surface-to-volume ratio are unable to detoxify hydrogen sulphide effectively enough but can only survive in sulphidic habitats due to their anaerobic capacity.

This argument leads us to postulate that not only post-settled *D. serra* but especially larvae may be affected even more than juveniles by hydrogen sulphide and that natural "sulphide eruptions" have to be considered as an important factor for *D. serra* recruitment failures.

The Namibian coastline can be separated into two zoogeographical provinces, the cool temperate southwest coast (Namaqua) and the cool temperate northwest coast (Namib) (Emanuel et al., 1992). Various explanations have been given for the differing species numbers and community structures, including the Lüderitz upwelling cell acting as a barrier to northward larval dispersal. However, some species found in the area around Lüderitz are absent in the central Namibian region and reappear further north in a small pocket at Möwe Bay (Currie, 1999). Since the northern province correlates well with the post-upwelling cell characterised by oxygen-deficient bottomwater and opal-rich deposits, Currie (1999) hypothesises that the hypoxia and hydrogen sulphide associated with the Lüderitz upwelling cell could contribute considerably as a community structuring force. Various studies confirmed the structuring effect of hydrogen sulphide (Hiroki, 1977; Diaz and Rosenberg, 1995; Gamenick et al., 1996, 1998; Jahn et al., 1997). However, adult *D. serra* seem to be adapted to sulphidic conditions and can therefore be abundant in the southern and the northern province. Populations seem to withstand moderate sulphide eruptions even after recruitment failures, since the adults reproduce several times during their 5-year life span (Laudien et al., in press). Further research should include larval stages as well as adult animals from different provinces to confirm a possible structuring effect of naturally occurring hydrogen sulphide in the Benguela Current upwelling system.

5. Conclusion

The results of the present study show that juvenile *D. serra* respond to sulphide exposure conditions by moving to the sediment surface, which favours their transport by currents to nearby areas with better conditions. Initially, the clams are well adapted to detoxify hydrogen sulphate to non-toxic thiosulphate and thus keep concentrations of the toxin at very low levels within the valves. The juvenile clams are able to gain energy by switching to anaerobiosis during exposure. However, anaerobiosis and the reduction in energy requirements only supported time-limited survival, leading to a significantly reduced survivorship after 2 days. Therefore, we postulate that natural "sulphide eruptions", especially when they have large spatial and temporal extensions, have to be considered as an important factor for recruitment failures of the surf clam *D. serra*.

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