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Effect of hypoxia and anoxia on invertebrate behaviour: ecological perspectives from species to community level

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Abstract

Coastal hypoxia and anoxia have become a global key stressor to marine ecosystems, with almost 500 dead zones recorded wordwide. By triggering cascading effects from the individual organism to the community and ecosystem-level, oxygen depletions threat marine biodiversity and can alter ecosystem structure and function. By integrating both physiological function and ecological processes, animal behaviour is ideal for assessing the stress state of benthic macrofauna to low dissolved oxygen. The initial response of organisms can serve as an early-warning signal, while the successive behavioural reactions of key species indicate hypoxia levels and help assess community degradation. Here we document the behavioural responses of a representative spectrum of benthic macrofauna in the natural setting in the Northern Adriatic Sea, Mediterranean. We experimentally induced small-scale anoxia with a benthic chamber in 24 m depth to overcome the difficulties in predicting the onset of hypoxia, which often hinders full documentation in the field. The behavioural reactions were documented with a time-lapse camera. Oxygen depletion elicited significant and repeatable changes in general (visibility, locomotion, body movement and posture, location) and species-specific reactions in virtually all organisms (302 individuals from 32 species and 2 species groups). Most atypical (stress) behaviours were associated with specific oxygen thresholds: arm-tipping in the ophiuroid *Ophiothrix quinquemaculata*, for example, with the onset of mild hypoxia ($< 2\,\mathrm{mL\,O_2\,L^{-1}}$), the emergence of polychates on the sediment surface with moderate hypoxia ($< 1\,\mathrm{mL\,O_2\,L^{-1}}$), the emergence of the infaunal sea urchin *Schizaster canaliferus* on the sediment with severe hypoxia ($< 0.5\,\mathrm{mL\,O_2\,L^{-1}}$) and heavy body rotations in sea anemones with anoxia. Other species changed their activity patterns, i.e. circadian rhythm in the hermit crab *Paguristes eremita* or the bioherm-associated crab *Pisidia longimana*. Intra- and interspecific reactions were weakened or changed: decapods ceased defensive and territorial behaviour, and predator-prey interactions and relationships shifted. This nuanced scale of resolution is a useful tool to interpret present benthic community sta-

tus (behaviour) and past mortalities (community composition, e.g. survival of tolerant species). This information on the sensitivity (onset of stress response), tolerance (mortality, survival), and characteristics (i.e. life habit, functional role) of key species also helps predict potential future changes in benthic structure and ecosystem functioning. This integrated approach can transport complex ecological processes to the public and decision-makers and help define specific monitoring, assessment and conservation plans.

1 Introduction

The increasing intensity and diversity of anthropogenic stressors is triggering unprecedented habitat and biodiversity loss in marine ecosystems worldwide (Halpern et al., 2008). The effects, ranging from population depletion to species extinction and community homogenization, raise concerns whether ecosystem function and ecosystem goods and services can be maintained (UNEP, 2006; Worm et al., 2006; Cardinale et al., 2012). A key issue is understanding how species respond to such rapid human-induced environmental change (Sih et al., 2011; Tuomainen and Candolin, 2011).

Eutrophication is one of the most striking ecological threats to shallow coastal seas and estuaries (Smith and Schindler, 2009). Excessive nutrient inputs have the potential to tip systems into hypoxia (here defined as dissolved oxygen [DO] concentrations $< 2\,\mathrm{mL\,L^{-1}}$, equivalent to $2.8\,\mathrm{mg\,L^{-1}}$ or $91.4\,\mu\mathrm{M}$; Diaz and Rosenberg, 2008) and anoxia or exacerbate conditions in predisposed areas (Gooday et al., 2009; Howarth et al., 2011). Currently, the number of coastal eutrophic and hypoxic sites nearly touches the 500 mark (Diaz et al., 2010), with devastating effects for marine biodiversity and ecosystem functioning (Zhang et al., 2010). Ocean warming is expected to additionally increase the frequency, duration, intensity and extent of hypoxia/anoxia in certain zones (Keeling et al., 2010; Gruber, 2011), pushing coastal ecosystems toward tipping points (Conley et al., 2009; Rabalais et al., 2010) (Fig. 1).

Physiological and metabolic adaptions to hypoxia are the initial organismic responses (Grieshaber et al., 1994; Hagerman, 1998), mortality the endpoint (Vaquer-Sunyer and Duarte, 2008; Riedel et al., 2012). Exposure to oxygen concentrations far above the lethal threshold already adversly affects growth, reproduction, locomotion and feeding (e.g. summarized in Gray et al., 2002; Wu, 2002; Levin et al., 2009). Importantly, basic behavioural activities are the link between individual responses and population changes (Boyd et al., 2002). Intraspecifically, for example, hypoxia affects mate choice or courtship behaviours in fish, impacting reproduction and development (Wu, 2009). Change in one behaviour may influence the outcome of others, e.g. altered locomotion can affect reproduction and/or food finding behaviour (Boyd et al., 2002). Interspecifically, shifts in locomotion – migration to a new region, shallower burial depths or exposure atop elevated substrates – may change predator–prey relationships (Pihl et al., 1992; Riedel et al., 2008a; Long and Seitz, 2008). Behavioural parameters thus integrate direct and indirect responses across several levels of biological organisation, from individual fitness to community composition, reflecting cascading changes in biodiversity, biogeochemical processes and ecosystem function (e.g. Solan et al., 2004; Middelburg and Levin, 2009; Ekau et al., 2010; Zhang et al., 2010).

Such intimately nested responses call for more holistic approaches. Unexpected onsets of hypoxia, poorly predictable extents and the often rapid course of events are a challenge in gaining a better insight into when ecosystems approach critical points. This calls for recognizing perturbations at the earliest possible stage, when adaptive capacity and resilience are still functional. The initial short-term reactions and atypical behaviours of benthic macrofauna are a suitable avenue (summarised in Diaz and Rosenberg, 1995; Rabalais et al., 2001; Levin et al., 2009). For example, avoidance and altered migration patterns generally start when oxygen falls below 3.0 $\mathrm{mg\,L^{-1}}$ (equivalent to 2.1 $\mathrm{mL\,L^{-1}}$). Such initial behavioural responses of benthic macrofauna integrate processes in the environment (from the sediment to the water body), yielding information beyond prevailing oxygen concentrations and species tolerance ranges to encompass overall ecosystem stress (e.g. Xu et al., 2006). This makes behaviour a suitable early-warning indicator that also increases our understanding of longer-term ecosystem consequences (Tuomainen and Candolin, 2011).

Our in situ approach complements laboratory experiments, which provide detailed information on the individual organism-level (e.g. respiration, growth rate and reproduction) but typically concentrate on captured animals of single-species in artificial settings (Biro, 2012). The benthic chamber not only rivals the level of resolution in laboratory studies but also documents the complex biological interactions that accompany and define ecosystem responses to stress (Adams, 2003). Such multi-species analyses provide a very realistic and more holistic understanding of the complex structural and functional processes and potential species interactions in a community setting – a prerequisite for understanding ecosystem integrity (Culp et al., 2000; Sibley et al., 2000).

We hypothesized that certain (atypical) behaviours of benthic invertebrates are associated with distinct oxygen thresholds and that the behaviour of key species reliably indicats the status of benthic communities during hypoxia. We continuously recorded the multiple direct and indirect responses, including altered behaviours, inter- and intraspecific interactions and mortality sequences, of a sublittoral macrobenthic assemblage to hypoxia/anoxia in a realistic scenario. The benthic chamber induced small-scale anoxia in a benthic soft-bottom community setting in 24 m depth in the Northern Adriatic Sea. A time-lapse camera and sensors (oxygen, hydrogen sulphide, temperature, pH) enabled continuous documentation of environmental parameters and biological responses. The focus was on the benthic macrofauna as the first component to visibly react to coastal hypoxia and the compartment that fundamentally defines such ecosystems and their functioning. The sediment-geochemical processes (Koron et al., 2013; Metzger et al., 2013), the accompanying meiofauna responses (foraminiferans: Langlet et al., 2013a, b; harpacticoid copepods: Grego et al., 2013a, b; De Troch et al., 2013), and immediate post-anoxia scavenging and longer-term recovery (Blasnig et al., 2013) are treated elsewhere in this special issue.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀◀ | ▶▶
◀ | ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

2 Material and methods

2.1 Location and dates

The Northern Adriatic Sea (Mediterranean) is a recognised area for seasonal low DO events (Malej and Malačič, 1995; Diaz and Rosenberg, 2008), here often associated with massive marine snow events (Danovaro et al., 2009 and references therein). The study site is located within and representative for a wide-ranging, suspension-feeding macroepifauna community consisting of interspecific aggregations (multi-species clumps or bioherms) described as the *Ophiothrix-Reniera-Microcosmus* (ORM) community by Fedra et al. (1976).

Eleven deployments with a specially designed benthic chamber, were made in 2005 (September) and 2006 (August to October) in a soft-bottom habitat (poorly sorted silty sand) in 24 m depth in the Gulf of Trieste (45°32′ 55.68″ N, 13°33′ 1.89″ E). This position, adjacent to the oceanographic buoy of the Marine Biology Station Piran, is not directly affected by bottom fisheries and has not experienced hypoxia for at least 5 yr (V. Malačič, personal communication, 2005) before our experiment.

2.2 Experimental procedure

A benthic chamber was used to experimentally create small-scale anoxia and quantify macrobenthic responses to decreasing oxygen concentrations (for a detailed description of the method see Stachowitsch et al., 2007). The design involves the successive use of two interchangeable bases (both 50 cm × 50 cm × 50 cm): an aluminum frame plus separate instrument lid (“open” configuration) and a plexiglass chamber plus this lid (“closed” configuration). Initially, the open configuration was positioned above a benthic assemblage for ca. 24 h to document macrobenthic behaviour during normoxia. Then, in a second step, the closed configuration was positioned for another 3 to 4 days above the same assemblage to document the reactions to hypoxia and anoxia. The plexiglass chamber was pressed ca. 4 cm into the sediment and prevented water exchange with the immediate environment. The instrument lid houses a time-lapse camera, 2 flashes, a datalogger and the microsensor array for dissolved oxygen (DO), hydrogen sulphide (H_2S) and temperature recording (Unisense$^{®}$). Oxygen was measured 2 and 20 cm above the sediment to detect potential stratification, the H_2S sensor was positioned in 2 cm height. Bottom pH was measured at the beginning and the end of almost all deployments (WTW TA 197-pH sensor). Images were taken in 6 min intervals, microsensor values logged every minute.

Overall, ca. 10 000 images were produced, yielding a documentation time of 1003 h (hypoxia: 331.9 h, anoxia: 336.5 h). The flashes, interrupted current and food input never triggered any visible reaction in the benthic invertebrates and were thus not considered to have altered the course of the short-term experiments. Decreasing oxygen values in the chamber solely reflect natural respiration rates of the enclosed fauna (i.e. no macroalgae enclosed, photosynthesis by microflora under the low light conditions in 24 m depth negligible). The 11 deployments were similarly executed, but their durations varied somewhat due to weather/sea conditions. Four deployments started immediately with the closed configuration because of the highly turbid bottom water (for a summary of data and treatments in the 11 deployments see Table A1). After each deployment, living and dead organisms were collected and preserved in a 4 % formalin-seawater solution. The experiments followed the institutional guidelines of the University of Vienna, Austria, and the Marine Biology Station Piran, Slovenia.

2.3 Macrofauna data analysis

The behaviour in a total of 302 individuals was evaluated, representing 32 species and 2 species groups (i.e. individuals unidentifiable based on the photographs). Time-lapse movies (Adobe Premiere Pro CS4) produced from the still image series provided quick viewing of the overall sequence of events (sample 4 day film available at http://phaidra.univie.ac.at/o:87923). This yielded data on a representative spectrum of taxa, life habits, (stress) behaviours, interactions as well as on sensitivities (initial reactions) and tolerances (i.e. survival or mortality). Evaluated life habits include substrate relationship (cryptic and non-cryptic epifauna, infauna), mobility (mobile, sessile) and 3 major feeding types (predators, suspension feeders, deposit feeders) (Table A2). Species represented by only 1 or a few individuals were evaluated in full, those by many individuals selectively (those individuals most often visible and recognizable, for example the porcellanid crab *Pisidia longimana*). Each individual was analysed separately, image by image, and the behaviour recorded in categories (key categories of behaviours and responses listed below, for species-specific behaviours see Table A2) (in 8 cases until they were predated: see Sect. 3.3.2 Predator-prey interactions). The terms "typical/normal" behaviour refer to responses observed during normoxia (in this study and earlier observations). Additional information on the hermit crab *Paguristes eremita* is provided in Pretterebner et al. (2012), on decapods (except *Alpheus glaber*) in Haselmair et al. (2010). Mortality and/or survival in relation to hypoxia (DO concentration), anoxia (duration) and associated H_2S development was evaluated on overall 495 individuals (39 species and 1 species group) based on the images and specimens collected after the deployment. The time-point of death was typically unambiguous, e.g. collapse of soft-bodied forms and various combinations of overturning, clear-cut body postures, discoloration. For more information on mortality/survival and life habits see Riedel et al. (2012).

Key categories of behaviours and responses

1. Visibility: visible, non-visible (hidden in/under bioherm, in sediment).
2. Locomotion: change in location (horizontal and/or vertical. Subcategories: minor (displacement < 1 body length), major (> 1 body length), none).
3. Movement (other than locomotion): turn (change in direction), squirm, extremity movement (i.e. legs, antennae, eyes, chelipeds), body movement (i.e. body rotation in sea anemones, retraction into or stretching out of shell in hermit crabs, body contraction in ascidians).

4. Body posture: normal, atypical (posture never observed under normoxia).
5. Location: bottom (organism on sediment), middle (< 5 cm above sediment, on macroepifauna), high/top (> 5 cm/on top of bioherm).
6. Intra- and interspecific interaction: all encounters visibly triggering a change in behaviour, with focus on predator–prey relationships, atypical aggregation (> 3 individuals in direct contact), aggression.
7. Species-specific responses (details: Table A2): e.g., pharynx protrusion and acontia discharge in sea anemone *Calliactis parasitica*, camouflage discarding (i.e. shell fragments) in echinoid *Psammechinus microtuberculatus* or decorator crab *Ethusa mascarone*.

2.4 Statistical analysis

Behavioural data were assigned to 5 oxygen categories: normoxia ($\geq 2.0\,\mathrm{mL\,DO\,L^{-1}}$), mild ($< 2.0\,\mathrm{mL\,DO\,L^{-1}}$), moderate ($< 1.0\,\mathrm{mL\,DO\,L^{-1}}$), severe hypoxia ($< 0.5\,\mathrm{mL\,DO\,L^{-1}}$), and anoxia (no oxygen). For selected behavioural reactions to hypoxia and anoxia see Figs. A1–A33. Error bar graphs show the proportion of behaviours per oxygen category. The number of images evaluated per oxygen category (number below the x-axis) varies within and between species, depending on number of individuals evaluated per deployment, turbidity conditions (i.e. individual clearly identifiable), and deployment duration (including various oxygen category phases). In the pistol shrimp *Alpheus glaber* (both individuals in a deployment with an intermittent oxygen peak, see below in Results), the responses are depicted as histograms (Fig. A15), showing the number of behaviours observed per hour in relation to the oxygen curve (value averaged per hour). Significant H_2S refers to concentrations $> 14\,\mu\mathrm{M}$, as defined by Vaquer-Sunyer and Duarte (2010). The non-parametric Kruskal–Wallis test was used to determine potential differences in behaviours between oxygen categories, the Mann–Whitney *U* test to test differences in the means of behaviours across oxygen categories. Categories with < 5 images evaluated per oxygen category were excluded from statistical analysis. The significance level was $p < 0.05$. Analyses were performed using SPSS software package 20.0.

3 Results

3.1 Sensor data

During the open-frame configuration, oxygen concentrations within a particular deployment remained relatively constant, although the values across all deployments ranged from 2.8 to 8.9 $\mathrm{mL\,L^{-1}}$ 20 cm above the sediment and 2.6 to 5.6 $\mathrm{mL\,L^{-1}}$ in 2 cm height, reflecting the respective meteorological/oceanographic conditions. The “20 cm”-values were typically higher. After closing the chamber, oxygen values fell constantly, with both curves approximating each other. The in situ approach successfully mimicked the actual time course of oxygen depletion events documented earlier in the Northern Adriatic based on macrofauna behaviour (Stachowitsch, 1984): hypoxia was generated within ca. 1.5 days, anoxia within 3 days (Table A1). At the transition from severe hypoxia to anoxia, the sediment colour changed from brown-grey to darker grey and finally black with gradually increasing H_2S concentrations.

The rapidity of the oxygen decline varied between deployments, reflecting the biomass enclosed and initial oxygen concentrations. Accordingly, hypoxia duration ranged from 11.4 to 52.9 h, anoxia 5.2 to 81.8 h. In deployment 2 an intermittent oxygen peak created two phases of oxygen decline (Riedel et al., 2008b). H_2S development occurred in 10 of the 11 deployments after onset of anoxia, with final values (average over last hour of deployment) reaching 167.6 µM (except deployment 11 with an intermittent peak of 300 µM). Temperature within a particular deployment remained constant (range across all experiments 17.6 to 21. 4 °C), bottom salinity was 38 psu. Bottom water pH dropped from 8.2 to a mean of 7.8 (minimum 7.5). Figure 2 illustrates the course of a representative deployment based on average values from Table A1.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

3.2 Macrofauna behaviour

Hypoxia elicited clear, visible reactions in all organisms. These reflected increasing response to decreasing oxygen values and/or increasing duration of anoxia. Three main types of reactions were evident: altered activity levels and new (stress) behaviours; altered interactions; distinct sensitivities and tolerances.

1. Altered activity levels and atypical behaviours: The first reactions typically involved an increase and/or decrease in the level of normal activities and/or initiation of atypical stress behaviours. Almost all species showed a sequence of behaviours to decreasing oxygen concentrations. For details see Figs. A1–A33 and text below (Sect. 3.2.1–3.2.7), for a synthesis see Fig. 7. Importantly, within a species, almost all individuals' behaviours showed a nearly identical sequence. Most behaviours were clearly related to specific oxygen thresholds/categories (Fig. 3). This was supported by the results from deployment 2, where atypical behaviours were interrupted during the temporary re-oxygenation and then resumed again at renewed hypoxia (e.g. *Alpheus glaber* (Fig. A15); deployment details see Riedel et al., 2008b). Whereas epifauna typically showed first responses at early, mild hypoxia, the first visible response of the macroinfauna (emergence) started at moderate hypoxia (exceptions: bivalve *Corbula gibba*, brittle star *Ophiura* spp.).

2. Altered intra- and interspecific interactions: This included the weakening of defensive (i.e. hiding and covering behaviour) and territorial behaviour as well as altered predator–prey interactions, including unusual interactions and encounters (see Sect. 3.3).

3. Sensitivity and tolerance: our experiments revealed a distinct sequence of mortality, reflecting the sensitivity and tolerance of the species and higher taxa to decreasing oxygen concentrations and anoxia duration (see Sect. 3.4).

3.2.1 Anthozoa

All percent values below refer to mean percent of observations of the respective behaviour during the 5 oxygen categories.

***Calliactis parasitica* (Couch, 1842), *N* = 6**

Mutualistic symbiont associated with hermit crabs, with up to 3 specimens per occupied shell; sometimes also on living gastropods (*Hexaplex trunculus*). Column normally yellow/buff with darker vertical stripes. Non-selective omnivorous suspension feeder that also sweeps the sediment with its tentacles; main prey: gastropods and crustaceans.

- Normoxia: tentacle crown typically facing down, tentacles sweeping the sediment as the crab walks about.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: significant change in crown orientation from down- to upward (51 %; Fig. A1d). Marked increase in opened tentacle crowns in all individuals (90 %; Fig. A1b). Sporadic slight body contractions (i.e. column diameter constricted or inflated; Fig. A1f).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: peak upward orientation, tentacle crowns started to close again more often (Fig. A1c). Mouth occasionally “puckered”; increased pharynx protrusion (Fig. A1h).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: significant increase in body contractions and body rotations (Fig. A1g) (36 and 22 %, respectively); pharynx protrusion reached 43 %. Column turned greyish (Fig. A1a); at near-anoxia, periderm in one individual started to peel off and acontia discharged.
- Anoxia: initially (no H_2S present), peak body contraction and rotation. With increasing H_2S (ca. 30 µM), however, visible decrease in these activities: body relaxed, tentacle crowns (either half open or closed) slightly moved/pulsated, rotations almost ceased; only weak body contractions. Two individuals detached from

shell and fell to sediment, another individual discharged its acontia. Four individuals died after 47.4 to 65.6 h of anoxia (H_2S range: 56.3–96.1 µM), two survived 25.1 h of anoxia (final H_2S 124.5 µM).

Cereus pedunculatus **(Pennant, 1777),** ***N*** **= 17**

Sea anemone buried in sediment, with tentacle crown flat on sediment surface; some specimens integrated into epifaunal aggregations. Opportunistic omnivorous suspension feeder on organic detritus but also on mobile prey (mainly amphipods and decapods) that blunder into its tentacles.

- Normoxia: Specimens touched, e.g. by passing crabs, closed crown and briefly retracted into sediment.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: minor extensions (i.e. slightly above sediment surface; Fig. A2h); tentacle crowns increasingly stayed open when touched (57 %, Fig. A2b); retraction into sediment dropped from 33 to 20 % (Fig. A2d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: significantly increase in minor extension (21 to 60 %). Crowns largely open, increased body contractions (Fig. A2e). Gradual discolouration (paler) of tentacles and oral disc (Fig. A2a).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: major extension (parts of column clearly visible) significantly increased (51 %); strong body contractions and rotations pendulating in all directions (Fig. A2f); peak pharynx protrusion (29 %, Fig. A2g).
- Anoxia: sharp increase in half-open and closed crowns (combined: 61 %; Fig. A2c). Body contractions and rotations continued until end of deployments. All individuals survived (maximum anoxia duration 81.8 h; max. H_2S concentration 279 µM).

3.2.2 Nemertini and Sipunculida

Tubulanus sp., $N = 1$

Mobile, infaunal nemertean; predator on muddy sublittoral bottoms.

- Normoxia: not visible on sediment surface.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- Anoxia: One large individual (ca. 40 cm) emerged 18 h after onset of anoxia (H_2S 33.3 µM) (Fig. A3a) and then moved about on sediment or squirmed in place (75 and 21 %, respectively; Fig. A3b). On two occasions the nemertean was under and in direct contact with an emerged infauna sea urchin (*Schizaster canaliferus*; total interaction time ca. 1 h), and also crawled under a holothurian (*Ocnus planci*; > 12 min). At 26.4 h of anoxia (H_2S 41.7 µM) it disappeared from view (i.e. mortality or survival not determined).

Sipunculida indet., $N = 4$

- Normoxia: infaunal animal, not visible on sediment surface.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: one individual emerged at transition from severe hypoxia to anoxia (hypoxia duration 33.5 h) and showed minor movements and squirming in place.

- Anoxia: three individuals emerged 7.6, 17.5 and 34.2 h after onset of anoxia (H_2S 34.9, 83.2 and 243.2 µM) (Fig. A4a). Most moved only short distances (5 %; Fig. A4b), tending to then squirm in place (41 %; Fig. A4b) rather than move about. Two individuals disappeared from view only 30 and 108 min after emergence (i.e. anoxia 9.5 and 34.8 h) and may have reburied themselves; other two specimens visible on sediment surface until end of deployment (anoxia duration 24.2 and 25.1 h, final H_2S 18.2 and 124.5 µM, respectively).

3.2.3 Mollusca

***Diodora* sp., *N* = 4**

Keyhole limpets are herbivores and browsing carnivores on hard substrata and typical inhabitants of multi-species clumps.

- Normoxia: Only 2 of overall 4 specimens visible because of their cryptic position on multi-species clumps or cover by brittle star aggregations (Fig. A5a). Individuals quite stationary (Fig. A5b).

- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: no significant change in behaviour.

- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: visibility significantly increased to 72 % (Fig. A5a), with one individual emerging from under an ascidian at $0.7\,\mathrm{mL\,DO\,L^{-1}}$. Combination of foot extension (Fig. A5d) and arm-tipping brittle stars *Ophiothrix quinquemaculata* (see below) simplified recognition of limpets. One individual crawled onto a hermit crab (*Paguristes eremita*) inhabited shell occupied by 3 anemones (*Calliactis parasitica*). The limpet was in direct contact with the column of two of these anemones for 3.3 h. At this point, crab extended from shell and motionless. Then, limpet movement caused crab's shell to overturn over a period of 48 min.

- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: fourth specimen visible. All individuals started to flip over or fall off from their substrate, i.e. shell overturned in 33 % of observations (Fig. A5c).

Foot extension peaked (37 %; Fig. A5d). One individual climbed atop a sea urchin (*Psammechinus microtuberculatus*) for 12 min before falling off at border to anoxia. Second individual, on hermit crab shell, remained firmly attached for 26.2 h, again in contact with both the anemones and the hermit crab, which repeatedly touched the limpet with its pereopods and claws. First mortality shortly before onset of anoxia (45.7 h of hypoxia).

- Anoxia: Remaining three individuals flipped over (76 %). The limpet that fell off the sea urchin uprighted itself 1 h later, before again falling over and remaining so until end of experiment 4.2 h later. Individual on hermit crab gradually loosened its attachment and exposed most of its foot for 5.6 h, before falling off onto sediment, after which it no longer moved despite being touched repeatedly by crab and its anemones. Two individuals died (2.4 and 11 h of anoxia; H_2S 46.6 and 5.6 µM), one specimen survived (albeit in deployment with second shortest anoxia: 8.5 h, no H_2S).

Fusinus rostratus **(Olivi, 1792),** $N = 1$

Common predatory fasciolariid gastropod on Northern Adriatic sublittoral soft bottoms.

- Normoxia: The single detected individual well visible on sediment surface (Fig. A6a); horizontal locomotion negligible (3 %, Fig. A6c).
- < 2.0 mL DO L^{-1}: as under normoxia.
- < 1.0 mL DO L^{-1}: as under normoxia, but peak horizontal locomotion (29 %).
- < 0.5 mL DO L^{-1}: animal climbed higher on a multi-species clump; after short locomotion phase, remained stationary. Foot/head alternately extended and retracted. Ultimately fell down and less often visible (Fig. A6b).
- Anoxia: visibility drops further (to 50 %), with second peak in horizontal locomotion (17 %). In 51 % of observations on top of epifaunal aggregation; during stationary

phases, foot/head visible. After 40.6 h of anoxia (H_2S 191.6 µM), animal disappeared from view (mortality or survival not determined).

***Hexaplex trunculus* (Linnaeus, 1758), *N* = 23**

Abundant and widespread muricid gastropod in the Northern Adriatic, exhibiting multiple predation strategies on bivalve prey. One of the few species in which the experimental design itself played a role in the behavioural response: by crawling up the plexiglas walls, *Hexaplex* took advantage of the highest available place (chamber lid) to initially seek refuge; i.e. number of individuals on top is an experimental artefact. Nonetheless, results mirror the species-typical response reaction because at oxygen depletion, muricids climb atop bioherms (Stachowitsch, 1984), i.e. the avoidance reaction per se is the same.

- Normoxia: animals visible in 40 % of observations (Fig. A7a), mostly on sediment/under bioherms, i.e. do not crawl up plexiglas wall (Fig. A7b).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: first animals started to crawl up chamber wall: minimal increase in vertical movement (7 %, Fig. A7c) reflects the few but often major moves, leaving animals on chamber lid.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: increased tendency to be higher up in chamber.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: location on top of chamber peaked (71 %); the few individuals visible at the sediment surface started to protract and/or retract their foot/head (Fig. A7d).
- Anoxia: at end of deployments 16 animals not visible on lid, 7 individuals fell to sediment from lid or bioherm, i.e. increase in visibility to pre-hypoxia values (44 %); peak in foot protractions/retractions (11 and 24 %, respectively). *H. trunculus* was among the species with the highest survival rates, i.e. only two mortalities at 46.3 and 51.5 h of anoxia (117.1 and 199.9 µM H_2S, respectively). Survivors

(max. anoxia duration 81.8 h; final H_2S 167.6 µM) also included specimens which did not seek refuge at chamber top but remained on sediment surface or bioherms throughout.

Murex brandaris **(Linnaeus, 1758),** ***N*** **= 1**

Spiny dye-murex: occurs regularly on Northern Adriatic muddy bottoms.

- Normoxia: as in *Hexaplex trunculus*, visible in 43 % of observations (Fig. A8a). As in all categories, stronger horizontal than vertical movements (Fig. A8c).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: animal increasingly often visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: peak horizontal movement (69 %).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: significant decrease in horizontal movement to 9 %, but animal moved higher up on plexiglass wall (i.e. location high/top: 23 %, Fig. A8b).
- Anoxia: animal remained on plexiglass wall with no movement. Specimen survived (albeit in deployment with shortest anoxia: 5.2 h, final H_2S 5.5 µM).

Abra alba **(Wood, 1802),** ***N*** **= 2**

Infaunal, deposit-feeding bivalve common in Northern Adriatic.

- Normoxia: not visible.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: animals emerged (Fig. A9a) and made a series of short moves on the sediment (Fig. A9b). Siphons visible (35 %, Fig. A9c).

- Anoxia: animals on surface, locomotion ceased, siphon visible only in 1 individual, which was pushed aside by hermit crab (no predation/scavenging). Both individuals died (duration of anoxia 20.6 and 34.6 h, H_2S 33.3 and 244 µM).

Chlamys varia **(Linnaeus, 1758),** ***N*** **= 7**

Suspension-feeding, epifaunal bivalve, typically bysally-attached to bioherms.

- Normoxia: animals in feeding position with normal (open) shell gape and mantle tissue visible (97 and 99 %, Fig. A10a and c).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: feeding position maintained, mantle tissue increasingly swollen (Fig. A10d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: as above. One juvenile died at $0.8\,\mathrm{m\,DO\,L^{-1}}$.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: peak mantle tissue swelling (20 %), first individuals showed tissue retraction (Fig. A10e). Second individual died at DO $0.2\,\mathrm{mL\,L^{-1}}$.
- Anoxia: wide shell gape (60 %, Fig. A10b) and strongly retracted mantle tissue (64 %). Three individuals died after 14 to 31.7 h of anoxia (median H_2S 13.7 µM), two others survived 19.1 and 22.9 h of anoxia (final H_2S 19.3 and 5.2 µM, respectively).

Corbula gibba **(Olivi, 1792),** ***N*** **= 37**

Highly abundant, shallow infaunal, suspension-feeding bivalve.

- Normoxia: animals started to appear on surface at lower boundary of normoxia category (11 %, Fig. A11a) and showed peak minor and major horizontal movement (Fig. A11b; total horizontal locomotion 43 %).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: number of animals appearing at the surface increased, with significant decrease in horizontal locomotion.

- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: overall number of individuals increased, but some re-buried or moved under bioherms.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: number of animals unchanged.
- Anoxia: highest number of individuals on surface, no horizontal movement. One individual manipulated by hermit crab *Paguristes eremita* but then re-buried itself (no predation/scavenging). Another individual raised from sediment surface by emerging infaunal sea urchin (*Schizaster canaliferus*). All animals survived (max. anoxia duration 78.3 h; max. H_2S 124.5 µM).

***Venerupis* cf. *rhomboides*, *N* = 1**

Suspension-feeding, infaunal bivalve, widely distributed in the Northern Adriatic.

- Normoxia: not visible.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: First the sediment bulged, the siphon became visible (Fig. A12c) and finally the whole animal emerged (Fig. A12a). Siphon always visible, foot only occasionally, with peak horizontal locomotion (total 15 %, Fig. A12b).
- Anoxia: strong siphon vs. foot visibility points to greater energy investment in seeking more oxygenated water than in locomotion. Mortality after 24.5 h anoxia (H_2S 111.4 µM).

3.2.4 Polychaeta

Polychaeta indet. including *Glycera* sp., *N* = 17

- Normoxia: not visible.

- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: animals started to emerge (Fig. A13a), peak squirming in place (32 %, Fig. A13b).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: overall number of individuals increased, two individuals disappeared from view (DO $< 0.3\,\mathrm{mL\,L^{-1}}$) and may have reburied themselves, two others died at transition from severe hypoxia to anoxia. Peak horizontal locomotion (19 %) and squirming continued.
- Anoxia: peak in animals visible at the surface, some individuals tried to climb up plexiglass wall or along sensors, but locomotion and squirming gradually decreased. Two animals successfully re-buried, two disappeared from view (i.e. mortality or survival not determined), seven individuals died (median anoxia duration 9.4 h, H_2S ranged: 9–34.9 µM) and one survived (max. anoxia duration 25.1 h, max. final H_2S 124.5 µM). *Glycera* died 21.3 h after onset of anoxia (H_2S 32.8 µM), discolouring the immediate sediment red.

Protula tubularia **(Montagu, 1803),** ***N*** **= 8**

Solitary tubeworm in calcareous tubes on hard substrate; head bears two sets of red slightly spiralled, pinnate tentacles; no operculum.

- Normoxia: tentacle crown typically extended in filter-feeding position (Fig. A14a). Contact with mobile organisms (e.g. crabs crawling on top of tube) caused crown closure and partial or full retraction into tube.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: tentacle crowns increasingly extended (80 %), retraction into tube gradually ceased (18 %, Fig. A14b).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: crown condition unchanged.

- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: retraction sharply increased from 20 to 68 %. One individual died shortly before onset of anoxia (oxygen concentration $0.2\,\mathrm{mL\,L^{-1}}$, no H_2S).
- Anoxia: Remaining seven individuals died between 2.2 and 42.3 h of anoxia (max. H_2S 253.2 µM); 2 individuals fully re-emerged from tube (anoxia 24 h, H_2S ∼ 40 µM) before mortality (crown relaxation).

3.2.5 Decapoda

***Alpheus glaber* (Olivi, 1792), *N* = 2**

Small deposit feeding pistol shrimp inhabiting burrows in sediment. Predated mainly by fish and larger decapods, i.e. *Nephrops norvegicus*.

- Normoxia: images show only one burrow opening of the two pistol shrimps (probable male–female partners; P. Dworschak, personal communication, 2013). No individual visible at opening or elsewhere on sediment surface. Opening visibly larger shortly before onset of hypoxia ($2.4\,\mathrm{mL\,DO\,L^{-1}}$).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$ (hypoxic phase 1, before intermittent oxygen peak): opening further enlarged after 4 h of hypoxia (oxygen concentration $1.6\,\mathrm{mL\,L^{-1}}$). Shortly before onset of moderate hypoxia ($1.3\,\mathrm{mL\,DO\,L^{-1}}$), one individual partly visible (chelipeds and carapace; Fig. A15a). The shrimp appeared briefly in irregular intervals at the opening before retreating tail-first again (i.e. when disturbed by approaching hermit crab *Paguristes eremita*). During re-oxygenation (intermittent oxygen peak), no pistol shrimps visible.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$ (hypoxic phase 2, after re-oxygenation): one individual reappeared at opening.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: one individual left burrow, staying only a few body lengths away from opening. Subsequent burst in activity (i.e. constant horizontal locomotion

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀ ▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

across whole chamber area; DO $< 0.4\,\mathrm{mL\,L^{-1}}$, Fig. A15b). Fell over on its side (Fig. A15c) after briefly raising body ($0.2\,\mathrm{mL\,DO\,L^{-1}}$). Extremity movement continued for ca. 1 h, followed by mortality ($0.2\,\mathrm{mL\,DO\,L^{-1}}$). Second individual visible at $0.4\,\mathrm{mL\,DO\,L^{-1}}$, left burrow 7 h later ($0.3\,\mathrm{mL\,DO\,L^{-1}}$). Shortly thereafter it also fell on its side (but only in 1 image, i.e. < 6 min), followed by a brief locomotion phase on sediment.

- Anoxia: after ca. 30 min anoxia (H_2S 0.6 µM), second individual again fell over and died (4.5 h after specimen 1). Fragmention visible 6 h later.

Ebalia tuberosa **(Pennant, 1777),** ***N*** **= 3**

Cryptic, night-active crab, temporarily embedded in sediment, leaving anteriormost carapace tip protruding. Feeds on small invertebrates (mainly annelids and small crustaceans) but also on dead and moribund organisms.

- Normoxia: all individuals buried in sediment, i.e. not visible.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: first individual visible on sediment surface at $1.38\,\mathrm{mL\,DO\,L^{-1}}$, moving along plexiglass chamber walls.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: second individual emerged from sediment at $0.63\,\mathrm{mL\,DO\,L^{-1}}$, both crabs with strong horizontal locomotion (77 %, Fig. A16c) on sediment.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: all three individuals visible (Fig. A16a). Horizontal locomotion dropped rapidly to 42 %, small peak in vertical locomotion as crabs start to climb onto higher/top positions on bioherms (Fig. A16c), where they remained almost inactive (except for extremity movement, data not shown).
- Anoxia: total locomotion dropped to 10 % (Fig. A16b), while body movement peaked (40 %, Fig. A16d). Individuals on bioherms in 36 % of observations (Fig A16e). Two individuals survived 14.1 h and 23.6 h of anoxia and H_2S values of

12.8 and 19.2 µM, respectively; one individual died after 22.9 h of anoxia (H_2S 16.7 µM).

Ethusa mascarone **(Herbst, 1785),** ***N*** **= 3**

This crab camouflages its carapace with various objects, typically bivalve shells and living sponges, held in place with last two pairs of pereopods. Can bury itself to hide from predators, i.e. demersal fish.

- Normoxia: partly visible (i.e. including camouflage) in 79 % of observations (Fig. A17a); "moving camouflage" on sediment surface clearly indicated horizontal locomotion (19 %, Fig. A17c).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: hiding behaviour unchanged; total locomotion dropped to 13 %.
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: hiding behaviour unchanged; peak body movements (44 %, Fig A17d).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: peak total locomotion (27 %); all three individuals successively discarded camouflage (i.e. camouflage still visible in 66 % of observations; Fig. A17b). One individual predated by sea anemone *Calliactis parasticia* ($0.4\,\mathrm{mL\,DO\,L^{-1}}$, no H_2S) only 1.3 h after dropping its shell fragments (see also Sect. 3.3.2). The two other individuals, discarding their camouflage at 0.23 and $0.03\,\mathrm{mL\,DO\,L^{-1}}$, initially hid within bioherms.
- Anoxia: One individual climbed up a sensor and disappeared from view; locomotion and body movement of remaining specimen nearly ceased, with mortality 7.5 h after onset of anoxia (no H_2S).

Eurynome aspera **(Pennant, 1777),** ***N*** **= 2**

Decorator crab that attaches algae, sponges etc. onto its carapace.

- Normoxia: visible on sediment in 53 % of observations (Fig. A18a); horizontal locomotion clearly dominated over vertical locomotion (38 vs. 11 %; Fig. A18b).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: no significant change in locomotion; peak body and extremity movement (34 and 17 %, respectively; Fig. A18c and d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: locomotion largely unchanged, body and extremity movement slightly dropped.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: in 80 % of observations, individuals on high/top location (Fig. A18e), locomotion and extremity movements almost ceased, only body movements (18 %). One individual died at $0.2\,\mathrm{mL\,DO\,L^{-1}}$ (no H_2S).
- Anoxia: second individual disappeared from view 5.1 h after onset of anoxia (no H_2S; mortality or potential survival not determined).

Galathea **spp.,** ***N*** **= 3**

Small, cryptic squat lobsters in rocky subtidal and on secondary hard bottoms. Primarily night-active (hidden in small groups in interstices of bioherms during daytime).

- Normoxia: visible in 20 % of observations (Fig. A19a), otherwise hidden; peak horizontal locomotion (76 %; Fig. A19b).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: visibility increased to 50 %. Horizontal locomotion dropped to 54 %, individuals increasingly moved onto higher substrates (Fig. A19d). One individual disappeared from view after 5.6 h of hypoxia ($1.2\,\mathrm{mL\,DO\,L^{-1}}$).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: peak vertical locomotion and body movement (Fig. A19c); two remaining crabs retreated to base of bioherms.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: visibility 59 %, locomotion nearly ceased, body movement dropped to 14 %. One individual died at $0.3\,\mathrm{mL\,DO\,L^{-1}}$ (17.8 h of hypoxia),

the second individual 3 h later at transition from severe hypoxia to anoxia (0.03 mL DO L^{-1}, no H_2S).

Inachus sp., *N* = 1

Spider crab had sponges attached to its carapace and initially hid under tentacles of sea anemone *Cereus pedunculatus*.

- Normoxia: Visible in 49 % of observations, mainly on sediment surface (Fig. A20a). Horizontal locomotion prevailed over vertical (33 vs. 4 %, Fig. A20b); body and extremity movement 38 and 23 %, respectively (Fig. A20c and d).
- < 2.0 mL DO L^{-1}: visibility increased to 100 %. Total locomotion remained unchanged (37 %), but slight shift toward increasing vertical locomotion (crab moved onto base of a bioherm). Peak body movement (46 %), slight decrease in extremity movement (17 %).
- < 1.0 mL DO L^{-1}: visibility unchanged. Both horizontal and vertical locomotion dropped (total locomotion decreased to 15 %).
- < 0.5 mL DO L^{-1}: visibility unchanged. Crab retreated to top of bioherm (72 %). Body movement decreased to 30 %, extremity movement nearly ceased.
- Anoxia: sharp decrease in locomotion, body and extremity movement (visible in 84 % of observations). Moribund crab then dragged down to sediment by a dying brittle star and died shortly thereafter (12.1 h of anoxia, no H_2S).

Macropodia spp., *N* = 7

The 7 decorator crabs in this study were rarely covered (if so, then with sponges).

- Normoxia: crabs visible in 50 % of observations (Fig. A21a), mostly lower on a bioherm rather than on sediment. More horizontal than vertical locomotion (24 vs.

8 %, respectively; Fig. A21b), body movement moderate 33 % (Fig. A21c), extremity movement low (12 %).

- < 2.0 mL DO L^{-1}: sharp increase in visibility (88 %), accompanied by significant increase in locomotion (horizontal: 30 %, vertical: 24 %) and movement onto higher substrates (25 %, Fig. A21e).
- < 1.0 mL DO L^{-1}: crabs increasingly climbed to higher/top positions on bioherms (65 %) and stayed there, often in aggregation with/close to other crabs such as *Pisidia longicornis* and *Pilumnus spinifer* (see also Sect. 3.3.1 Aggregation and aggressive behaviour). Total locomotion dropped slightly (from overall 54 to 48 %), body movements peaked (35 %) while extremity movement gradually decreased.
- < 0.5 mL DO L^{-1}: significant drop in horizontal (from 31 to 7 %) and vertical locomotion (from 17 to 3 %), body and extremity movement (from 35 and 5 % to 15 and 3 %, respectively). In 69 % of observations, crabs positioned atop bioherms. Three individuals died at DO 0.4, 0.3 and 0.1 mL L^{-1} (14.9, 15.8 and 19.9 h of hypoxia, no H_2S), either falling dead to the sediment or being dragged down by moribund brittle stars and dying shortly thereafter on the sediment.
- Anoxia: locomotion, body and extremity movement of remaining 4 individuals almost ceased; all individuals died between 3.3 to 9.8 h of anoxia (H_2S recorded only once: 0.8 µM).

Nepinnotheres pinnotheres (Linnaeus, 1758), *N* = 2

Small parasitic pea crab typically inhabiting ascidians (in this study both female specimens inhabited *Microcosmus* spp.) and bivalves, feeding on the particulate food of its host as well as on other filtered organisms.

- Normoxia: not visible.
- < 2.0 mL DO L^{-1}: not visible.

- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: not visible.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: shortly before onset of anoxia, both crabs became visible (Fig. A22a) and moved (Fig. A22b) within the atrial siphon of the ascidians (Fig. A22d) but did not leave.
- Anoxia: after abandoning their hosts 13.6 and 19.4 h after anoxia onset (H_2S 18.3 and 93.9 µM, respectively), both individuals remained at a higher position (63 %) or hid for shorter periods elsewhere in bioherm. Locomotion (horizontal: 30 %, vertical: 28 %) and body movements (22 %, Fig. A22c) occurred throughout the anoxic period. Both individuals survived until the end of the respective deployment (anoxia duration: 24.6 h and 78 h, final H_2S: 126.1 and 111.6 µM).

Paguristes eremita **(Linnaeus, 1767),** ***N*** **= 25**

Abundant hermit crab species in this community. Highly mobile, day-active omnivore preferring juvenile macrofauna and typically carrying numerous symbionts.

- Normoxia: mainly on sediment or partly hidden under multi-species clumps. Shells oriented with aperture facing down, crabs only partially protruding from shell (except during intra- and interspecific interactions). More locomotion during day than at night (for details on circadian rhythm see Pretterebner et al., 2012).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: locomotion dropped from 23 to 14 % (Fig. A23b) and the day and night rhythm dampened. The crabs increasingly climbed onto bioherms (47 %, Fig. A23a). Body movement increased almost two-fold to 51 % (Fig. A23c); body posture changed (Fig. A23d), with single individuals starting to extend from shell (Fig. A23e).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: number of observations with individuals on sediment and on bioherms almost equal. Body movement peaked (70 %); extension from shell increased from 15 to 35 %. Typical shell orientation maintained (Fig. A23f).

- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: some individuals moved back down to sediment, but locomotion almost ceased (5 %). Marked drop in body movement (from 70 to 40 %). Shell orientation changed significantly, with overturned shells (aperture facing up) in 17 % of observations.
- Anoxia: extension from shell reached 40 %; 13 of 25 individuals emerged from shell (Fig. A23e) and walked around (sediment, bioherms). One specimen returned to its shell but re-emerged after 15 min and finally climbed on an exposed infaunal sea urchin *Schizaster canaliferus*. Overall, 9 crabs died (anoxia duration: 18.4 to 62.1 h, H_2S range 116.5 to 248 µM), 8 of which were outside their shell on sediment. Thirteen individuals survived (maximum anoxia duration 25.1 h; H_2S 124.5 µM), 9 of which were on sediment and 4 on bioherms. Death or survival of 3 individuals undetermined (i.e. disappeared from view, poor visibility).

Pilumnus spinifer **(H. Milne-Edwards, 1834),** ***N*** **= 17**

Territorial crab, cryptic resident in sponge-dominated bioherms, hiding during day and searching for prey at night. Feeds mainly on juvenile macroepibenthic organisms but also on small adult crabs (i.e. *Pisidia longimana*) or brittle stars.

- Normoxia: visibility typically reflects night-time activity (16 % of observations, Fig. A24a), with peaks in horizontal locomotion (48 %, Fig. A24b) and body movement (26 %, Fig. A24c). Movement mainly on sediment surface or lower on bioherms.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: visibility increased (49 %) because the normal day–night rhythm waned; horizontal locomotion declined significantly to 36 %, while vertical locomotion increased as individuals climbed higher up on bioherms (significant increase from 2 to 25 %, Fig. A24d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: visibility further rose to 79 %; significant increase in vertical locomotion reflected in peak values of individuals atop elevated substrates (51 %).

Intra- and interspecific aggression maintained, i.e. dominant individuals chased subordinate individuals or other crabs such as *Macropodia* spp. away.

- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: locomotion and body movement significantly decreased, intra- and interspecific aggression ceased and predator–prey relationships changed: *Pilumnus* no longer attacked closely adjoining prey; instead, they aggregated with other individuals, for example the crab *Pisidia longicornis* and small invertebrates atop bioherms (see also Sect. 3.3.1). Four individuals died at transition from severe hypoxia to early anoxia (no H_2S present): they turned over and fell from their elevated positions.

- Anoxia: locomotion and body movement of remaining 13 individuals almost ceased. Twelve individuals died (anoxia duration 1.4 to 23.6 h, H_2S from 0 to 72.8 µM); one individual survived brief anoxia (8.5 h; no H_2S).

Pisidia longimana (Risso, 1816), _N_ = 4

Small porcellanid crab constituting the major component of decapod community abundance in bioherms; hides in cavities during daytime; prey for bigger crustaceans (i.e. *P. spinifer*) and fish. Behavioural reactions are based on 4 larger and conspicuous individuals, mortality on 150 individuals.

- Normoxia: visible during night (21 %, Fig. A25a) and, in 44 % of observations, on high/top positions of bioherms (Fig. A25d). Initial high locomotion (total locomotion 76 %) reflects night-time activity (for details on circadian rhythm see Haselmair et al., 2010).

- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: day-night rhythm waned, i.e. visibility increased (to 37 %); crabs located in bioherm mid-height (up to 5 cm above sediment, data not shown) rather than at high/top positions.

- < 1.0 mL DO L^{-1}: daytime hiding abandoned, total locomotion dropped significantly to 40 % (horizontal: 30 %, vertical 10 %; Fig. A25b), whereas body movement peaked (30 %, Fig. A25c).
- < 0.5 mL DO L^{-1}: increasing aggregation on high/top positions, often atop vagile fauna (e.g. Fig. 4a) such as sea urchins or the holothurian *Ocnus planci*. 57 % of the individuals died at transition from severe hypoxia to early anoxia (maximum H_2S 0.7 µM), turning over or falling from bioherms to sediment.
- Anoxia: activity of remaining moribund 64 individuals ceased; all individuals dead after 11.2 h of anoxia, no significant H_2S concentration.

3.2.6 Echinodermata

***Ocnus planci* (Brandt, 1835), *N* = 4**

Suspension-feeding holothurian commonly attached to bioherms.

- Normoxia: tentacle crowns mostly upward orientated (Fig. A26c) and open (both 56 %), no tentacular movements. Otherwise, sideward/horizontal orientation and closed crown (Fig. A26b) dominated (43 and 40 %, respectively).
- < 2.0 mL DO L^{-1}: upward orientated crown positions peaked (64 %). Half-open tentacle crowns increased markedly from 5 to 18 % (Fig. A26a) and minor body elongation started, i.e. posterior body tapered and started to elongate (38 %, Fig. A26e).
- < 1.0 mL DO L^{-1}: significant drop in upward orientation, crowns predominantly half open or closed (24 and 42 %, respectively). Minor body elongation reached 61 %.
- < 0.5 mL DO L^{-1}: significant increase in major body elongation (64 %), with total elongation peaking at 85 % (Fig. A26f). Crowns predominantly side- or downward orientated (51 and 23 %, respectively).

- Anoxia: both downward orientation of tentacle crown and crown closure peaked (56 and 65 %). All individuals died: three after ca. 24 h of anoxia (H_2S range 56.7 to 91.2 µM), one individual 48.2 h after onset of anoxia. The latter started to decompose ca. 4 h after death (H_2S 199 µM): body started to inflate and become covered with vesicles/blisters.

Psammechinus microtuberculatus (Blainville, 1825), *N* = 17

Common epifaunal deposit-feeding sea urchin.

- Normoxia: Visible in 54 % of observations (Fig. A27a), the remeinder hidden under bioherms. All visible individuals with camouflage (Fig. A27b). One individual left frame of open configuration.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: peak visibility as animals move out from under bioherms. First individuals discard camouflage. One individual returned under bioherm (not visible until end of deployment).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: visibility decreased (3 individuals hid under bioherms, not visible until end of deployment).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: most animals discarded their camouflage (from 92 to 31 %), one after being climbed upon by predatory crab *Pilumnus spinifer*. Horizontal locomotion decreased. Eight individuals died (median DO $0.12\,\mathrm{mL\,L^{-1}}$).
- Anoxia: No individual was camouflaged, horizontal locomotion ceased, remaining 4 individuals died (median anoxia duration 5.9 h, max. H_2S 5.6 µM).

Schizaster canaliferus (Lamarck, 1816), *N* = 22

Infaunal, deposit-feeding echinoid present in high densities in muddy soft-bottoms of the Northern Adriatic.

- Normoxia: First sediment bulging visible when oxygen values approached $2\,\mathrm{mL\,L^{-1}}$.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: Sediment bulging increased significantly (Fig. A28a).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: Peak sediment bulging (25 %), first animals appeared on sediment surface (Fig. A28b) and peak total locomotion (Fig. A28c), whereby major movements (Fig. A28d) dominated.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: Strong increase in emerged individuals, drop in total horizontal locomotion, whereby minor movements peaked. Six *Schizaster* died (median DO $0.09\,\mathrm{mL\,L^{-1}}$).
- Anoxia: peak visibility, significant drop in locomotion. Some animals flipped over and spines began to droop. Eleven individuals died (median anoxia duration 14.3 h, median H_2S 12.9 µM), five survived (max. 13.9 h anoxia, max. final H_2S 11.8 µM).

Ophiothrix quinquemaculata (Delle Chiaje, 1828), *N* = 28

The epifaunal brittle star is one of three designating species in this benthic community. Densely aggregated on bioherms; mostly stationary with arms extending upward in typical suspension-feeding position.

- Normoxia: arms almost only in suspension-feeding habitus (91 %, Fig. A29a); moderate locomotion on bioherm substrate (Fig. A29d).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: suspension feeding significantly dropped to 28 %, individuals increasingly elevating their discs above substrate and standing on arm-tips (arm-tipping: 49 %, Fig. A29b). Both horizontal and vertical locomotion on bioherms increased; single individuals briefly moved down on sediment but then returned to bioherm.

- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: arm-tipping peaked (51 %) and, increasingly, motionless/downward-oriented arms were recorded (31 %, Fig. A26c).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: brittle stars gradually became motionless, clinging moribund to bioherm or lying on sediment; occasional arm movement. Fourteen individuals died at near anoxia (average DO $0.1\,\mathrm{mL\,L^{-1}}$), with most individuals first turning over.
- Anoxia: two individuals predated by sea anemones (*Cereus pedunculatus*), the remaining 12 died within the first 21.2 h after anoxia onset and before significant H_2S development ($> 14\,\mu\mathrm{M}$, Vaquer-Sunyer and Duarte, 2010). No fragmentation visible (see Sect. 3.3.2 for more details on predation of other *O. quinquemaculata* by sea anemones).

Ophiura spp., $N = 3$

Brittle stars shallowly buried in sediment.

- Normoxia: not visible.
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: one individual emerged after 4.1 h of hypoxia (Fig. A30a); locomotion on sediment 47 % (Fig. A30b).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: locomotion of this specimen dropped to 19 %; motionless specimen then touched and ingested by *C. parasitica* (13 h after hypoxia onset, see Sect. 3.3.2).
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: emergence of second *Ophiura* individual (after 26 h of hypoxia), locomotion 50 %.
- Anoxia: third individual emerged after 10 h of anoxia (H_2S 4 µM). Both specimens then gradually became motionless, i.e. drop in locomotion to 14 %. Occasional

arm movement visible before death (anoxia duration: 11 and 18.2 h, H_2S: 5.1 and 10.3 µM, respectively).

Amphiura chiajei (Forbes, 1843), _N_ = 10

Disc and arms of this infaunal brittle star positioned in mucus-maintained burrows, only distal ends of arms exposed.

- Normoxia: not visible.
- < 2.0 mL DO L^{-1}: not visible.
- < 1.0 mL DO L^{-1}: arms of one individual visibly extended above the sediment surface (Fig. A31a).
- < 0.5 mL DO L^{-1}: four individuals fully emerged, horizontal locomotion increased to 16 % (Fig. A31b). One specimen disappeared from view at DO 0.1 mL L^{-1} and may have re-buried itself.
- Anoxia: six more individuals emerged (peaked emergence, 37 %), horizontal locomotion increased to 27 %. After ca. 9 h of anoxia, 2 specimens disappeared from view (mortality/survival not determined); the remaining 7 individuals died by 19.1 h after onset of anoxia (max. H_2S 21 µM).

3.2.7 Ascidiacea

Microcosmus spp., _N_ = 10

Solitary ascidian, often major component of bioherms. Designating genus in this community.

- Normoxia: oral siphons mostly open (68 %, Fig. A32a).

- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: siphons remained open (65 %), but number of half-open siphon increased (20 to 28 %, Fig. A32b). Siphon extension (until end of the respective deployments). Significant increase in body contraction to 15 % (Fig. A32d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: the number of closed siphons dropped to 7 % (Fig. A32c), body contraction unchanged.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: no major change in siphon habitus, body contraction peaked at 21 %. One individual started to droop (Fig. A32e).
- Anoxia: number of open siphons significantly dropped and both half-open and closed siphon habitus increased to a combined 58 %. Contraction dropped to 10 % after anoxia, ceasing when the individuals became moribund; drooping (interpreted as mortality) increased to 6 %, i.e. two out of 10 individuals died (both specimens at 76.8 h after anoxia, H_2S 109.9 and 155.3 µM, respectively), 8 specimens survived (max. anoxia duration 78.3 h; max. final H_2S 124.5 µM).

Phallusia mammilata **(Cuvier, 1815),** $N = 9$

Solitary ascidian, often substrate for brittle stars and crustaceans.

- Normoxia: oral siphons predominantly open (86 %, Fig. A33a).
- $< 2.0\,\mathrm{mL\,DO\,L^{-1}}$: slight increase in number of half-open and closed siphons (Fig. A33b and c); body contraction increase to 15 % (Fig. A33d).
- $< 1.0\,\mathrm{mL\,DO\,L^{-1}}$: siphon closure dropped to almost zero, contractions peaked at 21 %.
- $< 0.5\,\mathrm{mL\,DO\,L^{-1}}$: open siphon habitus prevailed; contraction remained at 20 %.
- Anoxia: marked change in siphon habitus: open siphons dropped to 61 % while closed siphons increased to 36 %. Body contractions decreased. Drooping

(Fig. A33e) occurred in three individuals (= mortality) between 20.3 and 38.7 h of anoxia (H_2S range: 86.6 to 250.7 µM); six individuals survived (max. anoxia duration 24.2 h; max. final H_2S 19.3 µM).

3.3 Intra- and interspecific interactions

Numerous interactions and resulting behavioural reactions were recorded during normoxic conditions. The tube worm *Protula tubularia*, for example, retracted its tentacle crown after a hermit crab touched the tube; brittle stars interrupted their filter-feeding posture and changed position on bioherms when touched by passing hermit crabs (and occasionally by the crabs' symbiotic sea anemone *C. parasitica*), while the anemone *Cereus pedunculatus* retracted into the sediment when approached by other organisms.

With decreasing oxygen concentrations, however, "normal" behavioural interactions weakened or ceased and atypical interactions and reactions, never observed under normoxia, were documented. Most obvious was the change in decapod defensive and territorial behaviour. Atypical predator–prey interactions involving sea anemones and brittle stars also occurred.

3.3.1 Aggregation and aggressive behaviour

Because of the hypoxia-related upward migration of the redox layer towards the sediment surface and the often very steep oxygen gradients near the sediment surface, moving only a few centimetres can mean the difference between tolerable and lethal conditions. Thus, most mobile in- and epifaunal organisms sought refuge from increasingly severe bottom hypoxia by moving onto elevated, better-oxygenated substrates above the sediment surface. Macroinfauna typically emerged onto the sediment surface (e.g. the sea urchin *Schizaster canaliferus*, the bivalve *Corbula gibba*), with many polychaetes further moving to elevated positions on the multi-species clumps. Many epifaunal species climbed on adjoining multi-species clumps (e.g. hermit crabs) or higher up on the substrate they inhabited (e.g. see Fig. 4b). Cryptic epifauna, i.e. the bioherm-associated crabs *Pilumnus spinifer* or *Pisidia longimana* emerged from hiding and aggregated on top of sponges and ascidians. Other mobile benthic invertebrates positioned themselves on atypical substrates, for example on mobile organisms avoided under normal oxygen conditions (e.g. the brittle star *Ophiothrix quinquemaculata* on hermit crabs, the hermit crab *Paguristes eremita* on sea urchins). The greatest number of aggregations as well as the greatest number of involved individuals was recorded during severe hypoxia (19 % of observations, Fig. 4a).

The search for better oxygen conditions led to atypical aggregations atop the highest substrates. This reduced the safety distance between species and outweighed normal inter- and interspecific aggression. The predatory crab *P. spinifer*, for example, is solitary and highly territorial. The absence of brittle stars on a multi-species clump typically indicates a resident crab (Wurzian, 1977). During normoxia, all encounters of two *P. spinifer* or of *P. spinifer* with other crabs (i.e. *Macropodia* spp.) ended with the dominant individual chasing the subordinate one away. This behaviour ceased at severe hypoxia: *Pilumnus* specimens were observed close to each other and with heterospecifics without showing any signs of aggressiveness.

3.3.2 Predator-prey interactions

With ongoing hypoxic stress, common anti-predator strategies such as hiding in multi-species clumps, camouflage, or flight response, ceased. The shrimp *Alpheus glaber*, for example, is a common prey item for both fish and other decapod crustaceans (Rufino et al., 2006). At severe hypoxia, one *Alpheus* specimen was closely watched by the fish *Gobius niger* for 1 h from less than 5 cm distance. Were it not for the chamber's plexiglass wall, the fish would no doubt have consumed the shrimp, which was clearly unable to flee. After leaving the burrow, the shrimps also had several encounters with an already motionless *P. eremita*: for up to 12 min both decapods remained side by side, the body of *Alpheus* touching the claws of *Paguristes*. Similarly, the brittle star *O. quinquemaculata* or the porcellanid crab *P. longimana* no longer showed flight reactions but aggregated atop bioherms together with potential predators: neither *P. spinifer* nor *E. tuberosa*, for example, attacked the brittle stars. In these cases, the search for more oxygenated substrates by both predator and prey species – with similar hypoxia tolerance – apparently impeded potential predation events. In the case of dissimilar tolerances, more tolerant species benefited by taking advantage of prey that were more vulnerable.

Overall, 8 predatory interactions (plus one predatory attack) were observed during the 11 deployments. These involved two sea anemone species, *Cereus pedunculatus* and *Calliactis parasitica*, and, on prey side, 6 epifaunal brittle stars *Ophiothrix quinquemaculata*, 1 infaunal brittle star *Ophiura* spp., and 1 decorator crab *Ethusa mascarone*. Riedel et al. (2008a) describes five predatory interactions (*O. quinquemaculata*) in detail. The other feeding events recorded later in the study support those previous findings.

All predatory interactions occurred within a relatively narrow oxygen concentration window, at the transition from severe hypoxia to anoxia (H_2S concentration maximum 3.5 µM). At this time, the anemones showed a burst in activity, i.e. extension and body rotation in all directions. In contrast, the predated organisms were already in a moribund state and/or no longer showed flight reactions. *O. quinquemaculata*, for example, generally maintained a safety distance of at least 1 to 2 cm from nearby anemones under normal oxygen concentrations. If contact was made, the brittle stars immediately retracted their arms and quickly moved a few centimetres away. At the time of predation, however, most brittle stars (*O. quinquemaculata*, *Ophiura* spp.) were already moribund, either clinging to bioherms or lying on the sediment. Here, the feeding interaction involved three phases: feeding (contact, pulling in of organism, transfer to mouth and ingestion), digestion, and regurgitation of brittle star remains (the latter observed 3 times; Fig. 5a–e). The feeding process lasted 1.7 to 7.7 h; regurgitation (digestion 2.1 to 12.6 h) took maximally 60 min. Finally, one highly extended and rotating *C. pedunculatus* made contact with an emerged *Ophiura* 1.7 h after onset of anoxia (no H_2S). The brittle star was attached to the anemone's tentacle crown for 36 min before it fell on the sediment with all arms fragmented. It moved on the surface a few centimetres by "rolling" from the the aboral to the oral side; 4.1 h after the predatory attack, only weak arm movement was recognizable. The animal died 4.6 h later (H_2S 2.8 µM).

Another example for reduced responsiveness to predation risk is *Ethusa mascarone* (Fig. 5f and g). The crab, which shortly before discarded its protective camouflage, moved directly towards the widely opened tentacle crown of *C. parasitica* and was eaten (0.4 mL DO L^{-1}, no H_2S). 8.3 h later, the anemone regurgitated part of the crab; another 4.2 h later the remaining exoskeleton followed (anoxia duration 2.6 h, H_2S 0.6 µM).

3.4 Sensitivity and tolerance

While hypoxia primarily affected behaviour and the abundance of individual species, anoxia significantly reduced the number of species. The overall number of individuals dropped from initially 495 at normoxia to 362 under hypoxia and ultimately to 196, amounting to a 61 % decrease in individual abundance. The corresponding decrease on the species level was from 40 to 38 at hypoxia (loss of the two decapod species *Eurynome aspera*: 1 individual at DO 0.2 mL L^{-1}; *Galathea* sp.: 2 individuals at DO 0.3 and 0.03 mL L^{-1}; data not shown) to 24 at anoxia (overall decrease 40 %). First mortalities started at severe hypoxia: the most sensitive species was the bivalve *Chlamys varia* (2 individuals at DO 0.8 and 0.2 mL L^{-1}). Mortality then increased rapidly at < 0.3 mL DO L^{-1} and centred at the transition from severe hypoxia to early anoxia (Fig. 6). The species that began to die under hypoxia were predominantly decapods (*Ethusa mascarone*, *Macropodia* spp., *Alpheus glaber*, *Pisidia longimana*, *Pilumnus spinifer*) and specimens of the two echinoid species *Psammechinus microtuberculatus* and *Schizaster canaliferus* and the brittle star *Ophiothrix quinquemaculata*. *S. canaliferus* was the first infaunal species to emerge and die (6 out of 17 individuals during hypoxia, median DO 0.09 mL L^{-1}; 11 ind. during anoxia, see below). Peak mortality (94 individuals) was reached within 10 h of anoxia (e.g. polychaetes, mortalities ranging from 7.3 to 13.2 h duration of anoxia) and markedly dropped until 40 h of anoxia.

More tolerant species (i.e. mortalities centred after 40 h of anoxia) included *Paguristes eremita* (median duration of anoxia 42.8 h; median H_2S 135 µM), *Hexaplex trunculus* (2 individuals dying at anoxia h 46.3 and 51.5, H_2S 117.1 and 199.9 µM), *Calliactis parasitica* (median duration anoxia 52.5 h, median H_2S 63 µM) and *Microcosmus sulcatus* (2 individuals, both dying at 76.8 h of anoxia, H_2S 109.9 and 155.3 µM, respectively).

The results show a differentiated sequence and range of mortality at the higher taxon level (Fig. 6). Among the most tolerant, i.e. those dying during anoxia only, were molluscs, anthozoans, and ascidians, with certain individuals surviving up to 80 h of anoxia and a maximum H_2S concentration of about 279 µM. In contrast, survival rates were lowest in polychaetes, decapods and echinoderms. Importantly, all individuals that died during hypoxia, and most of those that died during anoxia, did so before significant hydrogen sulphide development. Thus, the average H_2S concentration across all 11 deployments within the first 10 h of anoxia was low, i.e. 9.3 µM. Accordingly, 229 of the 299 mortalities (77 %) occurred before significant sulphide development. In general, species with the highest tolerance to anoxia also tolerated high sulphide values.

Overall, 196 individuals representing 24 species (8 higher taxa: Mollusca, Anthozoa, Ascidiacea, Polychaeta, Decapoda, Echinodermata, Scaphopoda) survived. In 13 species, of which 9 are molluscs, all individuals survived. These included: the pea crab *Nepinnotheres pinnotheres*, the sea anemone *Cereus pedunculatus*, the scaphopod *Dentalium* sp., the bivalves *Corbula gibba*, *Nucula nucleus*, *Fusinus rostratus*, *Parvicardium papillosum*, *Timoclea ovata*, *Tellina serrata*, as well as the gastropods *Apporhais pes-pelecani*, *Murex brandaris* and *Nassarius* cf. *pygmaeus*. Note, however, that based on individuals, 77 % of the survivors were represented by only 2 species: the infaunal bivalve *Corbula gibba* (66 individuals) and the sea anemone *Cereus pedunculatus* (28 individuals).

Among the distinguished life habit categories (substrate relationship, mobility, feeding type), epifaunal, mobile and predator species were generally more sensitive and affected earlier (i.e. around DO $< 0.2\,\mathrm{mL\,L^{-1}}$) than their infaunal, sessile, deposit and suspension-feeding conspecifics. The overall epifauna decrease was 42 % (data not shown), with the cryptic fauna (i.e. the decapods *E. mascarone*, *Galathea* sp., *Pisidia longinmana*) being particularly sensitive to decreasing oxygen (overall decrease 60 %). In contrast, in infauna the overall decrease was 36 %, with a major drop after ∼ 10 h of anoxia (e.g. *S. canaliferus*, indet. polychaeta, *Glycera* sp., *Amphiura chiajei*, *Ophiura* spp., *Venerupis* cf. *rhomboides*, *Abra alba*). The overall decline in mobile life-forms was twice as high (43 %) as in sessile forms during late anoxia (20 %), i.e. > 40 h (mortality of 2 *M. sulcatus*, 4 *C. parasitica*, 1 *Ocnus planci*, 2 *Protula tubularia*). Finally, among the 3 major feeding types, predators (among those particularly decapods, see above) were generally most vulnerable (overall decrease 53 %), followed by more tolerant deposit- and suspension-feeding species. In the latter two, overall reduction accounted for 36 and 33 % (starting after ∼ 10 and 20 h of anoxia), with loss in echinoderm species mainly responsible for the drop (deposit feeders: *A. chiajei*, *Ophiura* spp., *P. microtuberculatus*, *S. canaliferus*; suspension feeders: *O. planci*, *O. quinquemaculata*).

4 Discussion

The responses to hypoxia/anoxia act at various levels, and in concert, calling for a holistic, ecological approach to better understand and respond to this phenomenon. The present study emphasizes two key conceptual points: benthic macrofauna behaviour as a visible, integrated tool to monitor the full range of critical oxygen thresholds in the field, and the strong potential of field studies to quantify population- and community-level endpoints. Combined, they provide a realistic basis for assessing and predicting impacts on ecological integrity.

The macrobenthos in the North Adriatic Sea reacted rapidly and successively across the entire range of declining oxygen conditions. Overall, the 11 deployments revealed a consistent and predictable pattern of reactions and phases, both within and among species (Fig. 7). Moreover, the results go beyond individual behaviours to document sequences of reactions and interactions of representative soft-bottom species. Beyond supporting and refining the reactions to hypoxia/anoxia observed elsewhere, we also report on species whose sensitivities/tolerances are poorly described (i.e. pea crabs: Stauber, 1945) or that have rarely if ever been continuously observed in situ (e.g. the infaunal shrimp *Alpheus glaber*, P. Dworschak, personal communication, 2013). Most behavioural changes and mortalities were attributable solely to oxygen depletion: H_2S had not yet developed or was present in negligible concentrations.

4.1 Faunal responses: the individual- to community-level

4.1.1 Macrofauna behaviour

The initial reactions of the infauna and epifauna differed considerably compared to behaviour under normoxia, beginning with emergence/exposure and flight/avoidance at oxygen values below 2 $\mathrm{mL\,L^{-1}}$. This clearly identifies this oxygen concentration as a critical key threshold for Northern Adriatic communities (Fig. 7). This corresponds to values reviewed in Diaz and Rosenberg (1995) and Wu (2002). It is also in line with Vaquer-Sunyer and Duarte (2008) or Gray et al. (2002), who considered the 2 $\mathrm{mg\,DO\,L^{-1}}$ threshold (equivalent to 1.4 $\mathrm{mL\,DO\,L^{-1}}$ or 63 µM; e.g. Rabalais et al., 2001) as being potentially too low because certain organisms begin to react beforehand. In this study, the onset of hypoxia triggered reactions in almost half of the species and higher taxa analysed (exceptions: nematodes, sipunculids and polychaetes). This included representatives of both the epi- and the infauna i.e. the anemone *Cereus pedunculatus* began to extend from the sediment, the buried crab *Ebalia tuberosa* emerged from hiding in the sediment and the bivalve *Corbula gibba* appeared on the sediment surface. Also, all three major feeding types were affected: the suspension-feeding brittle star *Ophiothrix quinquemaculata* began arm tipping, the deposit-feeding sea urchin *Psammechinus microtuberculatus* discarded its camouflage, and the predatory crab *Pilumnus spinifer* emerged from hiding places and climbed atop bioherms. These visible signs of stress render “internal” physiological adaptions (Hagerman, 1998) into “external” behavioural reactions. After exposure or emergence, the avoidance strategies aimed at increasing oxygen supply involved, initial peaks in locomotion and movement to higher, more oxygenated areas. This is because oxygen concentrations increase logarithmically with height above the bottom (Jørgensen, 1980; Diaz and Rosenberg, 1995). Accordingly, the infauna typically emerges onto the sediment surface (Nilsson and Rosenberg, 1994; Pihl et al., 1992; Hrs-Brenko et al., 1994), the epifauna climbs onto elevated substrates, and sessile species attempt to stretch upward (e.g. Baden et al., 1990; Rosenberg et al., 1991). On the flat sublittoral soft-bottoms of the Northern Adriatic Sea, hypoxia can affect several hundred to thousands of km^2 (Stachowitsch, 1984). Less mobile benthic invertebrates cannot escape horizontally, potentially explaining the relatively short phase of increased activity. Multi-species clumps represent the sole refuge from short-term bottom-hypoxia. A few centimetres above the sediment surface can mean the difference between tolerable and lethal conditions. Thus, the ascidian *Microcosmus sulcatus* extended its siphons, paralleling observations on *Molgula manhattensis* by Sagasti et al. (2001). The anemone *Cereus pedunculatus* combined body elongation, upward tentacle extension, body rotation, swaying and contraction, probably to increase exposure to sea water both through the body wall and coelonteron, maximize the surface area to volume ratio, minimize the diffusion distance within the tissues (Sassaman and Mangum, 1972; Shick, 1991), and helping transport oxygen back to tissues attached to the bottom (Sagasti et al., 2001). Similar behaviours were observed for *Calliactis parasitica* (present study), for *Cerianthus* sp. in the Gulf of Mexico (Rabalais et al., 2001), as well as for *Diadumine leucolena* (Sagasti et al., 2001) and *Metridium senile* (Wahl, 1984) in laboratory studies. The latter two also released their pedal disc from the substrate (*D. leucolena*: $< 1\,\mathrm{mg\,DO\,L^{-1}}$; *M. senile*: anoxia). In our study, two *C. parasitica* individuals also detached from the hermit-crab shell at anoxia. In an earlier mortality event in the Northern Adriatic, numerous anemones – including *Cerianthus* sp. – were observed on the sediment surface with exposed pedal discs (e.g. Fig. 16 in Stachowitsch, 1984).

We interpret several subsequent behaviours as strategies to decrease oxygen demand once emergence, increased locomotion or climbing upwards fail. While covering the body with shells, stones or algae provides antipredator benefits or protection from

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

desiccation and UV-radiation (e.g. Dumont et al., 2007; Hultgren et al., 2008), establishing and maintaining this camouflage can also be costly (Berke and Woodin, 2008). Thus, discarding of camouflage by sea urchins and crabs (*P. microtuberculatus* and *Ethusa mascarone*) may represent a cost-benefit trade-off in favour of losing "additional ballast" during ongoing environmental stress (Dumont et al., 2007). Similarly, the hermit crab *Paguristes eremita* emerged from their shells and moved about fully exposed. This was first observed here by Stachowitsch (1984) and is outlined in greater detail in Pretterebner et al. (2012). Such behaviour also occurs under thermal stress and desiccation (Taylor, 1981; Bertness, 1982), when the crabs are pursued (Greenaway, 2003) or after sedimentation (Shives and Dunbar, 2010).

The responses of macrofauna in one system can support interpretations of benthic health elsewhere, even across large geographical and bathymetric ranges. Thus, laboratory and field observations underline the similarity of key macrofauna responses. A case in point is arm-tipping in ophiuroids, whereby the disc and respiratory structures are raised above the sediment surface. The same posture has been observed for *Amphiura chiajei, A. filiformis*, and *Ophiura albida* in the Kattegat (Baden et al., 1990; Rosenberg et al., 1991; Vistisen and Vismann, 1997), *Ophiura texturata* in the North Sea (Dethlefsen and von Westernhagen, 1983), *Ophiothrix quinquemaculata* in the Northern Adriatic (Stachowitsch, 1984, and the present study), *Ophiura* sp. in the Lower St. Lawrence Estuary, Canada (Belley et al., 2010), and brittle stars in the Gulf of Mexico (Rabalais et al., 2001). This would be a new and intriguing extension of the early concept of "parallel level-bottom communities" (Thorson, 1957). Thus, the tiered behavioural shifts we have documented can help draw conclusions about future oxygen conditions and community status – early-, mid-, late-term perturbation and successive community/ecosystem degradation – both in the Northern Adriatic and elsewhere.

4.1.2 Intra- and interspecific interactions

Community-level changes emerge from processes operating at the individual level that trigger cascading direct and indirect effects (O'Gorman et al., 2011). Weakened or altered functionally important biological interactions, such as predator–prey dynamics, can derail community dynamics long before extensive mortalities occur (Brante and Hughes, 2001; Tallqvist, 2001; Seitz et al., 2003). Hypoxia-induced change – shifts in spatial distribution on the sediment or atop elevated substrates, abandoned defensive strategies such as decoration – adversely affect survival by increasing the vulnerability to predation. Thus, extended siphons or palps of bivalves and polychaetes above the sediment surface can be bitten off by predators (Sandberg et al., 1996). The relative tolerance of predator and prey governs predation efficiency (Breitburg et al., 1994). Nesterlode and Diaz (1998) hypothesized that relative tolerance may lead to selective predation, whereby the most sensitive prey will be exploited (Sandberg, 1994). In our experiments, the more hypoxia-tolerant sea anemones consumed moribund brittle stars (*Ophiothrix quinquemaculata*) and stressed decorator crabs *Ethusa mascarone*. We attribute this to the predators' increased activity and radius of activity (elongation and rotation in all directions), to the prey's reduced flight reaction, and to the chemical stimuli released by moribund, damaged and/or dead individuals that initiate a foraging response in most cnidarians (Elliott and Cook, 1989; McFarlane and Lawn, 1991). Chintiroglou and Koukouras (1991, 1992), who studied the diet of *Calliactis parasitica* and *Cereus pedunculatus*, never identified brittle stars (or other echinoderms) as prey items, perhaps due to high handling and assimilation time and low energy content (Thayer et al., 1973). Nonetheless, the time slot for such alternate predation is relatively narrow – at the transition from severe hypoxia to anoxia. Munari and Mistri (2011) studied the effect of short-term hypoxia on predation by the muricid gastropod *Rapana venosa* (hypoxia-tolerant) on three bivalve species with different tolerances: prey species were switched during hypoxia from the tolerant *Scapharca inaequivalvis* to the less tolerant and therefore more easily-to-catch (decreased burial depth and siphon extension) *Tapes philippinarum*.

Mutual interference and agonistic behaviour or cooperative foraging between predator species during perturbation can also alter consumption rates and prey density (Taylor and Eggleston, 2000). The present study revealed a new facet: the aggregation of potential predator-prey partners atop multi-species clumps clearly negated safety distances within and between species. Thus, oxygen deficiency outweighed normal intra- and interspecific aggression: the territory crab *Pilumnus spinifer* aggregated with con-/heterospecifics (see also Haselmair et al., 2010) and did not react to its normal prey (juvenile brittle stars or smaller crabs; Wurzian, 1977). We attribute this largely to the relatively similar hypoxia-tolerance of crustaceans and echinoderms (i.e. Sunyer-Vaquer and Duarte, 2008), represented here by *P. spinifer* and *O. quinquemaculata* (see also Riedel et al., 2012). The fact that crustaceans compensate for short-term hypoxia by increasing ventilation at the expense of other energetically demanding activities such as locomotion and feeding (Taylor et al., 1977) may also play a role. Thus, by changing behaviour, hypoxia can alter trophic pathways and derail community dynamics.

4.1.3 Sensitivity and tolerance

Beyond disrupting life cycles, altering behaviour and changing biological interactions, hypoxia significantly reduces abundance, biomass and diversity (e.g. Diaz and Rosenberg, 1995; Levin et al., 2009). Generally, fish are considered to be the most hypoxia-sensitive group, followed by crustaceans, polychaetes, echinoderms, sea anemones, molluscs, hydro-/scyphozoans and ascidians (Gray et al., 2002; Vaquer-Sunyer and Duarte, 2008). Our results broadly confirmed this pattern, with ascidians, anthozoans and molluscs being distinctly more tolerant than polychaetes, decapods, and echinoderms. Our in situ approach, however, provided a more nuanced picture by highlighting the complex intra- and interspecific variability in sensitivity, for example immediate behavioural reactions vs. survival/mortality. The generally more tolerant molluscs (metabolic depression and energy utilization; Larade and Storey, 2002) represented 13 of the 24 species with surviving individuals (see also Riedel et al., 2012). Nonetheless, some molluscs reacted immediately (e.g. the infaunal bivalve *Corbula gibba* emerging from the sediment at the onset of hypoxia), others died early (*Chlamys varia* and *Diodora* sp. at moderate and severe hypoxia), whereas others showed high survival rates (*Hexaplex trunculus*). Some species showed broader intraspecific tolerance. The hermit crab *P. eremita* tolerated between 18 and 62 h of anoxia. The infaunal sea urchin *Schizaster canaliferus* suffered 6 mortalities at hypoxia, starting at 0.3 mL DO L^{-1}, whereas 11 other individuals died within the first 24 h of anoxia. Such intraspecific ranges probably reflect different weight, size or life stage (e.g. Breitburg, 1992; Shimps et al., 2005) and ability to seek refuge atop elevated substrates. In later mortalities, H_2S development might also play a role. Such variability can provide an evolutionary advantage by buffering the population against stochastic, extreme events (e.g. Denny et al., 2011). Finally, the data also showed that high sensitivity and high tolerance are not necessarily mutually exclusive. *Corbula gibba* is a case in point: it was among the first to react to hypoxia, but by closing its valves hermetically and lowering its metabolism, it also withstood prolonged anoxia and sulphidic conditions (Hrs-Brenko, 2006 and references therein): all specimens survived our deployments.

The mortality peak at the transition from severe hypoxia to anoxia suggests that weak or short-term hypoxia (i.e. 1 to 2 days) primarily involves quantitative losses – in the sense of punctuated, species-specific mortalities – rather than fundamentally altered overall community structure and composition. Longer-lasting or more intense oxygen depletion, however, depauperizes and taxonomically and functionally homogenizes the benthic community (Sala and Knowlton, 2006). The fact that most mortalities took place before significant H_2S development allowed a clear distinction of the roles played by hypoxia and H_2S: low oxygen conditions alone are sufficient to cause quick and widespread community collapse in the Northern Adriatic. In a worst-case scenario, the macrofauna is eliminated entirely (Fedra et al., 1976; Stachowitsch, 1984; Hrs-Brenko et al., 1994). Note that while the bioherms markedly increase habitat complexity and provide refuge for mobile species, they also potentially accelerate mortalities and ecosystem collapse in a positive feedback loop (e.g. emergence and death of associated species from decomposing bioherms). This supports the conclusions drawn by Metzger et al. (2013, this issue) that the input of organic material by decaying macrofauna is partially responsible for the deteriorating biogeochemical environment. Accordingly, the death of major bioherm constituents such as sponges may also help define tipping points (Fig. 1). Such hypoxia-induced community degradation has also been observed in other biogenic habitats (e.g. oyster beds, Lenihan and Peterson, 1998): domino effects lead to widespread ecosystem degradation (Lenihan et al., 2001).

Beyond extending and complementing earlier field observations (e.g. Jørgensen, 1980; Stachowitsch, 1984; Rabalais et al., 2001), recent reviews (e.g. Levin et al., 2009) and meta-analyses (e.g. Vaquer-Sunyer and Duarte, 2008), the detailed sensitivities and tolerances can provide insight into post-disturbance community compositions. Thus, coupled with additional information (e.g. on growth rates and immigration), the data can help interpret and reconstruct the severity and timing of past oxygen depletion events based on the composition and sizes of remaining species.

4.2 Biodiversity and ecosystem functioning: the ecological perspective

Diversity loss due to oxygen depletion is determined by multiple factors ranging from sensitivity to low DO to the presence of additional stressors (e.g. Vaquer-Sunyer and Duarte, 2010, 2011). The multiple interactions and feedbacks, hidden drivers and emergent properties complicate predicting the consequences of species loss for ecosystem function. One assumption has been that high diversity maintains a high complexity of interactions and feedbacks among species, supporting stability and resistance to disturbance (Cardinale et al., 2012). In the case of bioherms and anoxia, however, multi-tiered complexity may actually accelerate community collapse (see above). Moreover, most key ecosystem processes are performed by particular species (Solan et al., 2004; Hector and Bagchi, 2007). Even rare species can perform key functions and increase the level of functional diversity (Mouillot et al. (2013), sustaining ecosystem processes, Tilman et al., 1997; Mouillot et al., 2011). Clearly, ecosystem performance depends on both the quantity and quality of biodiversity (Thrush et al., 2006).

Oxygen depletion triggered a significant drop in both individuals (“quantitative” impact on function, see below) and species (“qualititative” impact). The unique functional roles of some species (Ellison et al., 2005) become evident when examining major life habit categories (substrate relationship, mobility and feeding type). In the Northern Adriatic, suspension feeding can be used in a process-orientated interpretation of ecosystem status. Particular species typically filter a certain range of particle sizes (e.g. Ward and Shumway, 2004). When some individuals of that species are missing, other individuals continue to feed on that particle size (quantitative loss of function). When, however, the species itself is lost, then that particle size range may no longer be filtered (qualitative loss). The relatively early loss of hypoxia-sensitive sponges and brittle stars (*O. quinquemaculata*), but survival of the more tolerant ascidian *Microcosmus sulcatus*, all filtering distinct particle sizes (Gili and Coma, 1998), probably represents a qualitative shift in filter-feeding capacity. Moreover, the loss of two key suspension feeding components at the hypoxia/anoxia transition may markedly compromise the overall regulatory role of the filter-feeding compartment (natural eutrophication control; Officer et al., 1982). Loss of the stabilizing role of the ORM community – the benthos converts pelagic into benthic biomass and serves as a storage compartment (Ott and Fedra, 1977) – no doubt significantly impacts long-term and large-scale ecosystem functioning.

Importantly the impact on ecosystem integrity begins much earlier, long before such functionally important components are missing entirely (e.g. Thrush and Dayton, 2002). Here, behaviour is a sentinel. From the suspension-feeding perspective, the change from *O. quinquemaculata*'s the feeding posture to arm-tipping at mild hypoxia affects benthic-pelagic coupling (Graf, 1992) long before the animals die. From the bioturbation perspective, the upward movement (i.e. sediment bulging, Fig. 3) and emergence of the infaunal sea urchin *Schizaster canaliferus* means that particle reworking and burrow ventilation (Schinner et al., 1993; Kristensen et al., 2012) cease long before mortality.

In the Northern Adriatic, the complex bioherms create distinct “biodiversity hotspots” that provide substrate for larval settlement and epigrowth, shelter and food on the structurally less complex soft-bottom surfaces. The rapid mortality of their long-lived and slow-growing component species, such as larger sponges, stands in stark contrast to the slow recovery. The anoxic event in September 1983, for example, destroyed 50 % of the epifaunal biomass in only two days, and over 90 % within four days (Stachowitsch, 1984). The subsequent recolonization process was very slow, with both total biomass and the relative contribution of key species (*O. quinquemaculata*, *Microcosmus* spp., the sponge *Reniera* sp.) remaining distinctly below previously recorded values for years (Stachowitsch, 1991). Additionally, benthic recovery was (and presently continues to be) further impaired by harmful fishing activities and renewed oxygen depletions (e.g. Stachowitsch and Fuchs, 1995; Kollmann and Stachowitsch, 2001). The overall result is marked longer-term community degradation and decreased habitat complexity, offering new niches for opportunistic colonizers and non-indigenous species (e.g. Jewett et al., 2005; Dumont et al., 2011).

5 Conclusions

Coastal oxygen depletion raises many questions, among them how these events can be detected early, how their spatial extents, durations and frequencies can be monitored, and how the effects ameliorated. This requires knowing the state of the benthic community and the ecological impacts at multiple, organism to ecosystem levels.

As an integrative parameter, behavioural reactions and mortality help define critical oxygen thresholds and determine the immediate ecological status. The knowledge about sensitive (“losers”; Roberts and Brink, 2010) and more tolerant species (surviving post-disturbance “winners”) can help to pinpoint and define the spatial extension of past mortalities. Finally, the roles individual species play, help better gauge potential effects on ecosystem integrity, function and resilience. Our integrated approach is applicable across communities, habitats and biogeographical zones, and could also provide input into paleoecological interpretations. It also provides a useful tool to transport complex ecological processes to the public and decision-makers to define specific monitoring, assessment and conservation plans.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

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Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

Table A1. Summary of the 11 deployments. No: number of deployment; ww: wet weight; –: no data.

No	Date	Duration deployment (h)		Duration (h)		H_2S(μM)	pH	Temp.	Biomass (total)
		open (frame)	closed (chamber)	hypoxia	anoxia	average last h	final	°C	g ww 0.25 m^{-2}
2	17–22 Sep 2005	–	132.8	20.1	81.8	167.6	–	18.5	436.7
3	24–27 Sep 2005	–	69.4	46.1	5.2	5.5	–	17.8	682.1
4	27 Sep–1 Oct 2005	–	101.6	41.6	28.8	36.8	–	17.6	604.7
6	5–8 Aug 2006	22.4	48.3	12.5	22.9	5.2	7.9	18.8	–
7	17–21 Sep 2006	20.9	72.1	33.8	28.7	124.2	7.7	19.7	839.8
8	21–24 Sep 2006	21.7	41.9	22.7	8.5	0.0	–	20.4	526.3
9	25–29 Sep 2006	21.9	73	40.5	19.1	19.3	7.8	20.6	648.9
10	29 Sep–2 Oct 2006	22.7	40.3	16.7	13.9	11.8	7.9	21.4	629.2
11	5–10 Oct 2006	23.6	95.4	11.4	78.3	106.7	7.5	21.3	724.0
12	10–14 Oct 2006	25.4	75.2	33.6	24.2	18.2	7.8	21.3	631.6
13	17–21 Oct 2006	–	94.6	52.9	25.1	124.5	7.8	20.4	1042.7

Table A2. Macrobenthic species (32 species and 2 species groups) recorded. Group abbreviation: Ant: Anthozoa, Nem: Nemertini, Sip: Sipunculida, Gas: Gastropoda, Biv: Bivalvia, Pol: Polychaeta, Dec: Decapoda, Hol: Holothuroidea, Ech: Echinoidea, Oph: Ophiuroidea, Asc: Ascidiacea. Life habit: epi: epifauna, in: infauna, cry: cryptic fauna; SF: suspension feeder, P: predator, D: deposit feeder. Behaviour subcategories: [1] visible, non-visible; [2] horizontal, vertical (both minor, major), no locomotion; [3] open, half open, closed; [4] up-, side-, downward orientated; [5] bottom, middle (< 5 cm above sediment), high/top (> 5 cm); [6a] column; [6b] tentacles and oral disc: original, discoloured; [7] normal, "puckered"; [8] normal, protruded; [9] intact, peeled off; [10] minor, major; [11] overturned/moribund; [12] retracted, extended; [13] upright, overturned; [14] closed, half open, normal, widely gaping; [15] normal, swollen, retracted; [16] extended, partially retracted, fully retracted; [17] inside burrow, outside (on sediment); [18] normal, discarded; [19] normal, extended, out of shell; [20] suspension feeding, arm tipping, arms down/moribund; – not evaluated/applicable.

Table A2. Continued.

Group	Species	*N*	Life habits	Visibility[1]	Locomotion[2]	Movement	Body posture	Location[5]	Interaction	Species-specific responses
Ant	*Calliactis parasitica*	6	epi, ses, SF	+	–	rotation, detachment	contraction, crown habitus[3] and orientation[4]	–	+	discolouration[6a], mouth[7], pharynx[8], acontia[1], periderm[9]
Ant	*Cereus pedunculatus*	17	epi, ses, SF	–	–	rotation	contraction, crown habitus[3], retraction, extension[10]	–	+	discolouration[6b], mouth[7], pharynx[8]
Nem	*Tubulanus* sp.	1	in, mob, P	+	+	squirm	–	–	–	–
Sip	indet. species	4	in, mob, D	+	+	squirm	–	–	–	–
Gas	*Diodora* sp.	4	epi, mob, P	+	+	–	orientation[11], foot habitus[12]	–	–	–
Gas	*Fusinus rostratus*	1	epi, mob, P	+	+	–	foot habitus[12]	+	–	–
Gas	*Hexaplex trunculus*	23	epi, mob, P	+	+	turn, shell orientation[13]	foot habitus[12]	+	–	–
Gas	*Murex brandaris*	1	epi, mob, P	+	+	turn	–	+	–	–
Biv	*Abra alba*	2	in, mob, D	+	+	–	siphon[1]	–	–	–
Biv	*Chlamys varia*	7	epi, mob, SF	–	+	–	valve gape[14]	–	+	mantle tissue[15]
Biv	*Corbula gibba*	37	in, mob, SF	+	+	–	–	–	–	–
Biv	*Venerupis* cf. *rhomboides*	1	in, mob, SF	+	+	–	foot habitus[12], siphon[1]	–	–	–
Pol	indet. species	17	in, mob, D/P	+	+	squirm	–	–	–	–
Pol	*Glycera* sp.	1	in, mob, P	+	+	squirm	–	–	–	–
Pol	*Protula tubularia*	8	epi, ses, SF	–	–	–	crown extension[16]	–	+	–

Table A2. Continued.

Group	Species	*N*	Life habits	Visibility[1]	Locomotion[2]	Movement	Body posture	Location[5]	Interaction	Species-specific responses
Dec	*Alpheus glaber*	2	epi, mob, D	+	+	body and extremity, turn	orientation[11]	+[17]	+	–
Dec	*Ebalia tuberosa*	3	epi, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Ethusa mascarone*	3	epi/cryp, mob, P	+	+	body and extremity, turn	–	+	+	camouflage[18]
Dec	*Eurynome aspera*	2	epi, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Galathea* spp.	3	epi/cryp, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Inachus* sp.	1	epi, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Macropodia* spp.	7	epi, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Nepinnotheres pinnotheres*	2	epi/cryp, mob, P	+	+	body and extremity, turn	–	+	+	host abandonment
Dec	*Paguristes eremita*	25	epi, mob, P	+	+	body, turn	posture[19]	+	+	shell orientation[13]
Dec	*Pilumnus spinifer*	17	epi/cryp, mob, P	+	+	body and extremity, turn	–	+	+	–
Dec	*Pisidia longimana*	4	epi/cryp, mob, P	+	+	body and extremity, turn	–	+	+	–
Hol	*Ocnus planci*	4	epi, mob, SF	–	+	–	contraction, elongation[10], crown habitus[3]and orientation[4]	–	+	–
Ech	*Psammechinusmicrotuberculatus*	17	epi, mob, D	+	+	spine movement	–	–	–	camouflage
Ech	*Schizaster canaliferus*	22	in, mob, D	+	+	spine movement	orientation[11]	–	–	sediment bulging
Oph	*Ophiothrix quinquemaculata*	28	epi, mob, SF	+	+	–	body habitus[20]	+	+	fragmentation
Oph	*Ophiura* spp.	3	inf, mob, D	+	+	–	–	–	+	fragmentation
Oph	*Amphiura chiajei*	10	inf, mob, D	+	+	–	–	–	+	armtips[1], fragmentation
Asc	*Microcosmus* spp.	10	epi, ses, SF	–	–	–	siphon habitus[3]and elongation, contraction, drooping	–	+	–
Asc	*Phallusia mammilata*	9	epi, ses, SF	–	–	–	siphon habitus[3], contraction, drooping	–	+	–

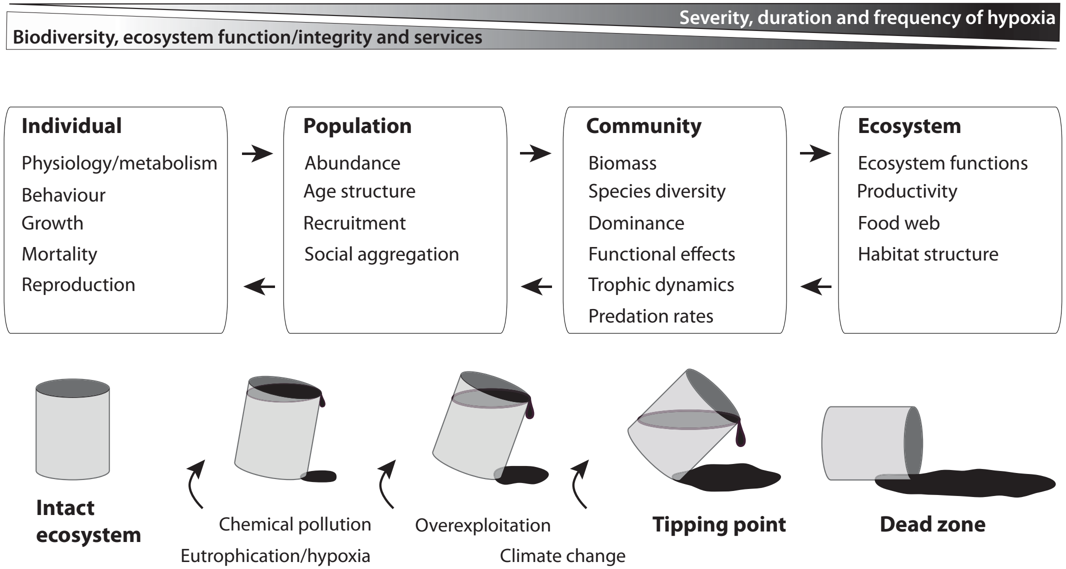

Fig. 1. Conceptual link between hypoxia and the multi-level effects in marine ecosystems. Hypoxia induces individual responses, which in turn affect population attributes, community dynamics and ultimately biodiversity and ecosystem integrity. The interactive and cumulative effects of multiple human stressors increasingly force the system to a critical tipping point and qualitatively altered state.

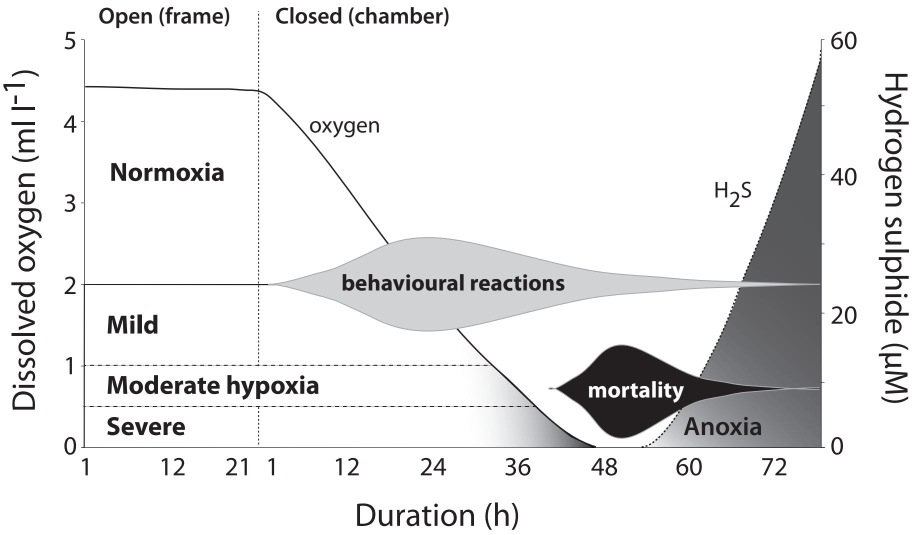

Fig. 2. Schematic course of all 11 deployments (oxygen, H_2S, behaviour, mortality) based on averaged values in Table A1. Hypoxia threshold 2 mL dissolved oxygen [DO]L^{-1}; dashed lines (1 and 0.5 mL DOL^{-1}) separate different stages of hypoxia.

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀I
▶I
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

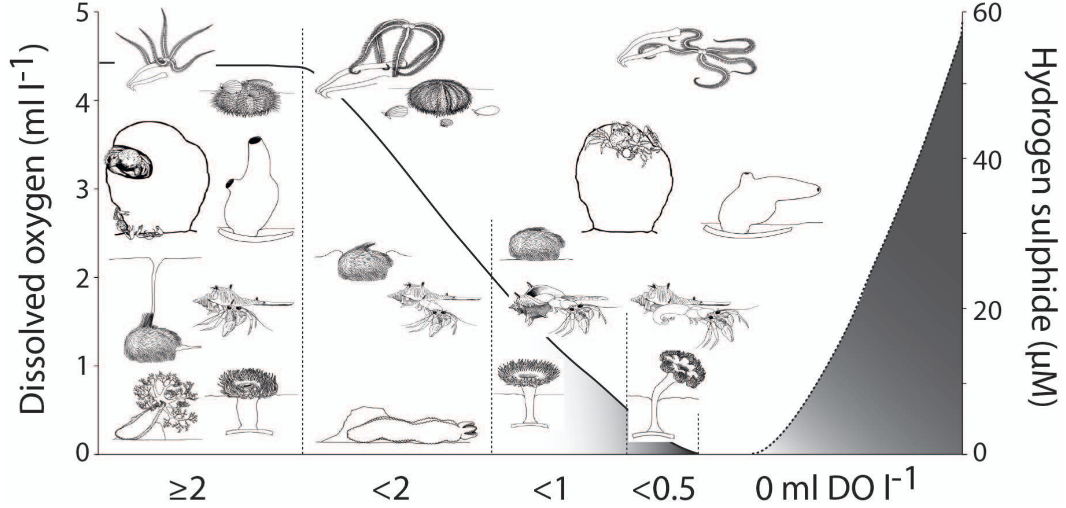

Fig. 3. Behavioural reactions of key species across oxygen concentrations/thresholds.

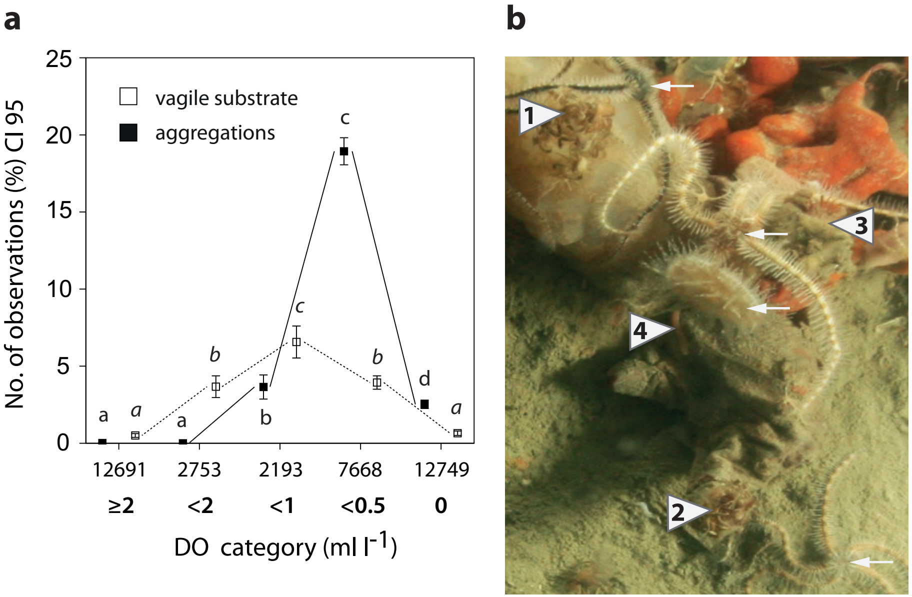

Fig. 4. (a) Mean percentage of observations of crustacean aggregations (black squares) and of investigated specimens atypically using vagile species as substrates (open squares) in the 5 oxygen categories. Numbers below x-axes: number of photographs evaluated per oxygen category. Same letters above error bars: no significant difference between means. **(b)** Atypical aggregations during severe hypoxia ($0.2\,\mathrm{mL\,DO\,L^{-1}}$; no H_2S): Numerous *Pisidia longimana* aggregate on the ascidians *Phallusia mammilata* (1) and *Microcosmus* spp. (2). Stressed brittle stars (*Ophiothrix quinquemaculata*) cling to the substrate (top arrow), one individual already moribund on sediment surface in lower right corner (bottom arrow). The hermit crab *Paguristes eremita* (3) with symbiotic sea anemone *Calliactis parasitica* (third arrow from top) on shell, on a sponge. Note *O. quinquemaculata* (second arrow from top) using the crab's shell as elevated substrate. Freshly emerged polychaete on ascidian (4).

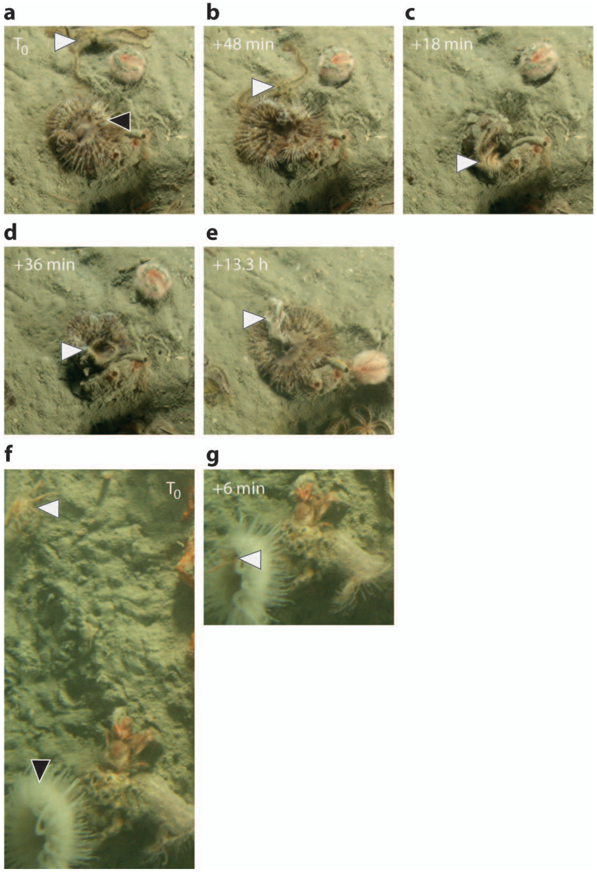

Fig. 5. Predation by *Cereus pedunculatus* on *Ophiothrix quinquemaculata* **(a–e)** and *Calliactis parasitica* on *Ethusa mascarone* **(f–g)**. **(a)** Moribund brittle star (white arrow) on sediment, *Cereus* (black arrow) with elevated tentacle crown, minor extension. **(b)** Anemone catches arm brittle star arm. **(c)** Brittle star pulled onto tentacle crown. **(d)** Prey ingested. **(e)** Regurgitation of remains; note still moving infaunal sea urchin *Schizaster canaliferus*. **(f)** *Ethusa* (white arrow), with recently discarded decoration, moving towards widely opened tentacle crown (black arrow). **(g)** *Calliactis* ingests crab, legs still protruding from mouth.

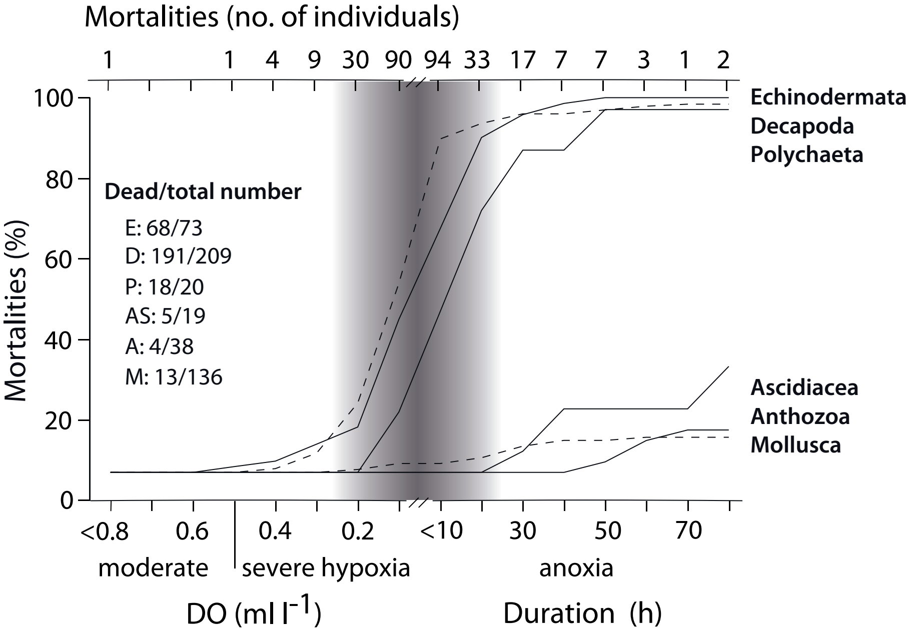

Fig. 6. Number of individuals dying ($N = 299$) across hypoxia (mL dissolved oxygen [DO]L^{-1}) and anoxia duration **(h)** and mortality curves of higher taxa (%) in all deployments. Rapid increase, peak and decrease in mortalities at transition from severe hypoxia to anoxia highlighted. Elongated vertical line on first x-axis separates moderate and severe hypoxia.

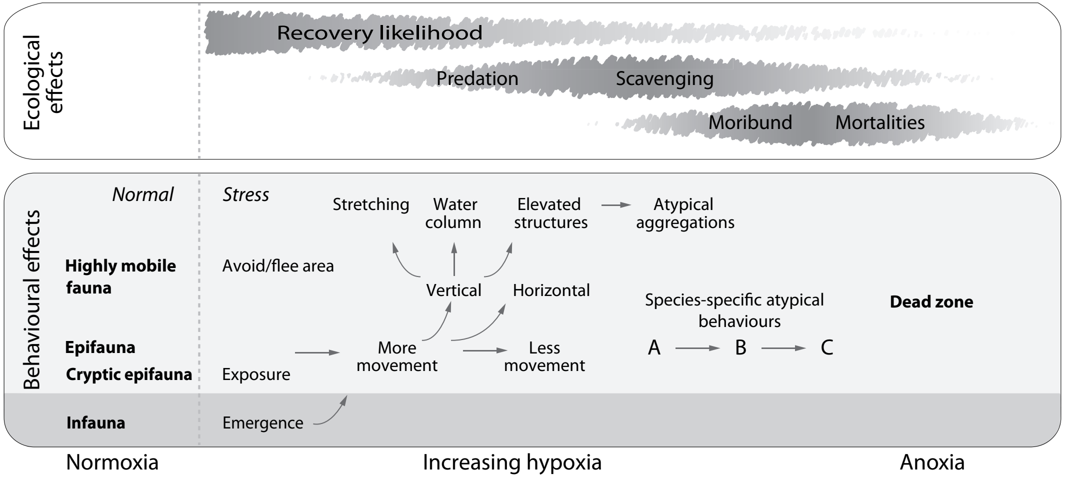

Fig. 7. Synthesis of behavioural reactions and accompanying ecological effects. See text for examples of each response category.

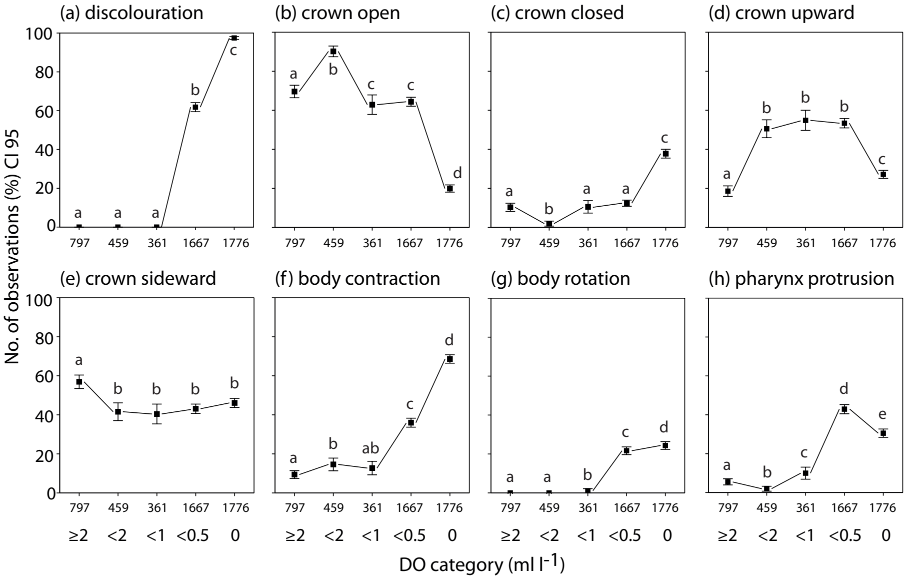

Fig. A1. A1–A33. Species-specific changes in behaviour in the 5 oxygen categories. Numbers below x-axes: number of photographs evaluated per dissolved oxygen [DO] category. Analyses are by Mann–Whitney *U* tests ($p < 0.05$). Same letters above error bars indicate no significant difference between means of the respective oxygen categories. The proportion of subcategory behaviours do not necessarily total 100 % because not all subcategories are depicted. *Calliactis parasitica* ($N = 6$).

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

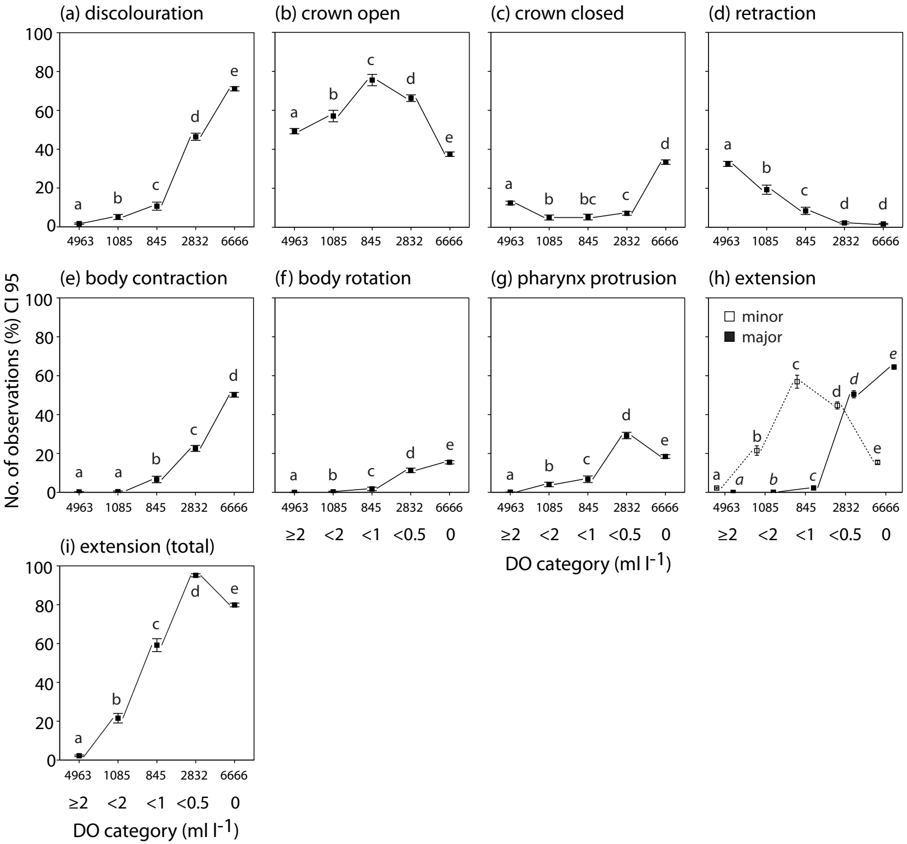

Fig. A2. *Cereus pedunculatus* (*N* = 17).

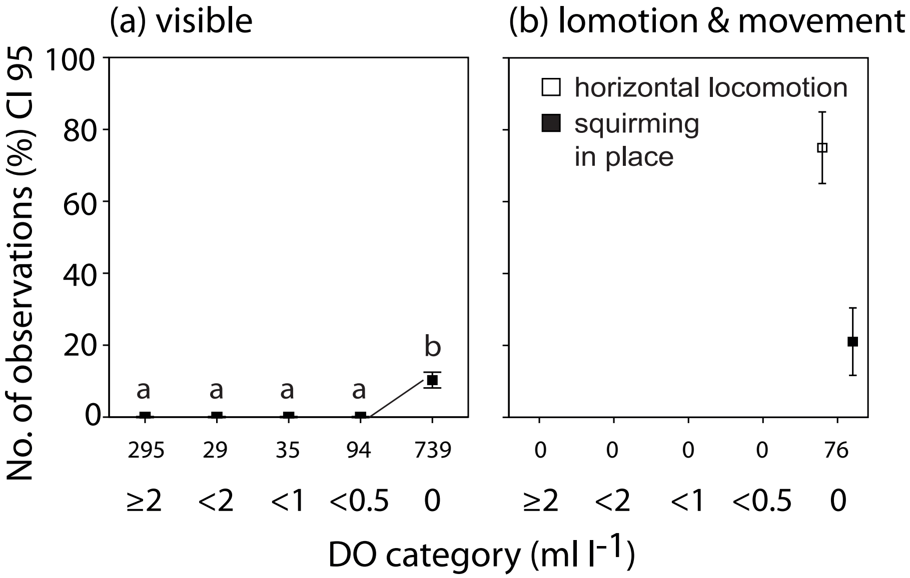

Fig. A3. *Tubulanus* sp. ($N = 1$).

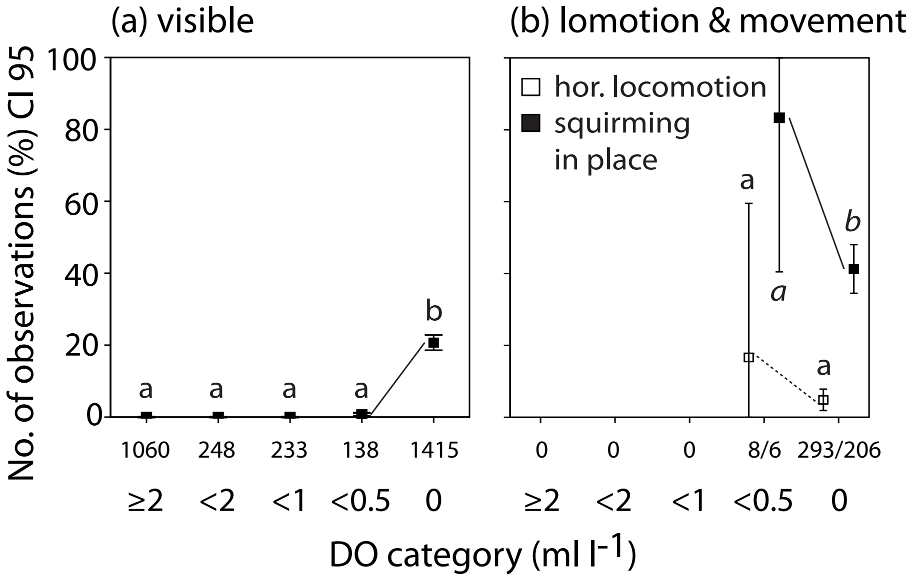

Fig. A4. Indet. Sipunculida ($N = 4$).

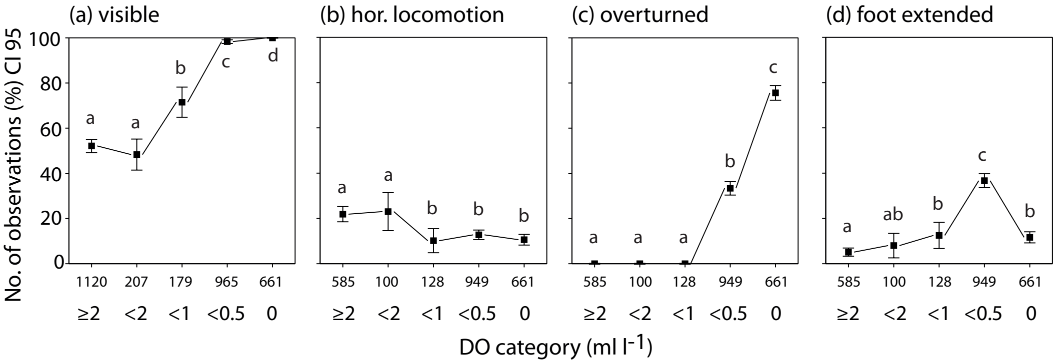

Fig. A5. *Diodora* sp. ($N = 4$).

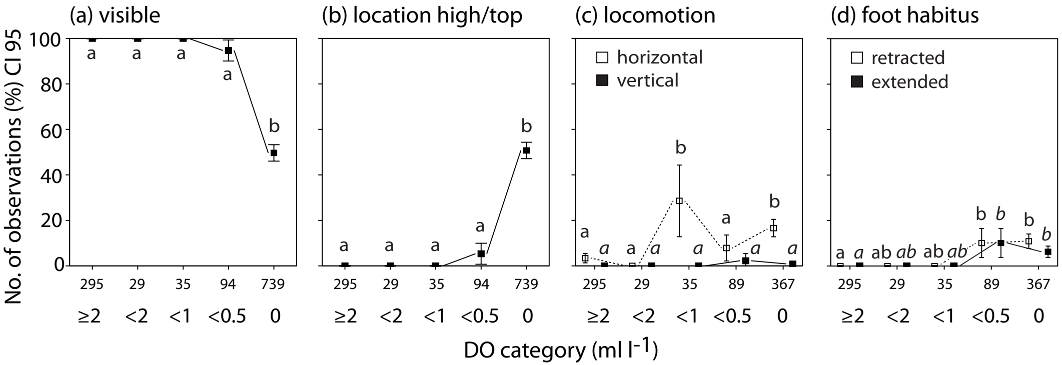

Fig. A6. *Fusinus rostratus* (*N* = 1).

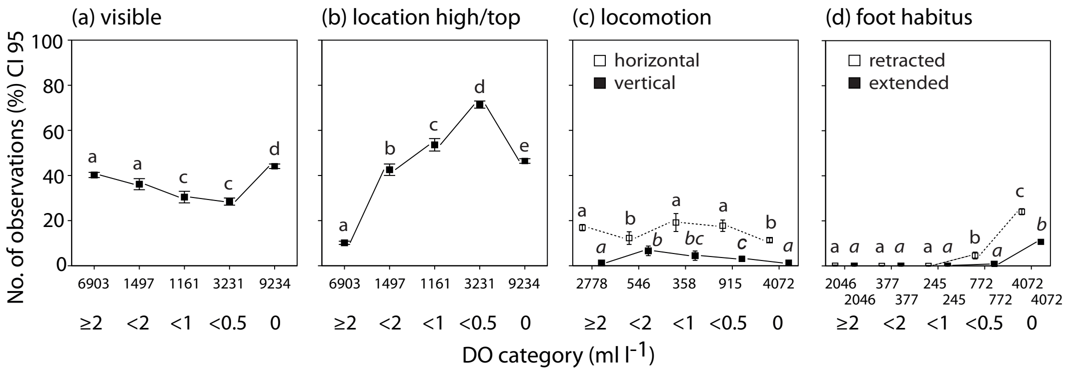

Fig. A7. *Hexaplex trunculus* (*N* = 23).

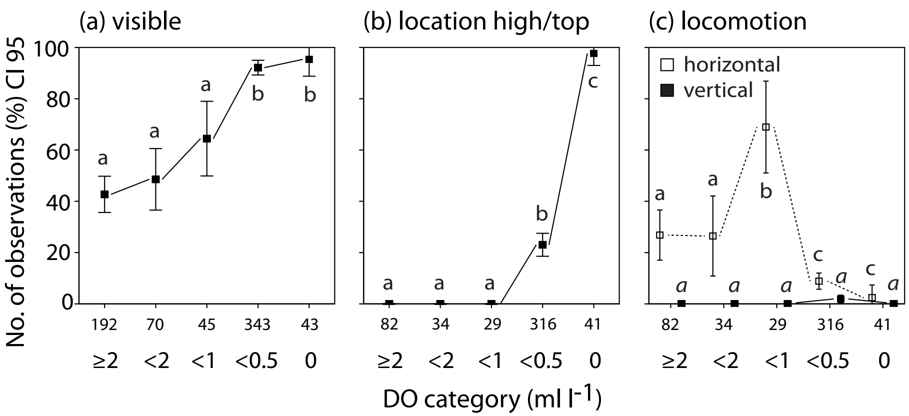

Fig. A8. *Murex brandaris* (*N* = 1).

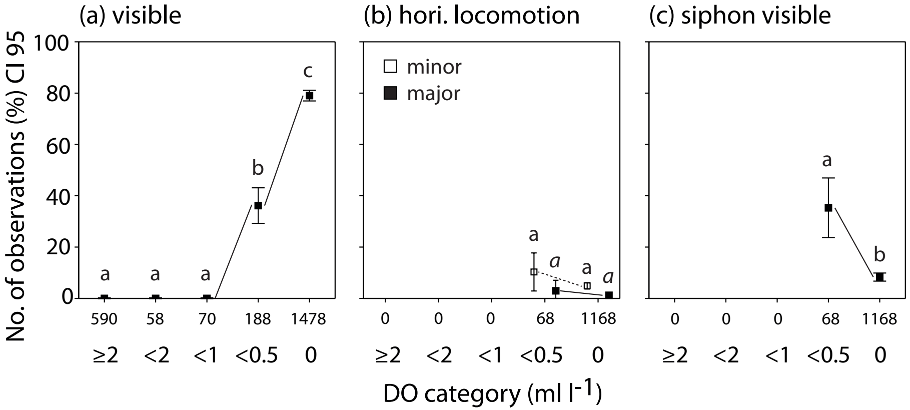

Fig. A9. *Abra alba* (*N* = 2).

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀|
|▶
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

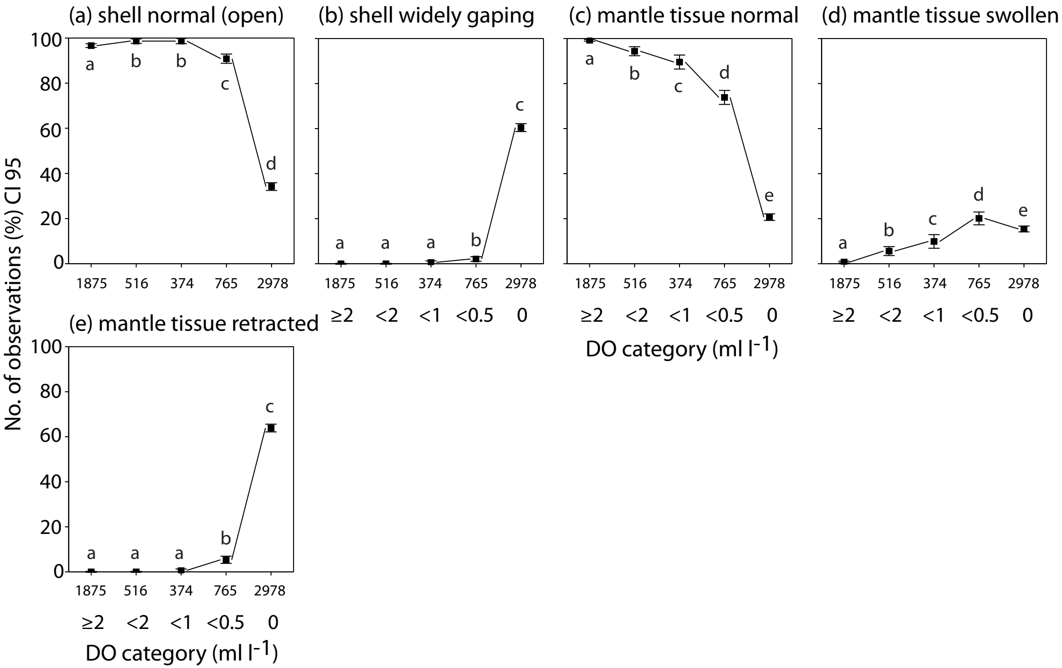

Fig. A10. *Chlamys varia* ($N = 7$).

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀|
|▶
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

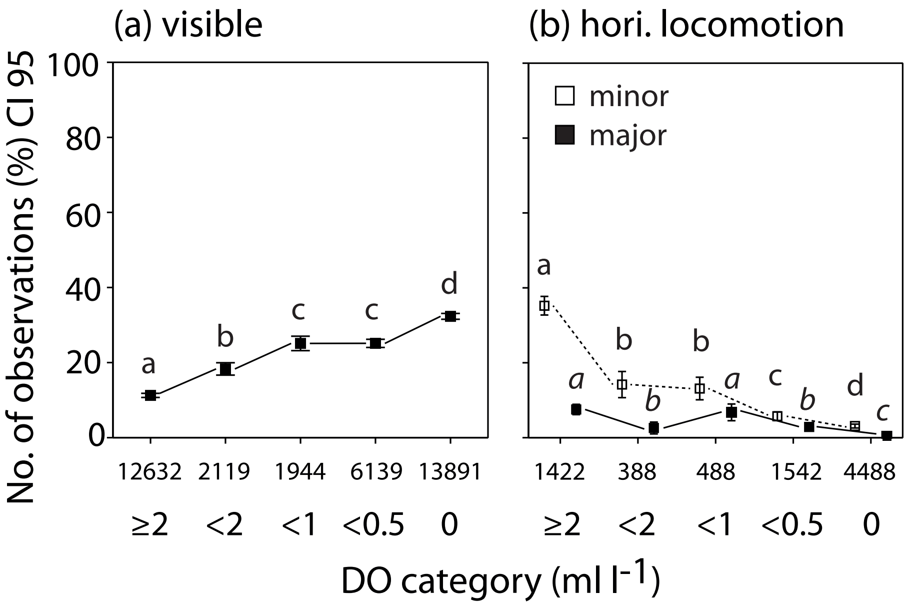

Fig. A11. *Corbula gibba* ($N = 37$).

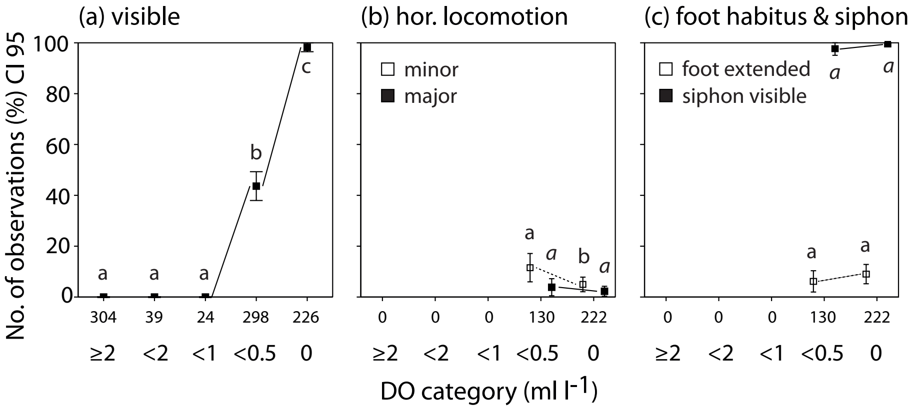

Fig. A12. *Venerupis* cf. *rhomboides* (*N* = 1).

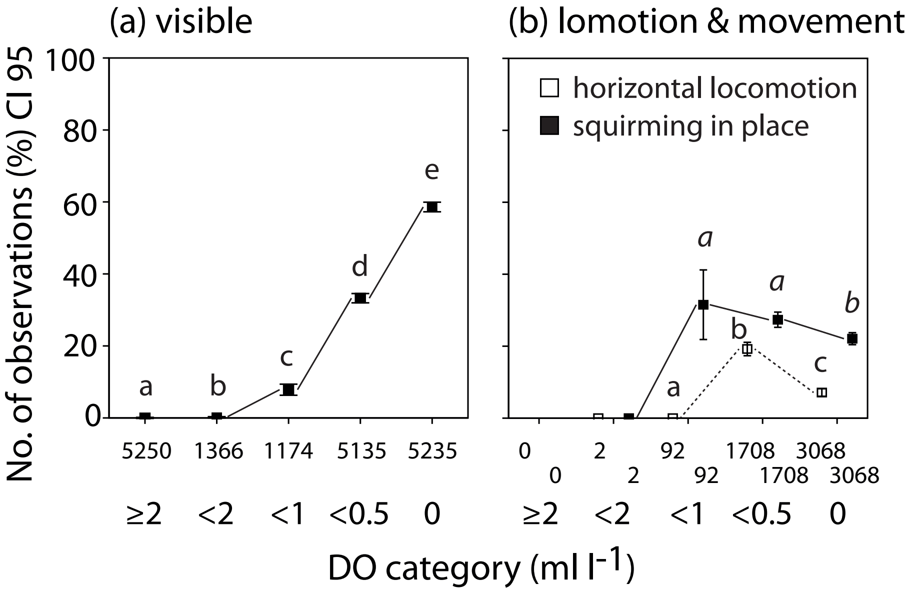

Fig. A13. Indet. Polychaeta ($N = 17$) and *Glycera* sp. ($N = 1$).

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀ ▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

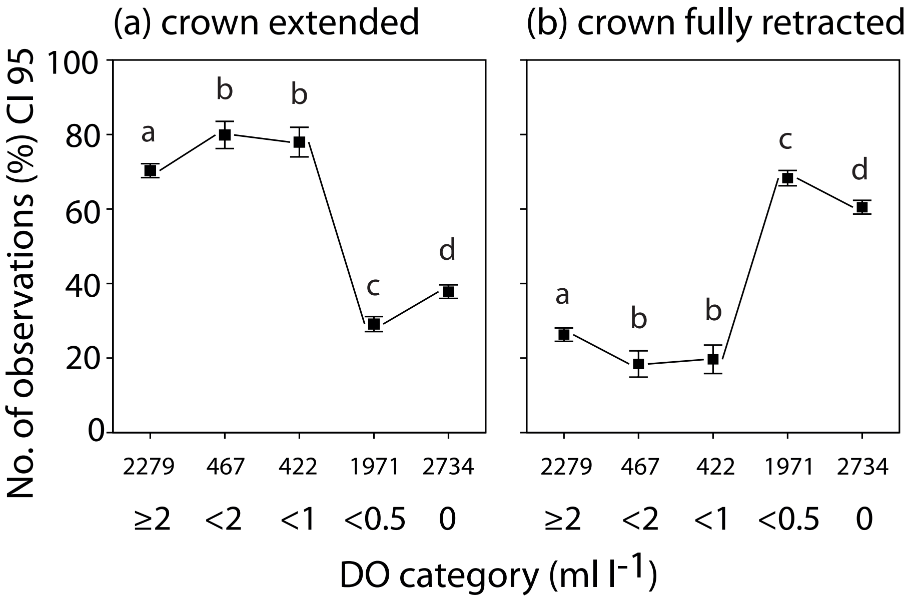

Fig. A14. *Protula tubularia* (*N* = 8).

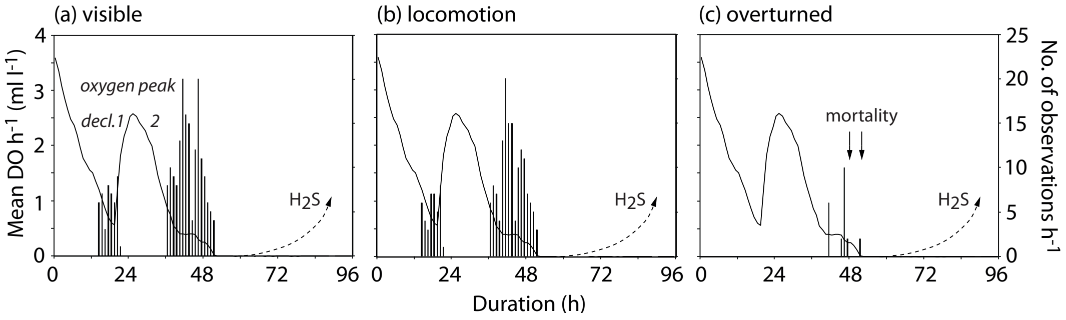

Fig. A15. *Alpheus glaber* (*N* = 2).

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

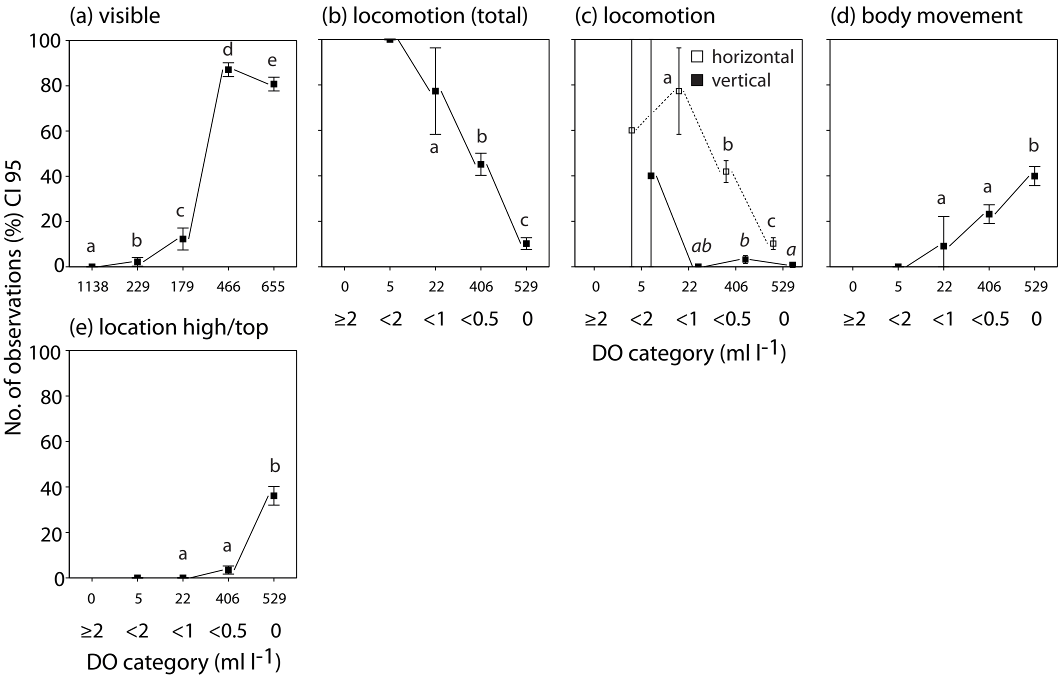

Fig. A16. *Ebalia tuberosa* (*N* = 3).

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

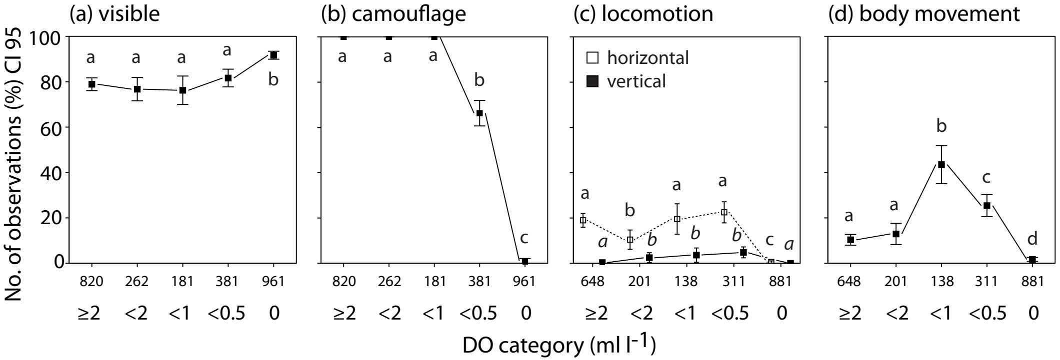

Fig. A17. *Ethusa mascarone* ($N = 3$).

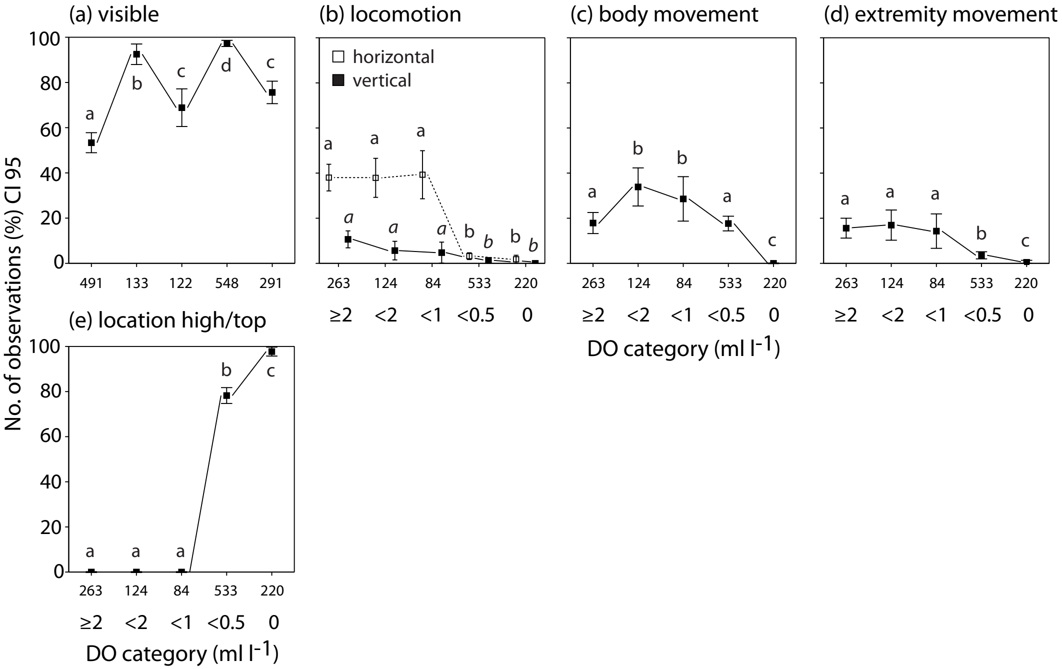

Fig. A18. *Eurynome aspera* ($N = 2$).

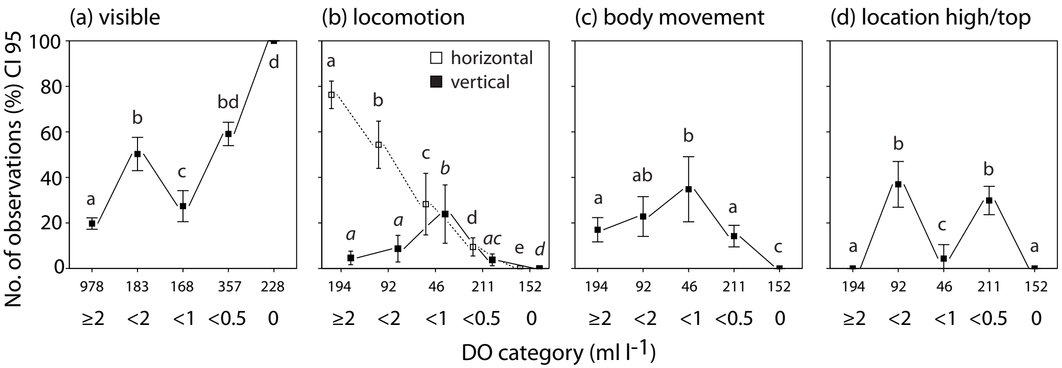

Fig. A19. *Galathea* spp. ($N = 3$).

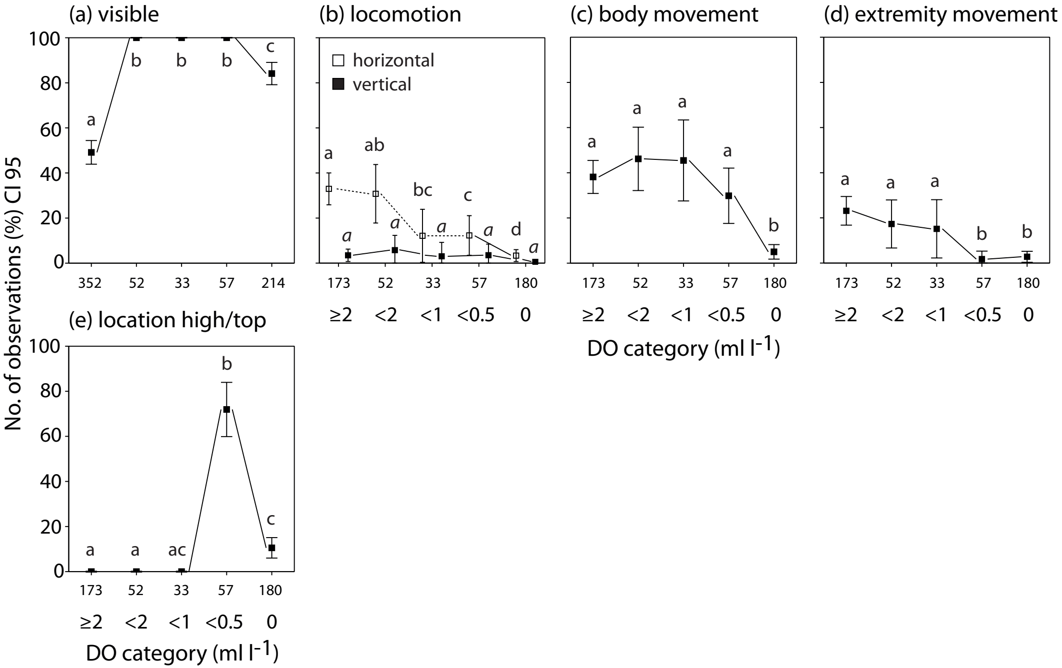

Fig. A20. *Inachus* sp. ($N = 1$).

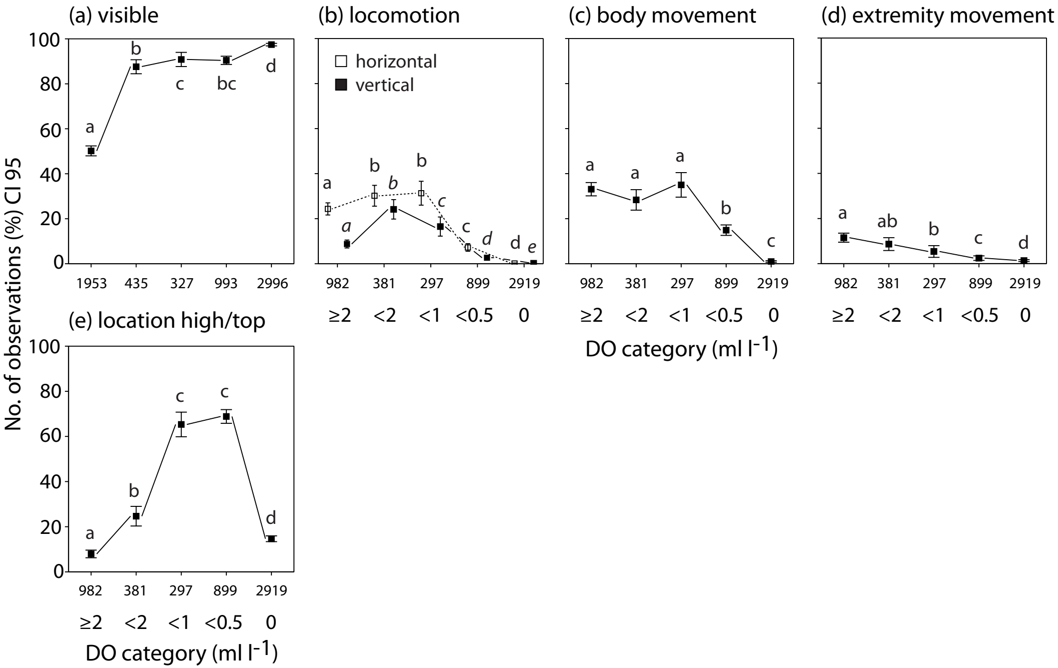

Fig. A21. *Macropodia* spp. (*N* = 7).

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀◀ ▶▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

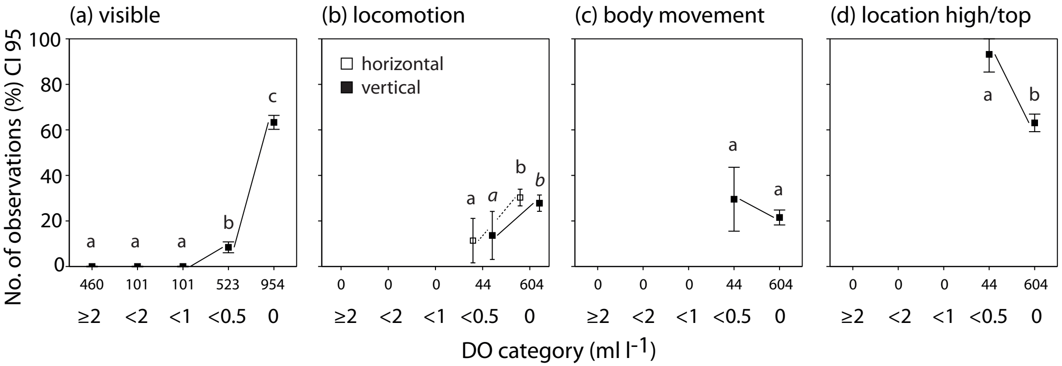

Fig. A22. *Nepinnotheres pinnotheres* ($N = 2$).

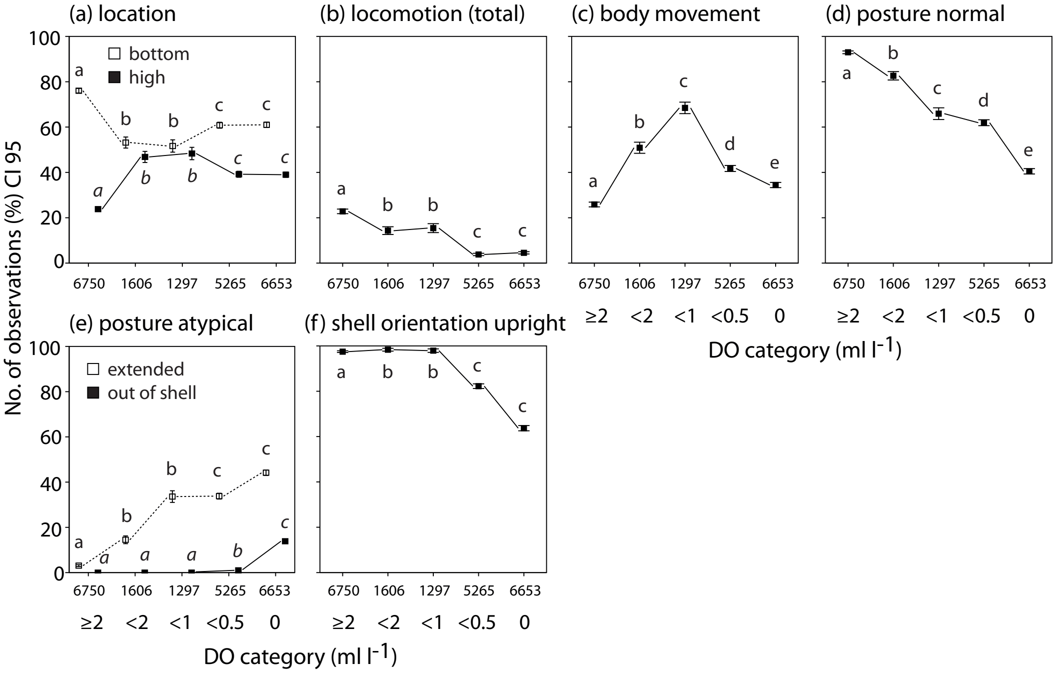

Fig. A23. *Paguristes eremita* (*N* = 25).

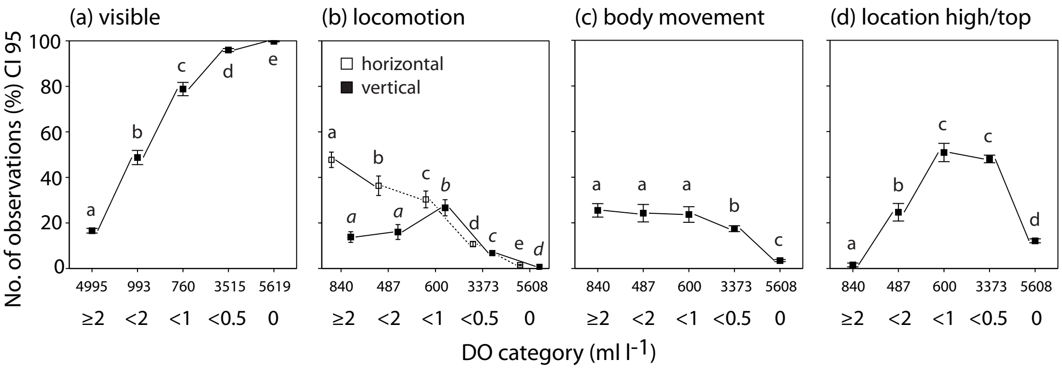

Fig. A24. *Pilumnus spinifer* (*N* = 17).

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◀ ▶
◀ ▶
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

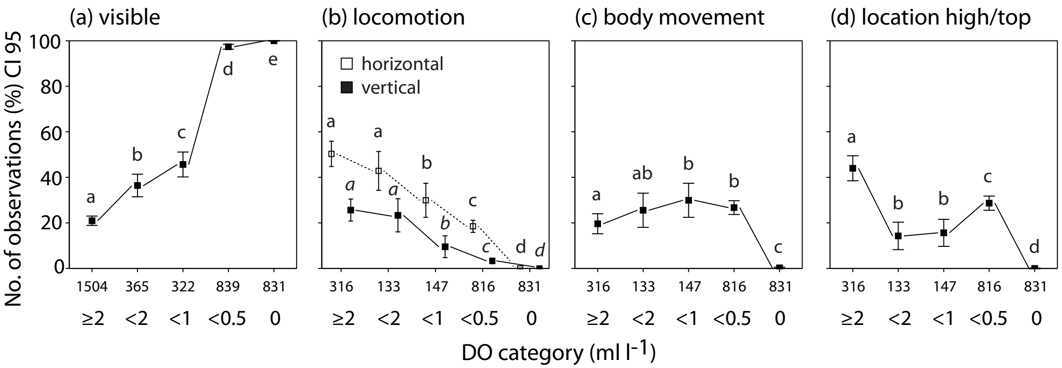

Fig. A25. *Pisidia longimana* ($N = 4$).

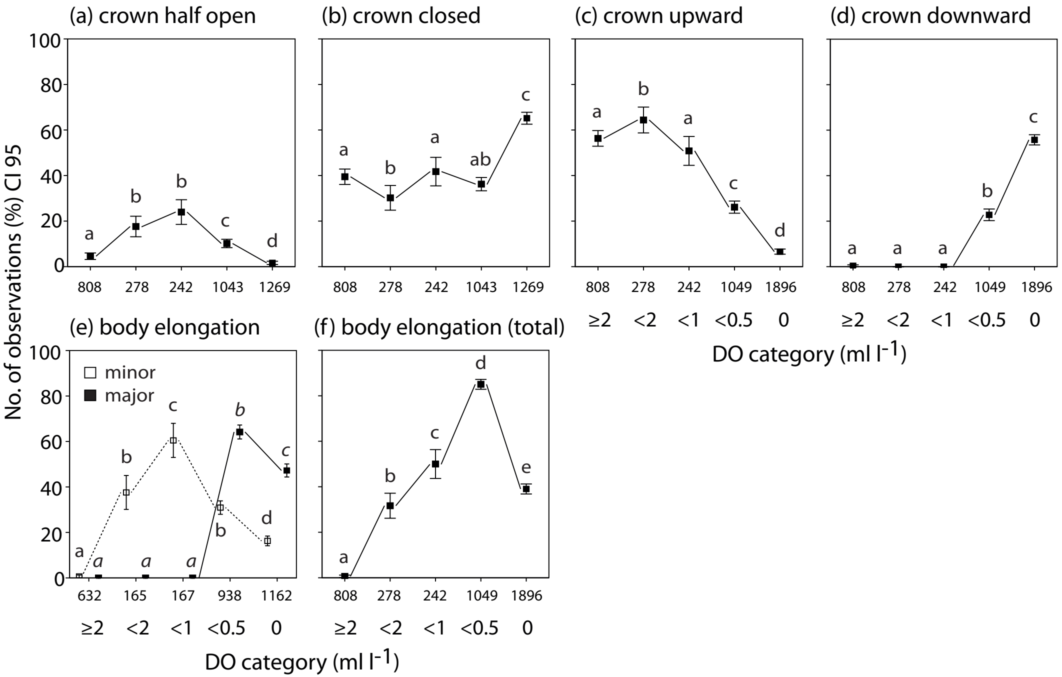

Fig. A26. *Ocnus planci* (*N* = 4).

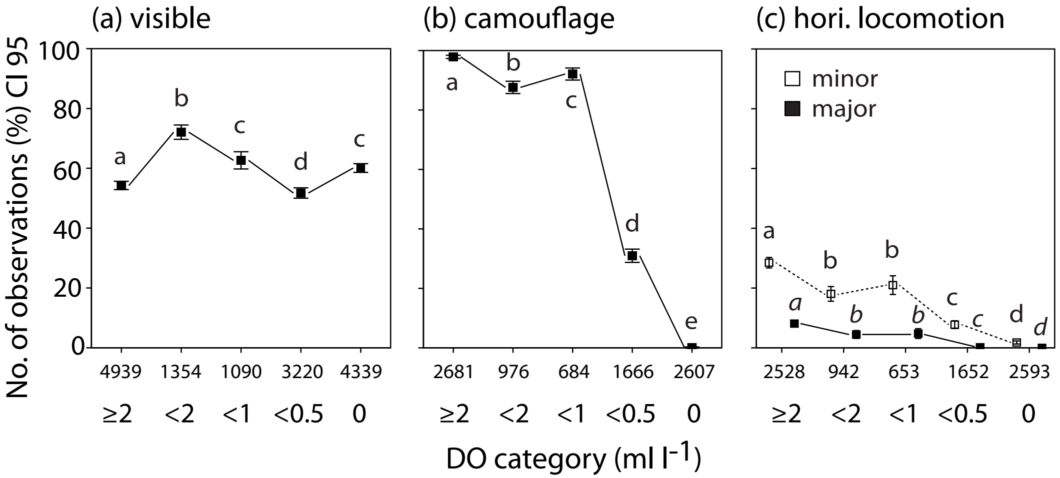

Fig. A27. *Psammechinus microtuberculatus* (*N* = 17).

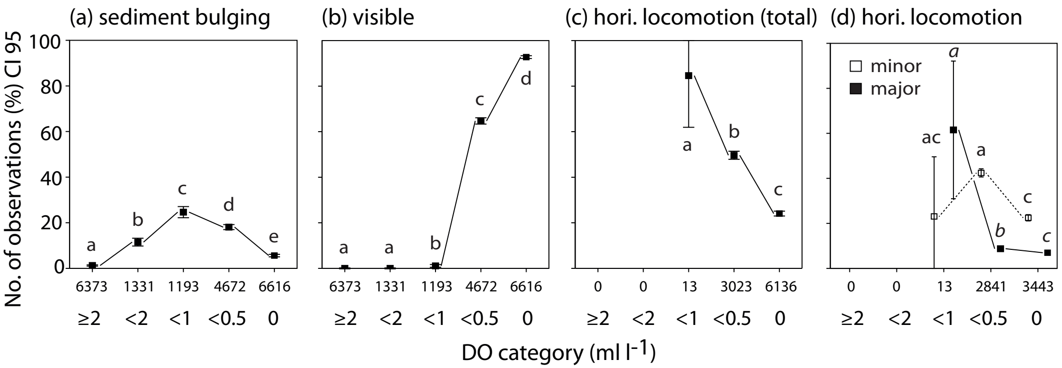

Fig. A28. *Schizaster canaliferus* (*N* = 22).

Title Page
Abstract
Introduction
Conclusions
References
Tables
Figures
◀◀
▶▶
◀
▶
Back
Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

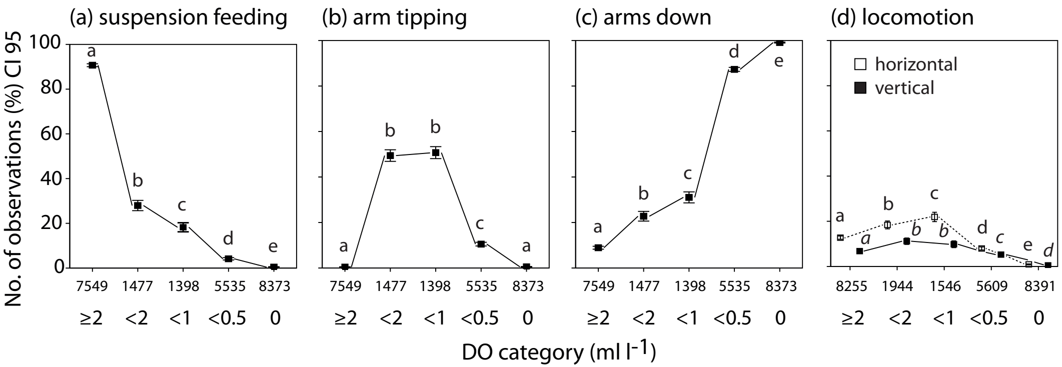

Fig. A29. *Ophiothrix quinquemaculata* (*N* = 28).

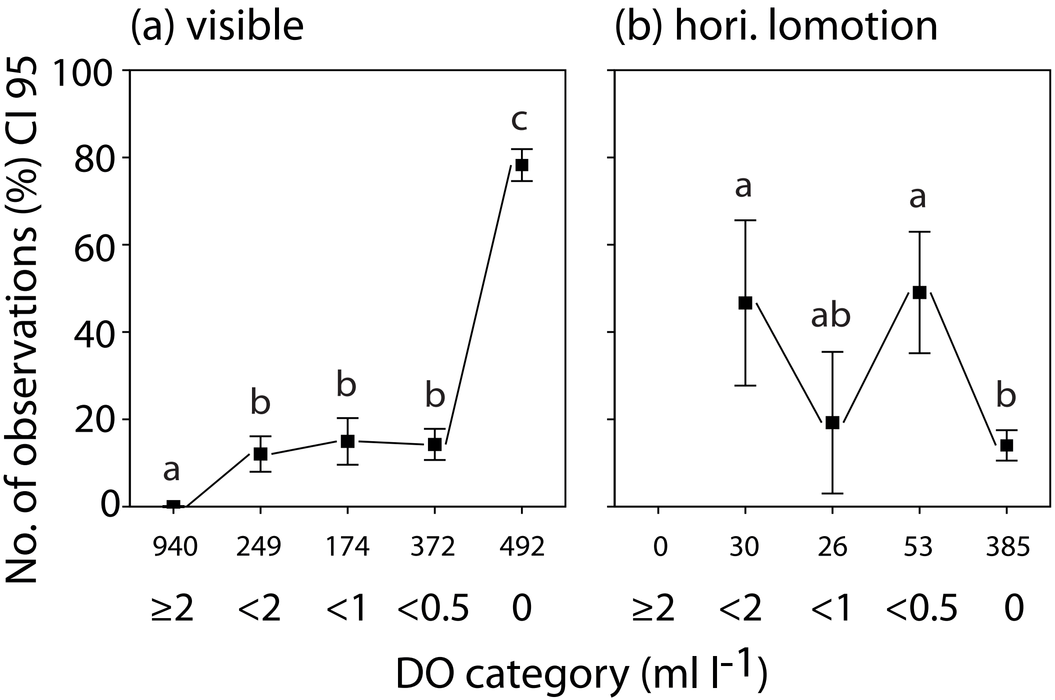

Fig. A30. *Ophiura* spp. ($N = 3$).

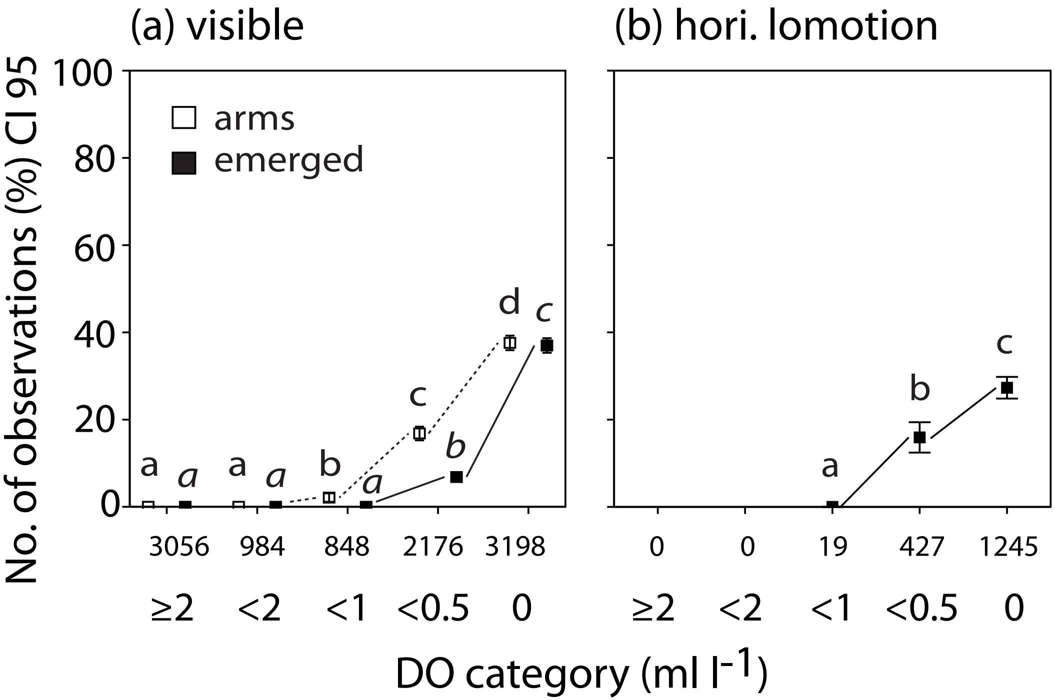

Fig. A31. *Amphiura chiajei* ($N = 10$).

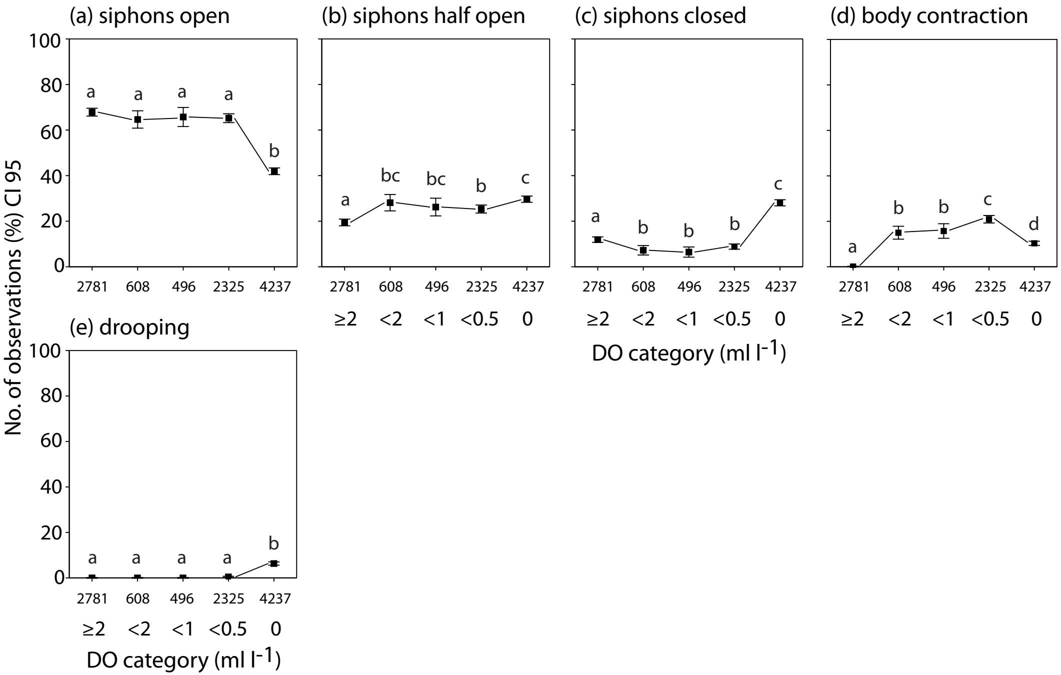

Fig. A32. *Microcosmus* spp. ($N = 10$).

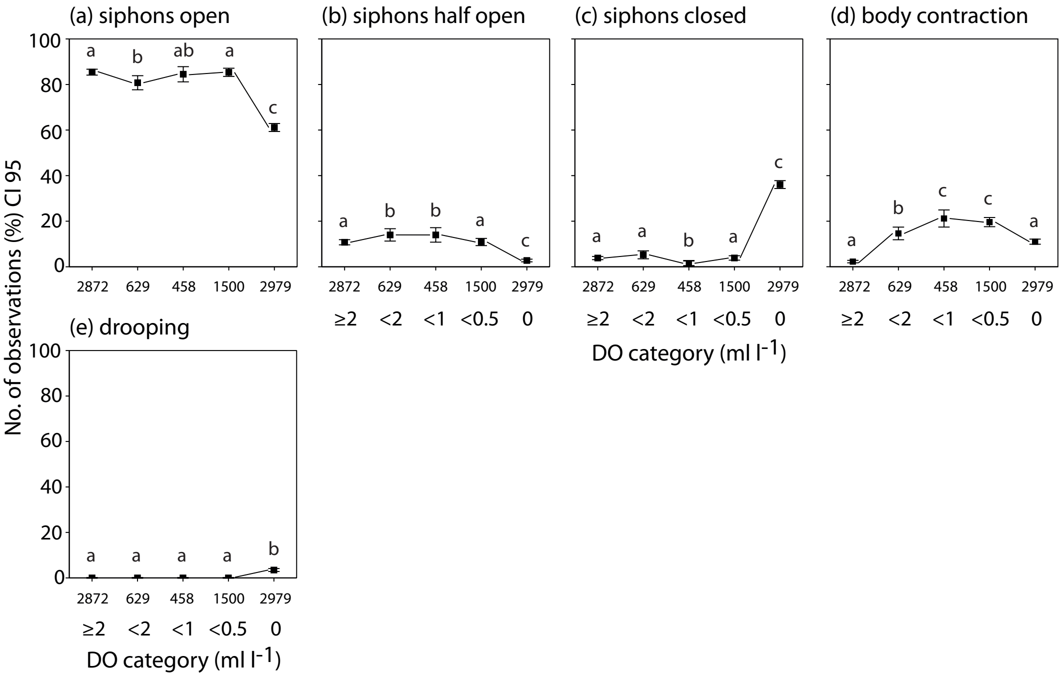

Fig. A33. *Phallusia mammilata* ($N = 9$).