



Behaviour and mortality of benthic crustaceans in response to experimentally induced hypoxia and anoxia *in situ*

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ABSTRACT: Crustaceans are sensitive to hypoxia and are therefore useful indicator organisms for oxygen depletions. We used the experimental anoxia generating unit in the northern Adriatic Sea to artificially induce and document hypoxia and anoxia on a small scale (0.25 m² per deployment). Behavioural responses and mortalities were documented for 9 crustacean species typical for the sublittoral soft bottom of the Gulf of Trieste. All species showed a similar succession of atypical responses, albeit at different thresholds. The first reaction to declining dissolved oxygen (DO) was avoidance by climbing to more oxygenated (higher) positions. The animals left their shelters, altered their activity patterns, and exposed themselves to a higher risk of predation at mild hypoxia (2 to 1 ml l⁻¹ DO). Moderate hypoxia (1 to 0.5 ml l⁻¹ DO) triggered changes in inter- and intraspecific interactions, resulting in aggregations of up to 27 individuals at the highest elevations. At severe hypoxia (0.5 to 0.01 ml l⁻¹ DO), sublethal responses such as discarding of camouflage (*Ethusa mascarone*) were recorded, and 54% of all crustaceans died. Anoxia left most remaining individuals immobile and moribund until death. Almost all responses were related to DO thresholds—hydrogen sulphide (H₂S) had not yet evolved. *Pisidia longimana*, *Galathea* spp., and *Macropodia* spp. were the most sensitive; *Pilumnus spinifer* and *Ebalia tuberosa* were the somewhat more tolerant species. Only *Nepinotheres pinnotheres* survived prolonged anoxia as well as high H₂S concentrations.

KEY WORDS: Dissolved oxygen · Hydrogen sulphide · Northern Adriatic Sea · Bioherms · Sublittoral · Eutrophication

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INTRODUCTION

Hypoxia and anoxia severely affect coastal benthic ecosystems, resulting in long-term changes in benthic communities and extensive so-called dead zones (Rabalais et al. 2002). Currently, dead zones have been reported in more than 400 systems worldwide (Diaz & Rosenberg 2008). Diaz (2001) describes water column stratification and eutrophication as the 2 main factors in the development of hypoxia (DO concentrations < 2 ml l⁻¹) and anoxia. Approximate equivalencies at 20°C and a salinity of 38 are: 1 ml l⁻¹ = 1.43 mg l⁻¹ = 4.18 kPa = 31.35 mm Hg. These conditions are met in the northern Adriatic Sea, which makes it a case study for intrinsically sensitive shallow coastal marine eco-

systems, both with regard to low DO events and to additional anthropogenic impacts (Lotze et al. 2006).

The effects of hypoxia depend on the severity, frequency, and duration of oxygen depletion. During hypoxia, benthic animals show a wide range of atypical responses and behaviours as well as physiological adaptations (Diaz & Rosenberg 1995, Burnett & Stickle 2001, Gray et al. 2002, Wu 2002). Prolonged oxygen depletion triggers mass mortalities of the benthic macroepifauna (for the northern Adriatic: Stachowitsch 1984). Altered benthic community structure and composition often mean a loss of ecosystem function and services (Sala & Knowlton 2006, Levin et al. 2009).

Anoxia in aquatic environments is always associated with elevated levels of H₂S, a toxicant for aerobic organ-

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isms (Reiffenstein et al. 1992). Anoxia and H₂S have synergistic effects on benthic organisms but are difficult to separate, and both should be considered when studying behavioural reactions to anoxia (Vismann 1991).

The present study focuses on crustaceans associated with bioherms or multi-species clumps—the macroepibenthic assemblages typical for the soft bottoms in the Gulf of Trieste, the investigated part of the northern Adriatic Sea. Vagile organisms here account for 7.9% of total biomass, with crustaceans in third position (Zuschin et al. 1999). This species-rich group comprises the main invertebrate scavengers and predators in this suspension-feeder-dominated environment (Fedra et al. 1976). Many are very sensitive to hypoxia and H₂S (Theede et al. 1969, Vaquer-Sunyer & Duarte 2008). We hypothesize that crustaceans will be among the first organisms to respond to low DO values and can therefore serve as indicators of developing hypoxia. We also hypothesize that different lifestyles will be reflected in differentiated responses and mortality times, with certain species functioning as early warning signals. We deployed the EAGU (experimental anoxia generating unit) (Stachowitsch et al. 2007) to investigate detailed behavioural responses, intra- and interspecific interactions, and mortalities in the natural environment. The DO and H₂S sensor data allowed individual behaviour—and community-level responses—to be linked to specific thresholds.

MATERIALS AND METHODS

Study site. The study area is situated approximately 2.3 km off Cap Madonna (Piran, Slovenia) in the Gulf of Trieste (45° 32' 55.68" N, 13° 33' 1.89" E) close to the oceanographic buoy of the Marine Biology Station Piran at a depth of 24 m.

The northern Adriatic Sea is a shallow (mean depth 35 m), semi-enclosed ecosystem with highly variable oceanographic and biological conditions due to the combined influence of freshwater discharges, mainly from the Po River, and meteorological and climatic conditions (Justić et al. 1993). These factors govern circulation and lead to summer water-column stratification, hindering oxygen exchange between surface and bottom water (Degobbis et al. 1995, Supić et al. 2002). During periods of persistent stratification, DO drops rapidly in the bottom water due to respiration, benthic recycling of nutrients, and bacterial decomposition (Zavatarelli et al. 1998, Russo et al. 2005).

The poorly sorted silty sand in the study area is colonized by a diverse infauna and high-biomass macroepibenthic assemblages forming so-called multi-species clumps or bioherms (Fedra et al. 1976). These bioherms consist of sessile species, mostly sponges,

ascidians, anemones, serpulids, and bivalves growing on hard substrata such as shells. The bioherms, in turn, harbor semi-sessile and vagile organisms such as brittle stars and crustaceans (Zuschin et al. 1999). The community is dominated by filter- and suspension feeders and occupies about 40% of the Gulf of Trieste. It was described as the 'ORM' community based on the most dominant genera: the brittle star *Ophiothrix quinque maculata*, the sponges *Reniera* spp., and the ascidians *Microcosmus* spp. (Fedra et al. 1976). Bioherm distribution is patchy on a background of low epifaunal density, represented mainly by mobile deposit-feeding or carnivorous species such as holothurians or hermit crabs (Zuschin & Stachowitsch 2009).

Experimental design and sampling. The EAGU consists of 2 interchangeable cubic bases, i.e. an open-sided aluminium frame and a plexiglas-walled chamber, both measuring 50 × 50 × 50 cm (see Stachowitsch et al. 2007 for detailed description). An instrument lid, placed on top of either of the 2 bases, houses a digital time-lapse camera, 2 flashes, and a datalogger with a microsensor array to record oxygen, H₂S, and temperature (Unisense®). The Clark-type microsensors measure a current that reflects the partial pressure of the sulphide species H₂S and oxygen at the sensor tip. The 2 oxygen sensors were positioned at 2 and 20 cm above the sediment in order to detect potential oxygen gradients, and the H₂S sensor was positioned 2 cm above the sediment. pH was measured once a day with a WTW TA 197-pH sensor. Sensor values were logged every minute, and images were taken every 6 min.

Initially, the 'open' configuration (aluminium frame plus lid) was positioned above a bioherm (0.25 m² area) to document behaviour under normoxic conditions for 24 h. In the second step, the 'closed' configuration, the frame was exchanged for the chamber, which was repositioned over the same assemblage to document responses to decreasing DO. The chamber sealed off the enclosed water body, and oxygen depletion was induced solely by respiration by the enclosed fauna. After anoxia set in, the deployments were continued for another 1 to 2 d to document the reaction of less sensitive species. All remaining living and dead organisms were then collected and preserved in a 4% formalin:sea-water solution for species and biomass determination.

Nine out of 13 deployments contained bioherm-associated crustaceans and were evaluated here (Table 1). The overall documentation time of all 9 deployments was 832.2 h (158.6 h open, 673.6 h closed configuration), encompassing 8322 images. The fieldwork was conducted in September 2005 and from July to October 2006.

Investigated taxa. The present study focused on crustaceans associated with bioherms: *Pisidia longimana* (Risso, 1816), *Pilumnus spinifer* (H. Milne-Edwards,

Table 1. Deployment overview. No.: number of deployment; OC: open configuration; CC: closed configuration

No.	Date (d.mo.yr)	Duration (h)		pH		Temp. (°C)	Crustacean abundance		Biomass (g wet wt 0.25 m ⁻²)	
		OC	CC	OC	CC		Species	Individuals	Total	Crustacean
2	17–22.09.05	–	132.8	8.2	–	18.5	4	57	436.7	1.7
6	05–08.08.06	22.4	48.3	8.2	7.9	18.8	5	48	–	5.3
7	17–21.09.06	20.9	72.1	8.1	7.7	19.7	2	20	839.8	3.3
8	21–24.09.06	21.7	41.9	8.2	–	20.4	6	25	526.3	0.9
9	25–29.09.06	21.9	73	8.2	7.8	20.6	2	36	648.9	1.2
10	29.09–02.10.06	22.7	40.3	8.2	7.9	21.4	2	14	629.2	0.9
11	05–10.10.06	23.6	95.4	8.2	7.5	21.3	4	51	724.0	4.9
12	10–14.10.06	25.4	75.2	8.3	7.8	21.3	3	17	631.6	1.1
13	17–21.10.06	–	94.6	8.3	7.8	20.4	6	36	1042.7	8.3

1834), *Eurynome aspera* (Pennant, 1777), *Macropodia* spp., *Galathea* spp., *Inachus* spp., and the mutualistic *Nepinnotheres pinnotheres* (Linnaeus, 1758); but it also included *Ebalia tuberosa* (Pennant, 1777) and *Ethusa mascarone* (Herbst, 1785), which mainly inhabit sediment areas. The former buries itself in the sediment during the day (Schembri 1979), the latter carries shells or other objects on its carapace for protection.

From each specimen, size (± 0.1 mm) and wet weight were determined (± 0.01 mg). For decomposed individuals, values were estimated from the images using formulas from the literature (see Haselmair 2008 for details). Juveniles and adults were distinguished according to Wurzian (1982) and Pallas et al. (2006).

Data analysis. The behaviours of each individual crab to declining DO and increasing H₂S concentrations were analysed image by image and recorded in categories (general and species-specific categories of behaviours and responses' are listed below). This was done for all crustaceans, except for the small and very abundant *Pisidia longimana*, where 4 larger and conspicuous individuals were selected.

General categories of behaviours and responses:

- Visible presence: sub-categories: completely hidden, partly visible (animal still protected), exposed
- Locomotion: change in location (>1 body length). Sub-categories: minor (1 to 3 body lengths) and major (>3 body lengths) locomotion both in vertical and horizontal directions
- Movement: activity without significant displacement. Sub-categories: turns (changes in direction), body movement (<1 body length), extremity movement
- Atypical posture: posture not observed under normoxia.
- Vertical position: 'ground' (on sediment), 'middle' (up to 5 cm), and 'high' (>5 cm above sediment)
- Atypical substrate: use of vagile organisms as a substratum
- Altered predator–prey relationship: predators fail to attack nearby prey, or prey does not flee from approaching predators
- Atypical aggregation: assemblage of more than 3

organisms (at least 1 per crustacean) in direct contact

Species-specific categories:

- Camouflage discarded: *Ethusa mascarone* discards shell carried on its back
- Host abandoned: *Nepinnotheres pinnotheres* leaves its ascidian host

The images were evaluated as long as the animal was visible and clearly identifiable. The time of mortality was defined as the last activity observed, and the duration of hypoxia until death was determined.

For statistical analyses, oxygen data were assigned to 5 DO categories: normoxia (≥ 2.0 ml l⁻¹), mild hypoxia (2.0 to 1.0 ml l⁻¹), moderate hypoxia (1.0 to 0.5 ml l⁻¹), severe hypoxia (<0.5 ml l⁻¹), and anoxia (0 ml l⁻¹). The duration of hypoxia tolerated, differences in lethal DO and H₂S concentrations as well as differences in behaviour between oxygen categories were tested using the non-parametric Kruskal-Wallis test. The Mann-Whitney *U*-test was chosen to test which oxygen categories are different from each other regarding single behaviours and to test differences between 2 taxa or deployments. The non-parametric Spearman's rank correlation was used to analyse whether the appearance of *Pisidia longimana* follows a diurnal rhythm during normoxia. Kendall partial rank-order correlation was chosen to investigate the association between decreasing DO and frequencies of the appearance of *P. longimana* during hypoxia and anoxia. The significance level in all tests was $p < 0.05$. Statistical analysis was performed using the software package SPSS 16.0 for Mac.

RESULTS

Sensor data

The EAGU induced anoxia within 1 to 3 d (Fig. 1). In deployments 6, 7, 8, 10, 11, oxygen declined rapidly, and anoxia was reached within 17.4 to 33.5 h. In all other deployments, anoxia was generated within 51.7

to 69.8 h (except for deployment 2, with an intermediate oxygen peak). The rapidity of oxygen decline had no influence on hypoxia-related behaviours but did affect mortality thresholds.

During each open configuration, DO concentrations remained relatively constant and the curves of the 2 sensors were parallel. Overall, the values ranged from 2.6 to 5.6 ml l⁻¹ on the bottom and from 2.8 to 8.9 ml l⁻¹ 20 cm above the sediment. Values measured 20 cm above the sediment were typically higher than those directly above the sediment (exceptions: deployments 6 and 7). The values of the 2 sensors during the open configuration differed less during calm (0.3 ml l⁻¹ DO) than during stormy weather (1.92 ml l⁻¹ DO). During the closed configuration, DO values dropped immediately and continuously. Both curves then approximated each other, but values typically remained slightly higher above than at the sediment until anoxia occurred.

Soon after anoxia occurred, H₂S concentrations rose steadily up to 21 µmol l⁻¹ in deployments with brief anoxia (6, 8, 9, 10, 12) and up to 304 µmol l⁻¹ H₂S when anoxia persisted for at least 2 d (2, 7, 11, 13).

Temperature remained nearly constant during a particular deployment and was similar between deployments (18.5 to 21.4°C; Table 1). Bottom water salinity was 38‰. Bottom water pH values during the open configuration were constant (8.1 to 8.3) and decreased to a minimum of 7.5 inside the chamber (deployment 11; Table 1).

Abundance and biomass

Three of the 9 investigated species were abundant and present in most deployments: *Pisidia longimana* (n = 265, average 29.4 ind. 0.25 m⁻²); *Pilumnus spinifer* (n = 17, average 1.9 ind. 0.25 m⁻²), and *Macropodia* spp. (n = 7, average 0.8 ind. 0.25 m⁻²) the others were represented by only 2 or 3 individuals each. The distribution of species across the deployments varied between 2 and 6 species. Three *Galathea* spp. were counted, but more were probably present.

Based on size, most individuals were adults. Juveniles of *Pilumnus spinifer*, *Macropodia* spp., and *Pisidia longimana* were present. The 2 female *Nepinotheres pinnotheres*, both inhabiting an ascidian, were immature.

Epibenthic biomass in the chambers was high (mean 685 g wet wt 0.25 m⁻²), largely because we specifically targeted multi-species clumps rather than free sediment surfaces. Mean crustacean biomass was 3.1 g wet wt 0.25 m⁻², ranging from 0.9 to 8.3 g wet wt 0.25 m⁻² within deployments, with the small and abundant *Pisidia longimana* accounting for about 28%. Crustaceans made up only 0.4% of total epifaunal biomass. These values agree with those reported in Wurzian (1982) and Fedra et al. (1976), although the observed density and biomass of *Pilumnus spinifer* and *P. longimana* here were considerably higher.

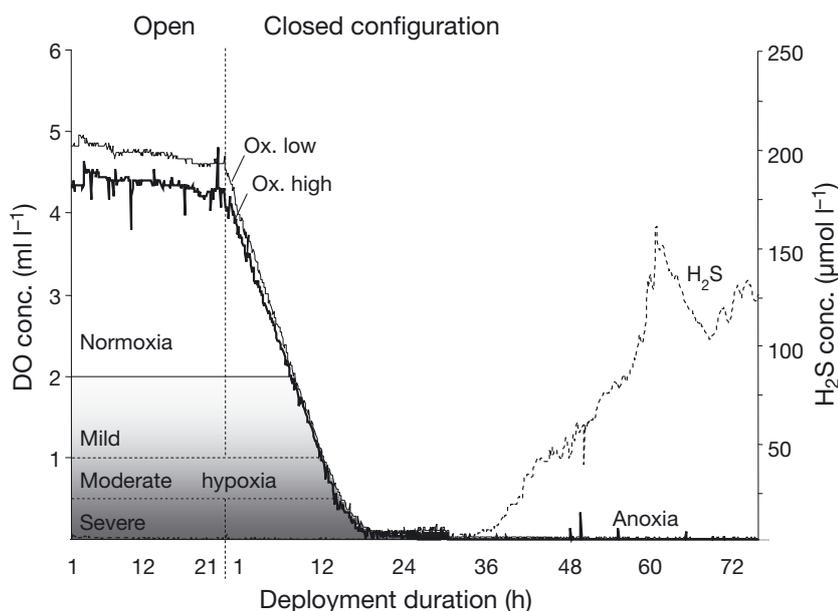


Fig. 1. Characteristic sensor data (deployment 7). White area under oxygen curve: normoxia; grey area: hypoxia. Threshold values for different stages of hypoxia indicated with dashed lines at 1 and 0.5 ml l⁻¹ dissolved oxygen (DO). Ox. low: values 2 cm above sediment; ox. high: values 20 cm above sediment (ml l⁻¹); H₂S: values 2 cm above sediment (µmol l⁻¹)

Behavioural responses

All species displayed a succession of behavioural patterns related to declining oxygen values (Fig. 2). Combinations of behaviours often occurred, and the timing of the responses varied interspecifically and, to a lesser degree, intraspecifically.

Avoidance

During normoxia, crustaceans were hidden from view in at least 83% of all observations because of their cryptic lifestyle or camouflage (Fig. 3). An exception was the majoidean crabs (*Macropodia* spp., *Inachus* sp., and *Eurynome aspera*). As oxygen decreased, their visibility increased significantly. *Pilumnus spinifer*, for example, was hidden 54% of the time during mild, 23% during moderate, and only 4% during

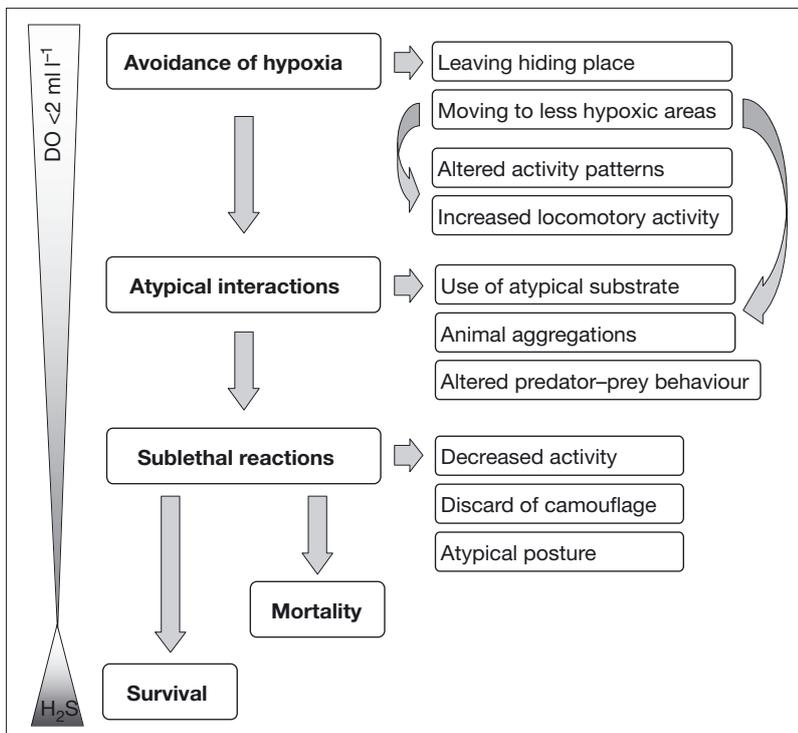


Fig. 2. Crustacean responses to decreasing DO values. Note that responses are elicited at different DO concentrations depending on species. Most atypical behaviours and mortalities were induced prior to H_2S evolution

severe hypoxia. This pattern was similar for *Galathea* spp. and *Pisidia longimana*. *Ethusa mascarone* began to move at moderate hypoxia. The 3 *Ebalia tuberosa* emerged from the sediment at 1.38, 0.63, and 0.45 ml l^{-1} DO. While all other species were already visible or dead during anoxia, *Nepinnotheres pinnotheres* remained hidden within its host until anoxia. One specimen emerged after 11.7 h of hypoxia and 13.6 h of anoxia, the other after 53.2 h of hypoxia and 19.4 h of anoxia. The corresponding H_2S concentrations were 18.3 and 93.9 $\mu\text{mol l}^{-1}$.

Crustaceans showed an activity peak at night. For *Pisidia longimana* (Fig. 4), for example, the correlation between the circadian rhythm and the percentage of visible animals was strong and significant (Spearman's r_s : 0.829; $p < 0.001$), whereas DO values and visible presence were initially not correlated. During mild hypoxia, normal patterns (daytime hiding) persisted, but from moderate hypoxia on, all individuals became visible permanently. The correlation with DO concentrations then became strong and significant (Kendall's τ : 0.8851; $p < 0.001$). All individuals emerged between 0.96 and 0.24 ml l^{-1} DO (medians, calculated for each deployment separately).

When hypoxia set in during the day, the first *Pisidia longimana* were visible at moderate hypoxia (median DO concentration of 0.96 ml l^{-1} ; Q_1/Q_3 [first and third

quartile] = 0.98/0.92); 50 % were visible at values below 0.65 ml l^{-1} DO ($Q_1/Q_3 = 0.77/0.53$), and 100 % at severe hypoxia, i.e. 0.34 ml l^{-1} DO ($Q_1/Q_3 = 0.38/0.30$). Normal nighttime activity masks hypoxia-induced emergence in deployments with hypoxia setting in at night. Usually about half of the individuals were visible simultaneously during any night; higher values point to a hypoxia effect. The median DO value for 100 % of animals visible during nighttime hypoxia onset (0.24 ml l^{-1} DO, $Q_1/Q_3 = 0.4/0.18$) also corresponds well with the daytime equivalent.

Hypoxic stress triggered a typical sequence of locomotory activity (locomotion, body, and extremity movement) in all species after emergence: a locomotion peak at the onset of hypoxia, a rapid decline during severe hypoxia, followed by inactivity during anoxia (Fig. 3). *Nepinnotheres pinnotheres* and *Ebalia tuberosa* differed: both continued to move during anoxia (in 50 and 60 % of all observations, respectively; Fig. 3), when most

other crabs were moribund or dead. *N. pinnotheres* was most active at anoxia, whereas *E. tuberosa* showed only body movements rather than locomotion.

When active during normoxia, crustaceans spent 65 % of their time on the sediment (Fig. 5), except *Pisidia longimana* and *Macropodia* spp., which largely remained on bioherms. Hypoxia induced all crustaceans to move to more oxygenated areas. Most species immediately climbed onto the next bioherm or higher up on the bioherm they inhabited during mild hypoxia. *Eurynome aspera*, *Ebalia tuberosa*, and *Ethusa mascarone* initially tried to escape horizontally on the sediment, then vertically. During mild hypoxia, crustaceans were on elevated sites in 64 % of all observations, rising to 83 % during moderate hypoxia (Fig. 5). Even those species that rarely (*E. aspera* and *Inachus* sp.) or never (*E. tuberosa* and *E. mascarone*) occurred on bioherms during normoxia, started to move up as DO (\pm SD), fell below 1.16 (± 0.6) and 0.15 (± 0.1) ml l^{-1} , respectively. At values persistently below 0.49 (± 0.32) ml l^{-1} DO, *Pilumnus spinifer* positioned itself at the highest place. One *E. mascarone* climbed up a sensor and disappeared from view. Ultimately, crustaceans left or dropped from their elevated perches and died on the sediment. Moribund animals apparently were unable to hold onto bioherms. Five of 8 *Macropodia* spp. and 1 *Inachus* sp. were dragged down by falling brittle stars.

Atypical interactions and sublethal responses

At normoxia, most crustaceans typically avoided each other. With decreasing oxygen concentrations, however, intra- and interspecific interactions gradually increased in number and duration. A common response during mild hypoxia was climbing on top of vagile or-

ganisms such as holothurians, echinoids, or ophiuroids, which are normally not used as a substratum (Fig. 6a). The most frequent interaction response was a diminished distance between individuals of one or different species. During mild hypoxia, dominant individuals of *Pilumnus spinifer* chased subordinate individuals or other crabs such as *Macropodia* spp. away. This behav-

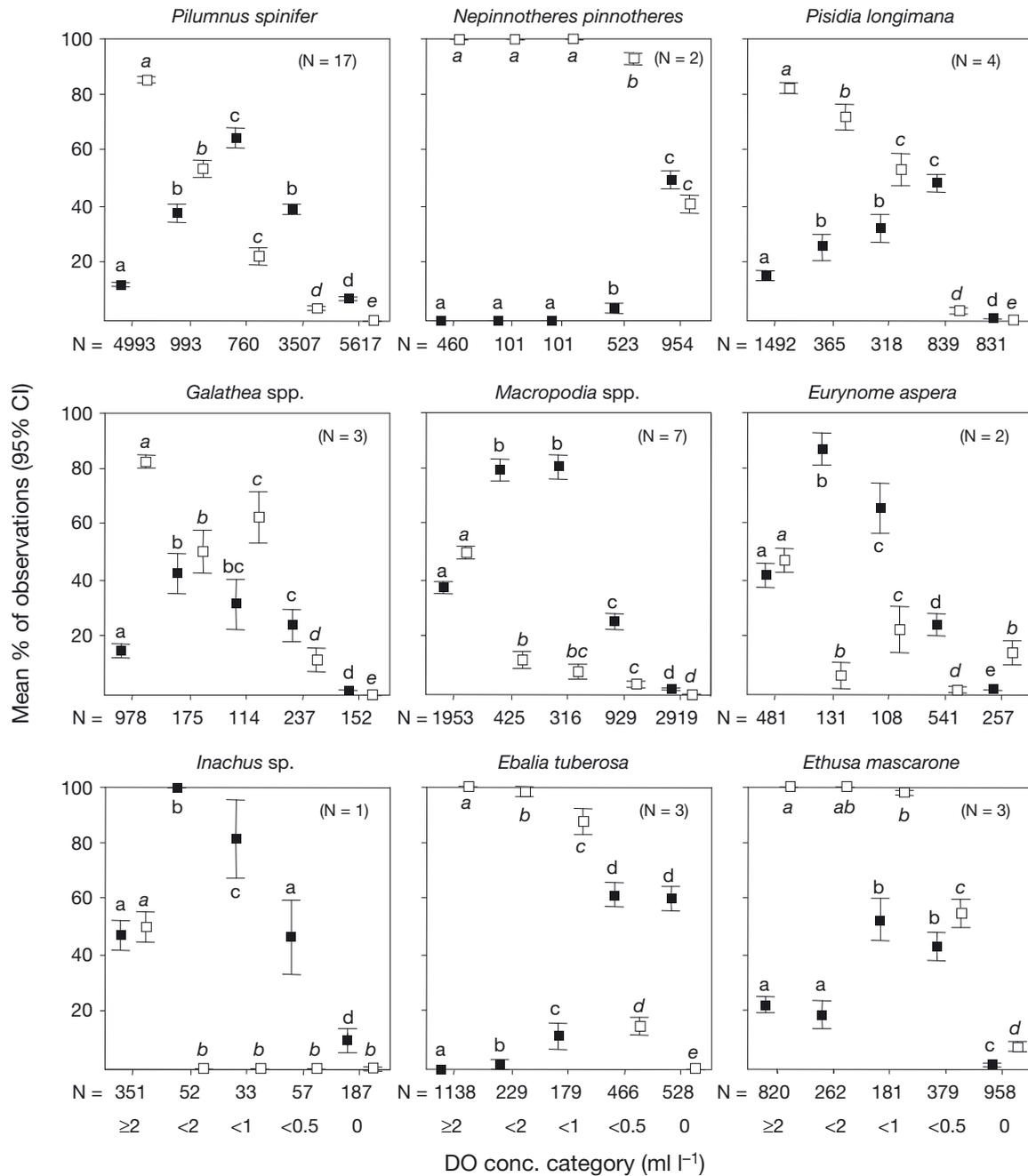


Fig. 3. Species-specific changes in hiding behaviour (□) and locomotory activity (■) in the 5 oxygen categories. Beyond visible presence, the following specific reactions were considered: *Nepinnotheres pinnotheres*, abandoning its host; *Ebalia tuberosa*, emerging from sediment; *Ethusa mascarone*, dropping its protective shell fragment. Numbers below x-axes: number of photographs evaluated per oxygen category. Analyses are by Mann-Whitney *U*-tests (p < 0.05). Same letters above error bars indicate no significant difference between means of the respective oxygen categories

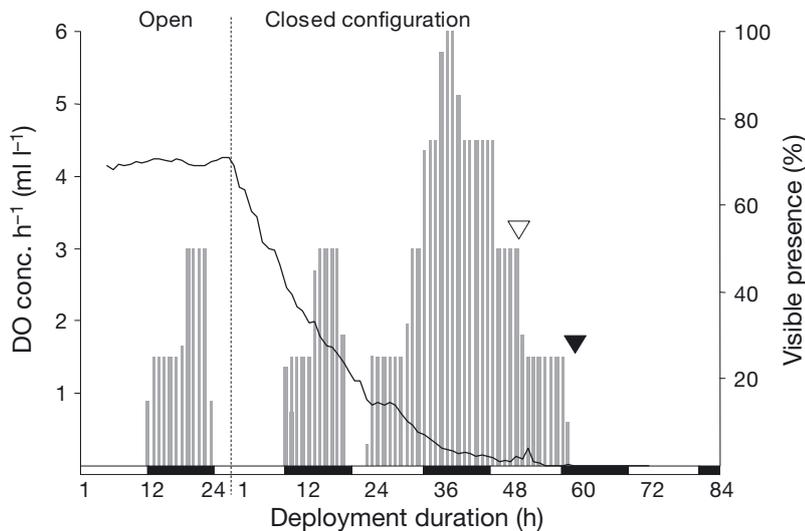


Fig. 4. Visible presence of *Pisidia longimana* during normoxia, hypoxia, and anoxia in selected deployments. Histograms: average number of observations per hour in relation to oxygen values (lower sensor). White and black arrows: 50 and 100% mortality, respectively. Dark horizontal bars: night; white horizontal bars: day

our ceased during severe hypoxia. For example, at DO below 0.43 ml l⁻¹ (deployment 7) and below 0.27 ml l⁻¹ (deployment 11), no intra- and interspecific aggression was observed between adjoining individuals. This behaviour, coupled with the restricted availability of higher substrata, resulted in atypical aggregations on the highest, most oxygenated sites. With ongoing hypoxia, aggregation sizes increased to a maximum of 27 crustaceans, mostly *Pisidia longimana* but also *P. spinifer*, *Macropodia* spp., and *Eurynome aspera*, along with other small organisms (shrimps, brittle stars, or flatworms; Fig. 6b). The greatest number of aggregations as well as the greatest number of individuals in such aggregations occurred during severe hypoxia (Fig. 6a).

Predator-prey relationships changed during severe hypoxia. Brittle stars never positioned themselves close to *Pilumnus spinifer* during normoxia, but did so during moderate and severe hypoxia. While prey still fled during moderate hypoxia, *Pisidia longimana* and juvenile brittle stars even climbed on top of potential predators at values below 0.5 ml l⁻¹ DO. One *Macropodia* spp. fled from *P. spinifer* at 1.2 ml l⁻¹ DO, but showed no escape reaction below 0.9 ml l⁻¹ DO. Another *Macropodia* spp. fled at 0.6 ml l⁻¹ DO. Conversely, *P. spinifer* or *Ebalia tuberosa* did not attack nearby prey (juvenile brittle stars, small crustaceans).

A common sublethal response was turning over shortly before death, with no righting response ever being observed thereafter. Among the species-specific sublethal responses, *Ethusa mascarone* discarded its camouflage at 0.4, 0.23, and 0.03 ml l⁻¹ DO. One individual was predated by the anemone *Calliactis*

parasitica shortly (1.3 h) after discarding its protective shell at 0.4 ml l⁻¹ DO and zero H₂S.

Mortality and survival

Mortality increased significantly during severe hypoxia and continued during anoxia (p < 0.001; Fig. 7a). Overall, 97% of all investigated individuals died, 54% of those during severe hypoxia, with *Galathea* spp., *Macropodia* spp., and *Pisidia longimana* among the first (Fig. 7b). Most crustaceans died before H₂S values began to rise. A total of 22 *P. longimana* individuals and 1 *Macropodia* sp. died at low H₂S concentrations (0.6 to 1.7 μmol l⁻¹). Most *Pilumnus spinifer* survived lower H₂S concentrations (<72.8 μmol l⁻¹; Table 2).

Pisidia longimana and *Pilumnus spinifer*, for example, died at significantly different mean (±SD) DO concentrations (0.1 ± 0.12 and 0.01 ± 0.01 ml l⁻¹, respectively; p = 0.004). The duration of hypoxia/anoxia that caused mortality also differed significantly between *P. longimana* and *P. spinifer* (p = 0.001) and between *P. spinifer* and *Macropodia* spp. (p < 0.001). The mean post-normoxia survival time for *Macropodia* spp. was 17.8 ± 2.7 h, for *P. longimana* 22.5 ± 8.3 h, and for *P. spinifer* 32.5 ±

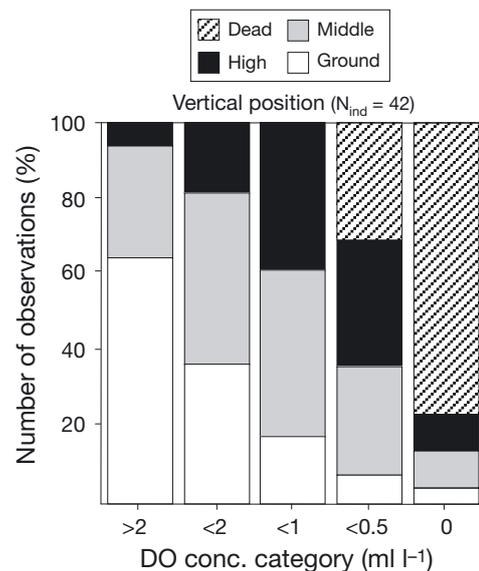


Fig. 5. Changes in vertical position (all species; n = 42) in the 5 oxygen categories. Percentage of observations of specimens positioned at different heights (ground: on sediment; middle: up to 5 cm; high: >5 cm above sediment)

9.6 h. The duration and severity of oxygen depletion influenced mortality thresholds. In deployments with a slow oxygen decline, *P. longimana* died at significantly higher concentrations ($0.17 \pm 0.15 \text{ ml l}^{-1} \text{ DO}$) than in those with a rapid decline ($0.06 \pm 0.07 \text{ ml l}^{-1} \text{ DO}$) ($p < 0.001$), a trend also shown by *Macropodia* spp. and *P. spinifer*.

Five individuals of 3 species survived (Fig. 7b). One *Pilumnus spinifer* and 2 *Ebalia tuberosa* were high up on a sponge but moribund at the end of the deployment. Both *Nepinnotheres pinnotheres* individuals survived extended oxygen depletion and high H_2S concentrations (Table 2) and continued to show very active horizontal and vertical locomotion.

DISCUSSION

Sensor data and biomass

The EAGU system captured the typical normoxic DO conditions during late summer and autumn in the northern Adriatic Sea and effectively created hypoxia and anoxia and promoted H_2S production. The occasionally low DO concentrations in the open configuration (minimum 2.6 ml l^{-1}) agree with previously recorded near-bottom autumn values (Stachowitsch

1991, Artegiani et al. 1997) and reflect the high oxygen demand of bacterial remineralisation and benthic respiration in periods with stratification (Herndl et al. 1989). Fluctuating concentrations and peak values reflect vertical mixing due to storms (Russo et al. 2005). Although DO concentrations are expected to be higher 20 cm above the sediment than directly at the bottom (Dade et al. 2001), in 2 deployments a reverse situation was observed. We attribute this to complex current patterns (open configuration) and to ventilation by burrowing organisms (closed configuration). The latter effect would be negligible in large-scale anoxia. The drop in pH values from 8.2 to 7.5 should not directly affect the epifauna (Knutzen 1981) but shifts the sulphide equilibrium towards H_2S (Jeroschewski et al. 1996).

Behavioural responses

Most investigations on the responses and adaptations of crustaceans to hypoxia and H_2S concentrations were conducted in laboratory settings and focused on physiological responses (Airriess & McMahon 1994, Vismann & Hagerman 1996, Burnett & Stickle 2001). Our approach yielded a full range of behavioural responses in a community-level setting. All crus-

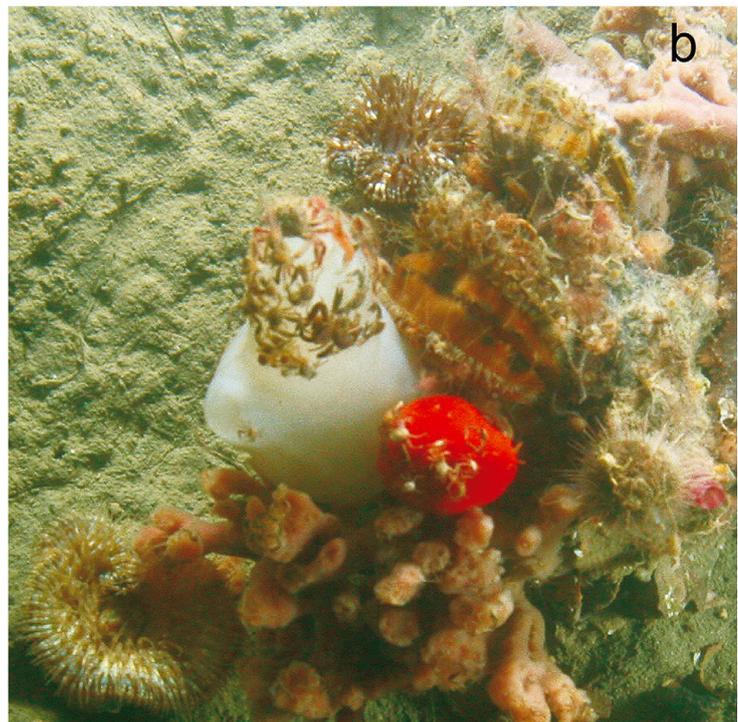
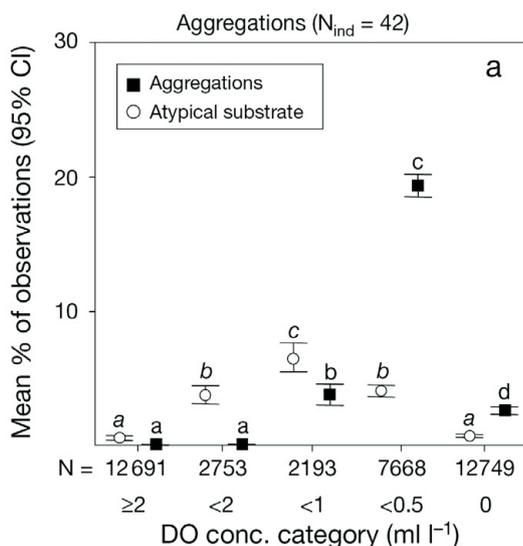


Fig. 6. (a) Mean percentage of observations of aggregations (■) and of investigated specimens atypically using vagile species as substrates (○) with 95% confidence intervals (all deployments). n = number of investigated individuals. See Fig. 3 for further details. (b) Aggregation during severe hypoxia (deployment 2: $0.4 \text{ ml l}^{-1} \text{ DO}$; zero H_2S). Numerous *Pisidia longimana* aggregate on *Phallusia mammillata* and on other parts of the bioherm. One *Pilumnus spinifer* and 2 shrimps are visible on the ascidian

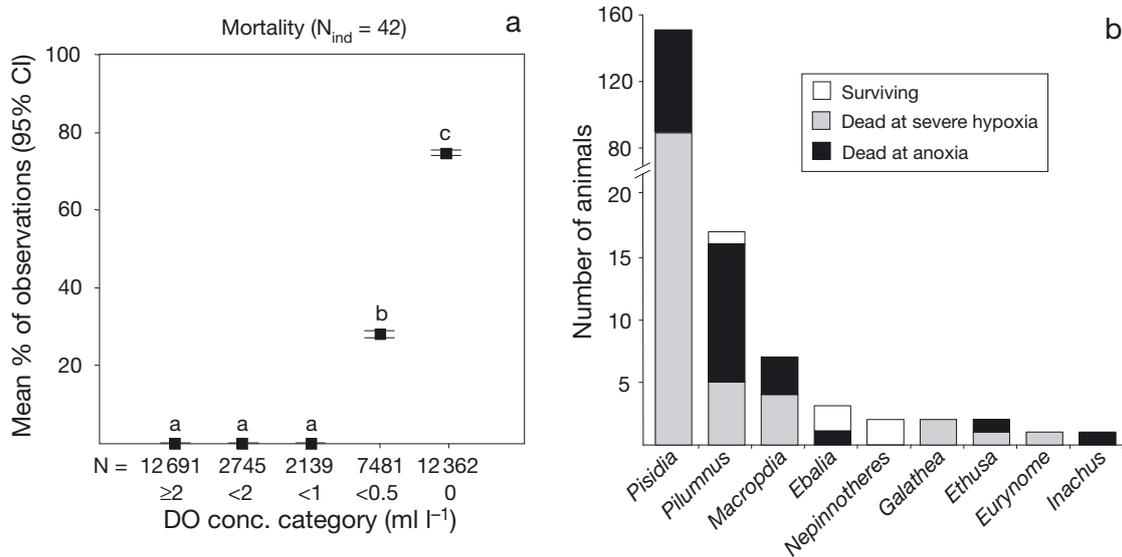


Fig. 7. (a) Mean percentage of observations of dead crustaceans during the 5 oxygen categories. All deployments evaluated. See Fig. 3 for further details. (b) Numbers of individuals of each taxon dying at severe hypoxia and anoxia as well as numbers of surviving taxa (n = 184)

taceans showed distinct responses and the respective patterns were repeated across deployments. Certain behaviours coincide with the observations reported elsewhere (Renaud 1986, Wannamaker & Rice 2000); others were previously unreported.

Avoidance

Avoidance, as an initial response, has been reported in numerous marine species (Pihl et al. 1991, Levin et al. 2009). In Mobile Bay (Alabama, USA) for example, vertical mass migrations of later moribund and easily capturable demersal fish and crustaceans to the shoreline are known as 'jubilees' (May 1973). The direction of escape in our deployments was either vertical or horizontal, probably reflecting the degree of association to bioherms. Based on survival times, all those species that initially fled horizontally were less sensitive to hypoxia. The higher DO concentrations at typical bioherm heights (ca. 20 cm) allow crustaceans to survive longer there. Such refuges are therefore important for short-term or near-ground hypoxia but would probably become insignificant at severe, prolonged, and large-scale anoxia.

Daytime presence of normally cryptic crustaceans (*Pilumnus spinifer*, *Pisidia longimana*, and *Galathea* spp.) indicates stress. Rosenberg et al. (1991) also observed the abandonment of protected positions in laboratory experiments at species-specific DO thresholds. In our deployments, most species emerged during mild hypoxia (median 1.6 ml l⁻¹ DO for the majoidean crabs and 1.4 ml l⁻¹ DO for *P. spinifer*). *Ethusa mas-*

carone and the less sensitive *Ebalia tuberosa* maintained their normal activity patterns longer, until moderate and severe hypoxia, respectively. *P. longimana* tended to hide longer than other crustaceans, possibly because such small crabs need to minimise the predation risk as long as possible, as reported for amphipods by Johansson (1997). When intraspecific behaviours were not correlated to one critical DO concentration—3 *E. tuberosa* emerged from the sediment at different stages of hypoxia during the night—normal nocturnal emergence (Schembri 1979) probably masked direct responses to hypoxia. Hypoxia forced all crustaceans to abandon their normal activity patterns regardless of the time of day. Circadian rhythms are widespread in crustaceans (deCoursey 1983, Aréchiga et al. 1993). *P. longimana*, *P. spinifer*, and *Macropodia* spp. are night active, probably a predator avoidance strategy (deGrave & Turner 1997). We identified 1 ml l⁻¹ DO as the species-specific threshold for visible presence in *P. longimana*. Once emerged, no crustaceans hid again unless DO temporarily increased (see Riedel et al. 2008b).

Avoidance reactions involved up to threefold greater locomotory activity in all species. Thereafter, activity decreased, typically ending in immobility at severe hypoxia. Such a pattern has also been reported in the blue crab *Callinectes sapidus* (deFur et al. 1990) and various shrimp species (Hagerman & Uglow 1984, Renaud 1986). Reduced locomotion is a common strategy to save energy, which is required for respiration (Johansson 1997, Mistri 2004). Declining activity may also involve switching to energy-conserving metabolic depression (Wu 2002). Reported

Table 2. DO and H₂S concentrations at time of death or end of deployment (surviving taxa) and the duration of hypoxia and anoxia until death or end of deployment. If n ≤ 2, range is replaced by measured value(s). Q₁: first quartile; Q₃: third quartile

Dead taxa	n	DO (ml l ⁻¹)		Duration of hypoxia (h)		H ₂ S (μmol l ⁻¹)	
		Median (Q ₁ /Q ₃)	Range	Median (Q ₁ /Q ₃)	Range	Median (Q ₁ /Q ₃)	Range
<i>Pisidia longimana</i>	150	0.03 (0/0.1)	0–0.3	21.3 (16.2/26.8)	9.5–45.0	0 (0/0)	0–1.68
<i>Pilumnus spinifer</i>	16	0 (0/0.01)	0–0.03	33.0 (23.4/37.4)	17.1–54.3	1.42 (0/10.77)	0–72.8
<i>Macropodia</i> spp.	7	0 (0/0.19)	0–0.39	16.4 (15.8/19.6)	14.9–22.3	0 (0/0.005)	0–0.84
<i>Ethusa mascarone</i>	2	–	0/0.4 ^a	–	30.2/10.3 ^a	–	0
<i>Galathea</i> spp.	2	–	0.03/0.33	–	21.8/17.8	–	0
<i>Eurynome aspera</i>	1	–	0.19	–	25.1	–	0
<i>Inachus</i> sp.	1	–	0	–	24.6	–	0
^a One predated animal							
Surviving taxa	n	DO (ml l ⁻¹)		Duration of hypoxia + anoxia (h)		H ₂ S (μmol l ⁻¹)	
<i>P. spinifer</i>	1	0		22.7 + 8.5		0	
<i>Ebalia tuberosa</i>	2	0		21.8 + 14.1/34.2 + 23.6		12.8/19.2	
<i>Nepinnotheres pinnotheres</i>	2	0		53.2 + 24.6/11.7 + 78		126.1/111.6	

species-specific thresholds for reduced activity are 2 ml l⁻¹ DO for *Crangon crangon* (Hagerman & Szaniawska 1986) and juvenile *Nephrops norvegicus* (Eriksson & Baden 1997), similar to the thresholds we found for *Inachus* sp. and *Eurynome aspera*. Nevertheless, most crustaceans in our study showed a first significant reduction in activity later, i.e. between 1 and 0.5 ml l⁻¹ DO. Two interpretations are possible: a greater tolerance in the natural, structurally complex setting (versus laboratory conditions), or a community composition adapted to low oxygen concentrations.

Atypical interactions and sublethal responses

Atypical interactions included using atypical substrata, multi-species aggregations, loss of habitat segregation, and altered predator–prey interactions. Examples for sublethal responses are atypical postures and discarding of camouflage.

In the northern Adriatic, the brittle star *Ophiothrix quinquemaculata* is known to climb onto and hitch rides with holothurians *Holothuria tubulosa* (Stachowitsch 1979). Crustaceans typically avoid mobile organisms as a substratum. During mild and moderate hypoxia, however, crustaceans crawled on any available structure or organism and formed multi-species aggregations. Intraspecific aggregations are known for non-territorial crustaceans like *Pisidia longimana* (Pallas et al. 2006), but are reported here for the first time

for the highly territorial (Wurzian 1977) *Pilumnus spinifer* as well. Loss of intraspecific aggression and territorial behaviour has also been described for *Mundia quadrispina*, but at a much lower threshold (<0.15 ml l⁻¹ DO; Burd & Brinkhurst 1984). These aggregations signal high hypoxic stress and are an indicator for severe hypoxia.

Hypoxia also affected intraspecific habitat segregation: juvenile majoidean crabs normally hid on bioherms, whereas adults were found on the sediment or hidden beneath an anemone or sponge. At hypoxia, both aggregated on bioherms.

Feeding behaviour and predator–prey interactions were also affected. *Pilumnus spinifer* normally feeds on small galatheid/porcellanid crabs and juvenile brittle stars (Štević 1975). The predatory crab determines the distribution of the brittle star *Ophiothrix quinquemaculata*, which normally maintains at least a 1 to 2 cm distance or avoids crab-occupied bioherms entirely (Wurzian 1977). If contact is made, brittle stars retract their arms and re-establish a safe distance (Riedel et al. 2008a). At severe hypoxia, however, brittle stars and crabs aggregated together. The absence of predator avoidance is common in oxygen-deficient environments (Johansson 1997) and was also recorded here for *Galathea* sp. and *Pisidia longimana*. The behaviour necessary to maintain aerobic respiration—exposure on higher substrates—is inappropriate for avoiding predation (Pihl et al. 1991, Mistri 2004). Whether moribund or inactive individuals are easy

prey depends on the relative hypoxic sensitivity of predator and prey. Hypoxia affects predator–prey interactions to the benefit of the more tolerant species (Sagasti et al. 2001). Predators may be less tolerant, in which case potential prey can attain a partial refuge at low oxygen concentrations (Sandberg 1994, Taylor & Eggleston 2000). This was the case here for the prey of *P. spinifer* and *Ebalia tuberosa*. Predation can also be interrupted to save energy, with hypoxia-induced starvation potentially posing a greater threat than low DO values (Baden et al. 1990). In contrast, more tolerant pelagic or opportunistic predators can exploit moribund or slowly recovering benthos (Karlson et al. 2002, Norkko et al. 2006). The anemone *Calliactis parasitica*, for example, took advantage of the situation (see also Riedel et al. 2008a) and predated one *Ethusa mascarone* at severe hypoxia after the crab discarded its protective shell. Discarding camouflage is a sublethal behaviour also observed in the sea urchin *Psammechinus microtuberculatus* (Riedel et al. 2008b). Holding a shell fragment is apparently energetically costly and may restrict mobility. One shell-less *Ethusa mascarone* climbed high up an oxygen sensor, perhaps extending its survival time. Nonetheless, *E. mascarone* discarded its camouflage relatively late (0.23 ml l⁻¹ DO). This indicates a trade-off between predation risk, mobility and obtaining sufficient oxygen.

Mortality and survival

While crustaceans are among the most sensitive taxa to low DO and to H₂S (Theede et al. 1969, Gray et al. 2002, Vaquer-Sunyer & Duarte 2008), those living in environments with permanently or temporarily low oxygen concentrations, such as thalassinideans, are remarkably tolerant (Atkinson & Taylor 2005). Mortalities typically occur below 1 ml l⁻¹ DO, and mass mortality below 0.5 ml l⁻¹ (Diaz & Rosenberg 1995). The mortality thresholds in our study were mainly below 0.5 ml l⁻¹ DO (overall median: 0.03 ml l⁻¹ DO). Mortality was rapid when prolonged severe hypoxia or anoxia occurred. After a median 21.5 h of hypoxia, almost all crustaceans were dead, indicating a general low tolerance. Many species that can cope with short-term hypoxia by changing their behaviour or adapting physiologically cannot survive extended or severe events (Hagerman & Szaniawska 1988, Airriess & McMahon 1994). Crustaceans may generally lack efficient physiological adaptations (Bernatis et al. 2007), promoting the initial vertical escape reaction. Limited anaerobic capacities may also help explain low survival rates (Hagerman & Vismann 1995).

The sequence of mortality was the same in all deployments. Thus, from a monitoring standpoint, dead

anomurans (*Pisidia longimana* and *Galathea* spp.) and majoidean crabs indicate severe hypoxia, whereas dead *Ethusa mascarone* and *Pilumnus spinifer* indicate anoxia. Juveniles and smaller individuals tended to die earlier than adults in the same deployment. This confirms the role of size and development stage, whereby juveniles and moulting crabs are more sensitive than adults (Stickle et al. 1989, Eriksson & Baden 1997), as are smaller individuals of a particular species (e.g. *Mundia quadrispina*; Burd & Brinkhurst 1984).

Finally, the course of hypoxia is known to influence mortality and survival times (e.g. polychaetes; Llansó 1991), which we observed here for crustaceans as well. A rapid drop killed the crustaceans at clearly higher DO concentrations and shortened survival times (e.g. *Pisidia longimana*). This scenario may limit the ability to flee, adapt, or otherwise compensate (McMahon 2001). For all species except *Pilumnus spinifer* and *Ebalia tuberosa*, mortality is directly attributable to oxygen depletion because they died before H₂S developed. We therefore identify hypoxia as the limiting factor. H₂S considerably decreases tolerance to oxygen depletion (Kang et al. 1993), reduces survival times by an average of 30% (Vaquer-Sunyer & Duarte 2010), and clearly promoted the mortality of crustaceans still alive at anoxia here. In *E. tuberosa*, for example, 12 to 21 µmol l⁻¹ H₂S were measured at the time of death. A similar concentration (16.2 µmol l⁻¹ H₂S) causes paralysis in the brown shrimp *Crangon crangon* (Vismann 1996).

Ebalia tuberosa and *Nepinnotheres pinnotheres* survived prolonged hypoxia as well as anoxia. The burying lifestyle of the former involves behavioural and morphological adaptations of the respiratory system, namely increased heart and scaphognathite activity and thus gill ventilation (Schembri 1979), which may provide benefits during hypoxia. Two *E. tuberosa* survived at least 14 h of anoxia (deployment ended), and one died after 19 h. Schembri (1979) found similar mortality thresholds (10 to 20 h) at anoxia for *E. tuberosa* in aquarium experiments. Nevertheless, this species tolerates hypoxic/anoxic conditions and low H₂S concentrations (max. 19.2 µmol l⁻¹) for at least 1 to 2 d.

Anoxia triggered the first visible response (host abandonment) in both *Nepinnotheres pinnotheres*. This points to high tolerance, but is nevertheless a very unusual behaviour for female individuals (Christensen & McDermott 1958). Pea crabs, especially species living in bivalves, are well adapted to low oxygen concentrations (Stauber 1945). Here, they also showed high tolerance to H₂S. In general, marine species with a high tolerance toward oxygen deficiency also cope better with sulphidic conditions (Theede et al. 1969). Based on its late reaction and

survival, *N. pinnotheres* can be classified as highly tolerant of hypoxia and anoxia and as H₂S tolerant for at least 1 to 3 d.

CONCLUSION

The present study highlights the complexity and interdependence of abiotic and biotic factors as well as inter- and intraspecific interactions in influencing and differentiating low-DO-related responses, survival times, and mortality rates of crustaceans *in situ*. The distinct behaviours and common patterns demonstrate the benefit of using this sensitive group as an early warning signal to gauge the severity of the low DO events that can lead to dead zones in shallow coastal seas.

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