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Oxygen crises in the North Adriatic: Effect on the structure and behaviour of the macroepibenthos

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“It's a magical world, Hobbes ol' buddy.

Let's go exploring!”

[Calvin and Hobbes]



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Zusammenfassung

Seichte Küstengewässer zählen durch anthropogene Eingriffe wie Überfischung, Eutrophierung oder Verbauung zu den meist gefährdetsten marinen Systemen [1] und könnten, sollte sich der Trend auch in Zukunft fortsetzen, einen massiven Biodiversitätsverlust erfahren [2]. Keine Umweltvariable von ökologischer Bedeutung hat sich während der letzten Jahrzehnte weltweit dramatischer verändert, als gelöster Sauerstoff [3]. "Todeszonen" am Meeresboden, verursacht durch Hypoxie ($<2.0 \text{ ml O}_2 \text{ l}^{-1}$) und Anoxie (kein Sauerstoff), stehen an der Spitze aufkommender Umweltprobleme [4], und könnten sich in den kommenden Jahren zunehmend verschärfen [5].

Die Nordadria, eines der anthropogen am stärksten beeinträchtigten Gebiete des Mittelmeeres [6, 7], weist aufgrund sommerlicher Stratifikation der Wassersäule eine lange Geschichte saisonaler Hypoxie/Anoxie auf [8]. Vermehrte Nährstoffzufuhr während der letzten Jahrzehnte hat auch hier die Häufigkeit und Schwere von Sauerstoffkrisen deutlich erhöht.

Dennoch, obwohl Vorkommen und Ausdehnung von hypoxischen/anoxischen Gebieten weltweit zunehmen, bleiben Voraussagen über deren Beginn und Auftreten schwierig und gezielte Feldforschung somit nahezu unmöglich. Unsere Arbeitsgruppe löst diese Aufgabe durch den Einsatz eines Unterwasserinstrumentes, welches kleinflächig Sauerstoffkrisen im Feld erzeugt und aufzeichnet. Mit Hilfe dieses Gerätes können komplexe Prozesse und Interaktionen innerhalb der benthischen Lebensgemeinschaft umfassend im natürlichen Lebensraum dokumentiert werden. Dazu zählen unter anderem Verhaltenreaktionen, intra- und interspezifische Interaktionen sowie Mortalitätsabfolgen, welche in Bezug zu bestimmten Sauerstoffgrenzwerten gesetzt werden.

Die Doktorarbeit umfasst vier Veröffentlichungen:

B. Riedel *et al.*, *CIESM Workshop Monographs no•35* (in Druck) gibt einen Überblick über Sauerstoffkrisen in der Nordadria und mögliche Auswirkungen vom Art- bis zum Ökosystemniveau. Potentielle Verbindungen zwischen Klimawandel und Eutrophierung werden diskutiert [9].

M. Stachowitsch *et al.*, *Limnol. Oceanogr.: Methods* **5**, 344 (2007) geht speziell auf das technische Design unseres Unterwassergerätes (EAGU; Experimental Anoxia Generating Unit) und dessen Handhabung im Feld ein [10].

B. Riedel *et al.*, *J. Exp. Mar. Biol. Ecol.* **367**, 17 (2008) beschreibt ein Experiment, in dem ein zwischenzeitlicher Sauerstoffanstieg durch unvorhergesehenen Wasseraustausch zu einer "2-Phasen-Hypoxie" führt. Alle initiierten Verhaltenreaktionen wurden während dieses Sauerstoffanstieges unterbrochen und erst bei hypoxischen Bedingungen wieder aufgenommen, womit die direkte Beziehung zwischen Sauerstoffwert und Verhalten demonstriert wird [11].

B. Riedel *et al.*, *Mar. Biol.* **153**, 1075 (2008) beschreibt zuvor nie beobachtete räuberische Beziehungen zwischen den Seeanemonen *Cereus pedunculatus* und *Calliactis parasitica* und dem Schlangensterne *Ophiothrix quinquemaculata* innerhalb eines knappen, fast-anoxischen Zeitfensters. Unsere Beobachtungen deuten darauf hin, dass Anoxie-tolerante Anemonen gegenüber empfindlicheren Ophiuriden einen Vorteil aus solchen kritischen Situation ziehen können [12].

Summary

In the marine environment, shallow coastal seas are the most endangered systems [1]. Through a series of impacts ranging from overfishing, eutrophication to coastal development, they are likely to experience the largest change in biodiversity should present trends in human activity continue [2]. No other crucial environmental variable has changed more drastically in shallow coastal marine ecosystems worldwide than dissolved oxygen (DO) [3]. So-called dead zones, extensive mortalities caused by hypoxia ($\text{DO} < 2.0 \text{ ml l}^{-1}$) and anoxia (no oxygen) in bottom-water layers, top the list of emerging environmental challenges [4], and the problem is likely to escalate in the coming years [5].

The Northern Adriatic Sea, the most impacted system of the entire Mediterranean [6, 7], has a long history of seasonal hypoxia and anoxia due to water column stratification [8]. Over the last decades, increasing nutrient and organic loads have triggered considerable environmental changes, with an enhanced frequency and severity of benthic low DO events.

Even though the frequency and extension of dead zones are increasing worldwide, their onset and extent remain difficult to predict and to study in the field. Our working group addresses this problem by deploying an underwater-chamber that artificially induces small-scale anoxia *in situ*. This approach fully documents the complex processes and interactions expected in a

community-level setting in the natural environment, e.g. behavioural reactions, intra- and interspecific interactions, and mortality sequences, all related to specific oxygen-thresholds.

This thesis encompasses four publications:

B. Riedel *et al.*, *CIESM Workshop Monographs no*35* (in press) provides a brief overview of hypoxia/anoxia in the Northern Adriatic and of responses from the species to the ecosystem level. The potential coupling between climate factors and coastal eutrophication is discussed [9].

M. Stachowitsch *et al.*, *Limnol. Oceanogr.: Methods* **5**, 344 (2007) introduces the underlying issues of documenting oxygen crises and the Univ. of Vienna's approach, specifies the technical design of our underwater-device and outlines the in situ procedures [10].

B. Riedel *et al.*, *J. Exp. Mar. Biol. Ecol.* **367**, 17 (2008) describes an experiment in which an intervening reoxygenation peak due to water intrusion created a two-phase oxygen decline. All initiated behaviours were interrupted during the reoxygenation and re-appeared in the second phase, demonstrating the direct relationship between oxygen levels and behaviour [11].

B. Riedel *et al.*, *Mar. Biol.* **153**, 1075 (2008) deals with previously unobserved predatory interactions between the sea anemones *Cereus pedunculatus* and *Calliactis parasitica* and the brittle star *Ophiothrix quinque maculata* in a narrow, near-anoxic window. Our observations suggest that the highly resistant sea anemones benefit by taking advantage of ophiuroids that are more vulnerable to anoxic conditions [12].

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Paper no° 1

B. Riedel *et al.*, *CIESM Workshop Monographs* no°35, (in press).

C I E S M W o r k s h o p M o n o g r a p h s



**Mediterranean biogeographic indicators of
global warming**

Helgoland, 28-31 May 2008

CIESM Workshop Monographs no° 35

To be cited as: CIESM, 2008. The Messinian Salinity Crisis from mega-deposits to microbiology - A consensus report. N° 33 in *CIESM Workshop Monographs* [F. Briand, Ed.], 168 pages, Monaco.

This collection offers a broad range of titles in the marine sciences, with a particular focus on emerging issues. The Monographs do not aim to present state-of-the-art reviews; they reflect the latest thinking of researchers gathered at CIESM invitation to assess existing knowledge, confront their hypotheses and perspectives, and to identify the most interesting paths for future action.

A collection founded and edited by Frédéric Briand.
Publisher: CIESM, 16 bd de Suisse, MC-98000, Monaco.

Dead zones: a future worst-case scenario for Northern Adriatic biodiversity

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Abstract

Shallow coastal seas are most endangered (Halpern et al., 2008) and, through a series of impacts ranging from overfishing, eutrophication to coastal development, they are likely to experience the largest change in biodiversity should present trends in human activity continue (Jenkins, 2003). No other crucial environmental variable has changed more drastically in shallow coastal marine ecosystems worldwide than dissolved oxygen (DO) (Diaz, 2001). “Dead zones”, caused by hypoxia ($DO < 2.0 \text{ ml l}^{-1}$) and anoxia (no oxygen) in bottom-water layers, top the list of emerging environmental challenges (UNEP, 2004), and the problem is likely to become worse in the coming years (Wu, 2002; Selman et al, 2008).

The Adriatic Sea is the most impacted system of the entire Mediterranean (Danovaro, 2003; Lotze et al., 2006). Over the last decades, increasing nutrient and organic loads have triggered considerable environmental changes, with an enhanced frequency and severity of benthic dystrophic events (Danovaro and Pusceddu, 2007).

We provide here a brief overview of low DO events in the Northern Adriatic and responses from the species to the ecosystem level. The potential coupling between climate factors and coastal eutrophication is discussed.

Northern Adriatic hypoxia

The Northern Adriatic Sea is a recognized area for long-term decreases in DO concentration and associated benthic community changes and mortalities (Stachowitsch, 1984, 1991; Justić et al., 1987). It combines many features known to be associated with low DO events (Stachowitsch and Avcin, 1988): it is semi-enclosed, shallow (<50 m) and is characterized by soft bottoms, a high riverine input (mainly from the Po River), high productivity and long water residence times (Ott, 1992). As elsewhere in the northern hemisphere, this constellation can be associated with seasonal hypoxia and anoxia in late summer/early fall. Moreover, the combination of certain meteorological and hydrological conditions such as calm weather

and/or reduced current circulation (Franco and Michelato, 1992; Malej and Malačič, 1995) can trigger hypoxia/anoxia.

Oxygen depletions, often associated with massive marine snow events, have been noted here periodically for centuries (Crema et al., 1991), but their frequency and severity have markedly increased during recent decades. High anthropogenic input of nutrients into the Northern Adriatic (Justić et al., 1995; Danovaro, 2003; Druon et al., 2004) has led to a higher production and deposition of organic matter than there is oxygen supply to allow its decomposition (Rabalais and Turner, 2001; Bishop et al., 2006). The average long-term decrease in water body transparency here over the 20th century, accompanied by decreasing bottom oxygen concentrations since the 1950s, has been convincingly outlined by Justić (Justić et al., 1987; Justić, 1988). Since the 1980s, severe oxygen deficiencies have been reported here on a regular basis (e.g. Fedra et al., 1976; Stachowitsch, 1984; Hrs-Brenko et al., 1994; Penna et al., 2004). The impacted areas range from restricted areas (several km²; Stachowitsch, 1992) to approx. 250 km² (Faganeli et al., 1985) to 4000 km² (Stefanon and Boldrin, 1982; D. Degobbi, pers. comm.), ultimately affecting every region (Fig. 1).

The Northern Adriatic is therefore a case study for recurring perturbations involving anoxia and marine snow events and shows profound effects on the species to community level (Šimunović et al., 1999; Barmawidjaja et al., 1995; Benović et al., 2000; Kollmann and Stachowitsch, 2001).

High-biomass suspension feeders and benthic control

Macroepifauna communities are widely distributed in the Northern Adriatic (Fedra, 1978; Zuschin et al., 1999) and largely consist of decimetre-scale, interspecific, high-biomass aggregations termed multi-species clumps (Fedra et al., 1976) or bioherms (Fig. 2): one or more shelly hard substrates provide the base for sessile, suspension-feeding colonizers (mostly sponges, ascidians, anemones or bivalves), which in turn serve as an elevated substrate for additional vagile and hemi-sessile organisms (mostly brittle stars and crabs) (Zuschin and Pervesler, 1996). The presence of a well-developed macroinfauna is expressed in the early designations (*Schizaster chiajei*-community) of the benthic communities here by Vatova (1949) and later authors (Gamulin-Brida, 1967; Orel and Menea, 1969; Orel et al., 1987; Occhipinti-Ambrogi et al., 2002).

The predominant, wide-ranging macroepibenthic community was named the ORM-community based on the biomass dominants, the brittle star *Ophiothrix quinquemaculata*, the

sponge *Reniera* sp. and the ascidians *Microcosmus* spp. The mean biomass, measured as wet weight, amounted to 370 (± 73) g/m² (Fedra et al., 1976).

In the shallow Northern Adriatic, the benthos is not merely a receiving compartment. Rather, complex feedback processes are in effect, with the benthic subsystem controlling and helping dampen oscillations in the pelagic subsystem (Ott, 1992). Ott and Fedra (1977) estimated that the suspension feeders here can remove all the suspended material in the water column every 20 days. This is on the same order of magnitude as calculated for the Oosterschelde (Herman and Scholten, 1990), Swedish waters (Loo and Rosenberg, 1989), the USA (Cloern, 1982) and France (Hily, 1991). Such communities have therefore been termed a “natural eutrophication control” (Officer et al., 1982) and play a key role in the stability of the entire ecosystem.

The repeated low DO events, coupled with commercial fishing activities during recent decades, however, have led to the destruction of epifauna-based benthic communities in many areas (Stachowitsch and Fuchs, 1995; Kollmann and Stachowitsch, 2001; Fig. 3). Their loss makes the system more sensitive to perturbations. Other key functional processes for the overall system, such as bioturbation and related sedimentary activities, may also be altered by hypoxia/anoxia and the corresponding loss of biodiversity (Snelgrove, 1998; Rosenberg, 2001; Levin, 2002). The current status of the ORM-community makes it unlikely that it fully fulfils its pre-mortality regulatory capacity.

Consequences on all levels

The point at which benthic animals are affected by low oxygen concentrations varies, but first indications of stress generally begin to appear when oxygen drops below 2.0-3.0 mg l⁻¹ (1.4-2.1 ml l⁻¹; Rabalais and Turner, 2001). Direct effects of exposure to hypoxia such as altered behaviour, physical inactivity and mass mortalities are well documented (Stachowitsch, 1984; Buzzelli et al., 2002; Montagna and Ritter, 2006). The larger, mobile benthos, for example, is often able to migrate out of the affected area, whereby the less mobile fauna – unable to escape or avoid hypoxic waters – exhibits a series of behavioural patterns in response to decreasing oxygen concentrations (Mistri, 2004). Infauna, for example, emerges from the sediment. Epifaunal organisms attempt to position themselves above the lowermost hypoxic bottom layer, either by moving onto higher substrates (Stachowitsch, 1991) or raising their bodies (i.e. arm-tipping brittle stars, siphon-stretching bivalves or tiptoeing crustaceans; reviewed by Diaz and Rosenberg, 1995).

Tolerance to hypoxia/anoxia in itself, however, is a question of physiological capacity and adaptability, which varies from species to species (Hagerman, 1998). Two “strategies”, depending on duration and intensity of the low oxygen bout, are possible. The first is to maintain aerobic respiration (e.g. increase in respiration rate, number of red blood cells, flow of blood through respiratory surfaces, or more effective use of respiratory pigments) as long as possible. The second is to resort to anaerobic respiration and reduce overall metabolism (e.g. resting, inactivity, down regulation of protein synthesis and certain regulatory enzymes) if severe hypoxia or anoxia prevails (Hagerman, 1998; Burnett and Stickle, 2001; Wu, 2002). However, once anaerobic conditions and H₂S develop, mass mortalities of nearly all organisms occur (Stachowitsch, 1984).

Diaz and Rosenberg (1995) reviewed the effects of hypoxia on benthic organisms. In general, fishes are more sensitive than crustaceans and echinoderms, whereby polychaetes and bivalves are the most tolerant. Within each taxon, however, there is considerable variability, dependent on the respective life habits (Gray et al. 2002).

In the Northern Adriatic, sea anemones are particularly tolerant to hypoxia due to a combination of physiological and behavioural adaptations (see Sassaman and Mangum, 1972; Shick, 1991). This is confirmed by other field and laboratory studies (Jørgensen, 1980; Wahl, 1984). In the 1983 mortality, for example, one week after the onset of the event, survivors predominantly included individual anthozoans such as *Ragactis pulchra*, *Cerianthus membranaceus* and *Epizoanthus erinaceus* (Stachowitsch, 1984). In our recent, artificially induced anoxia experiments in situ, *Cereus pedunculatus* was among the most tolerant species and survived more than 83 hours of anoxia and a final H₂S concentration of about 160 µM l⁻¹ (Riedel et al., in review). This information will be synthesized into a catalogue of behaviours, allowing indicator species to be defined and the status of benthic communities to be assessed.

Hypoxia may severely alter community composition by killing sensitive species but favouring a few tolerant forms (Dauer, 1993), and decreasing recruitment and growth (Breitburg 1992; Miller et al. 2002; Stierhoff et al. 2006). This will impact both the apparent and the potential biodiversity, e.g. pelagic resting stages in the sediment – important agents of local recolonization – will also be decimated (Boero and Bonsdorff, 2007; Danovaro and Pusceddu, 2007). Moreover, changes in functional types/groups (including ecosystem engineers; Crain and Bertness, 2006) occur along hypoxic gradients, influencing overall ecosystem properties (Pearson and Rosenberg, 1978; Diaz and Rosenberg, 1995): Suspension feeders might be replaced by deposit feeders, macrobenthos by meiobenthos, bioturbators may be lost,

phytoplankton communities can become dominated by nanoplankton and microflagellates. The result is an unbalanced community dynamics, altering both function and composition in unforeseen ways (Grall and Chauvaud, 2002).

Beyond these direct effects, there is increasing evidence for indirect effects (Eby et al., 2005). These include altered competition and predator–prey interactions, whereby predation rates increase or decrease depending on the relative tolerances of predator and prey to anoxia (Breitburg et al., 1994; Sagasti et al., 2001; Decker et al., 2004; Riedel et al., 2008). Thus, hypoxia also affects the trophodynamics of marine ecosystems. Wu (2002) suggests a general shift from *K*-selected to *r*-selected species, and from complex to simple food chains.

Such scenarios, which are increasingly unfolding in shallow coastal waters around the world (Selman et al., 2008), represent undisputable worst-case situations for biodiversity and ecosystem function. The result is local extinction (Solan et al., 2004) and large-scale homogenization at the lowest possible level (Sala and Knowlton, 2006). The ultimate reflection will be a total loss of ecosystem services beyond the seas as navigational highways.

Climate change – adding insult to injury?

For the Mediterranean, many models predict a temperature increase by an average 3 °C until the end of the 21st century, with a larger warming in summer than the global average. Mean precipitation is expected to decrease, especially in summer, mainly due to the northward extension of the descending branch of the subtropical Hadley circulation (Li et al, 2006). However, future impacts on the coastal system will vary greatly at regional scales (Scavia et al., 2002). Clearly, the trends will be determined by complex interactions between temperature, precipitation, runoff, currents, salinity and wind.

Climate change will influence hypoxia/anoxia both directly and indirectly. The mechanism involves changes in coastal eutrophication by two major pathways (Fig. 4):

- 1) Temperature-related changes in atmospheric circulation patterns will alter hydrological cycles, leading to shifts in precipitation, evapotranspiration and subsequent changes in river quantity and quality regimes (Miller and Russell, 1992). Specifically, changes in the magnitude and seasonal patterns of freshwater and terrestrially derived nutrient inputs will profoundly affect coastal salinity, turbidity, water residence time and primary production (Justic et al., 2005; Harley et al. 2006). Prolonged residence times during low-flow conditions will promote algal blooms (Relexans et al. 1988), whereas storm-related high river flows

result in higher nutrient inputs and stronger vertical salinity gradients. Both conditions favour the development of hypoxia/anoxia in bottom waters (Paerl et al. 1998; Scavia et al. 2002).

2) A warmer atmosphere leads to warmer water temperatures, which have a lower oxygen content available for respiration by aquatic organisms. Moreover, increased summertime surface temperatures, especially if coincident with reduced winds, will lead to more persistent stratification. This is a prerequisite for prolonged hypoxia/anoxia. (Justic et al. 2007; Thuiller, 2007). Finally, both photosynthesis and respiration are temperature-dependent processes and thus the rates of production, decomposition, and nutrient cycling are likely to increase (Kennedy et al. 2002; Harley et al. 2006).

In one of the few available models for the Northern Adriatic, Vichi et al (2003) predicted precisely such an overall enhancement of the water-column stratification on an annual basis, with stronger intensification during the summer. The diffusion of oxygen and nutrients between surface and bottom layers was reduced, and the transfer of organic matter through the food web shifted towards the smaller components of the microbial web.

Benthic and pelagic species will therefore be exposed to unusual temperature, salinity, and oxygen conditions. These factors will take most of the fauna to their physiological limits. Such stressed organisms, coupled with hypoxia-related denuded areas, will provide little resistance to disease and the immigration of alien species (Harvell et al., 2002; Osovitz and Hofmann, 2007).

Perspectives

Ecosystem stability is a crucial topic in modern ecology. In the Northern Adriatic, instability has been introduced by the recurring perturbations involving anoxia and marine snow events along with intensive dredging and trawling activities. Currently, the frequency of such disturbances greatly exceeds the duration of recolonization process. The situation in the Northern Adriatic has been described as “rapid death, slow recovery” (Stachowitsch, 1991).

Climate change is likely to affect hypoxia and anoxia in myriad ways and on different levels. Most of the anticipated changes will involve increased hypoxia/anoxia. Our current research (Stachowitsch et al., 2007) on artificially induce oxygen depletion events on the sea floor – including time-lapse documentation – provides a foretaste of what mass mortality, biodiversity loss and local extinction here will look like (www.marine-hypoxia.com).

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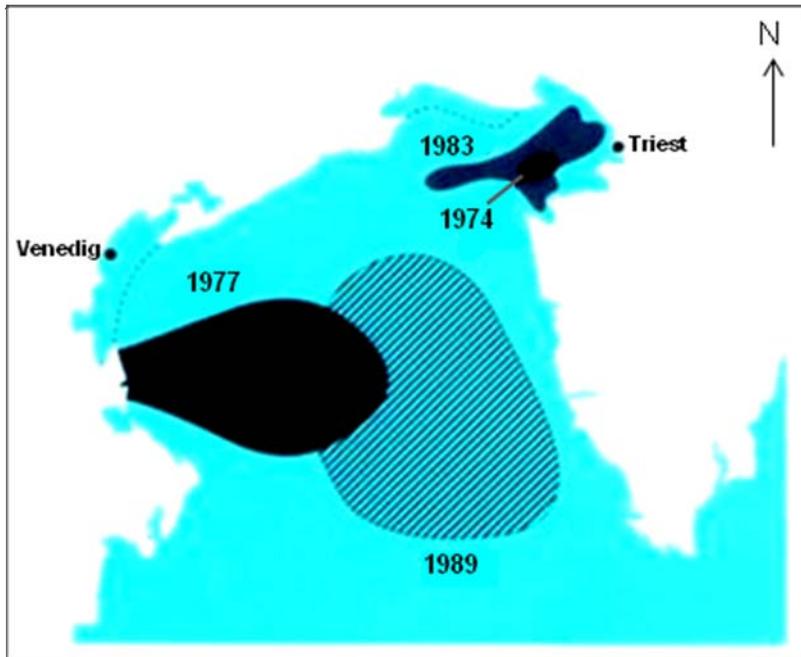


Figure 1. Bottom anoxias in the Northern Adriatic between 1974 and 1989. Virtually no area is unaffected and the number of unnoticed events is probably much higher (from Ott, 1992).

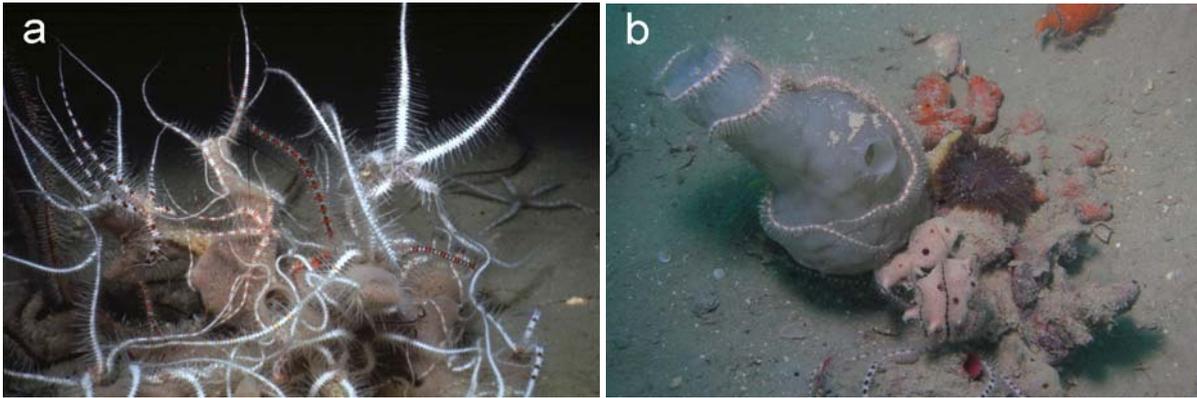


Figure 2. Typical aspect of ORM-community at 24 m depth, Gulf of Trieste. a) Dense aggregation of suspension-feeding brittle star *Ophiothrix quinquemaculata* on the sponge *Reniera* sp.; b) Multi-species clump, consisting of the ascidians *Phallusia mammilata* and *Microcosmus* spp., the sea anemone *Cereus pedunculatus*, various sponges, the sea cucumber *Ocnus planci* and *O. quinquemaculata*. [Photos: M. Stachowitsch and A. Haselmair].

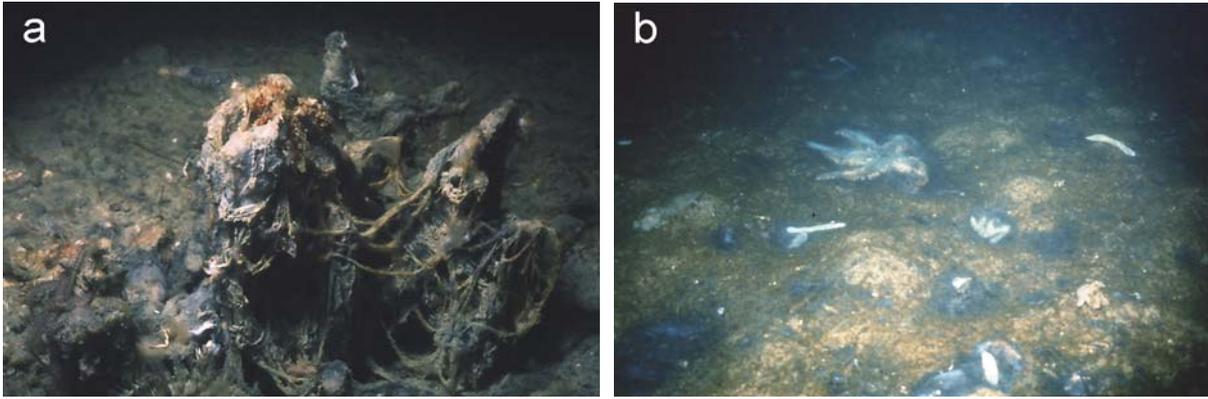


Figure 3. Mortality scenario after anoxia. a) Decomposing sponge bioherm with mucus cover and entangled crabs (*Pilumnus spinifer*, *Pisidia longicornis*); b) Typical late aspect of mass mortality. Decomposing sea star *Astropecten bispinosus* and sipunculids. Note lighter sediment mounds. [Photos: M. Stachowitsch].

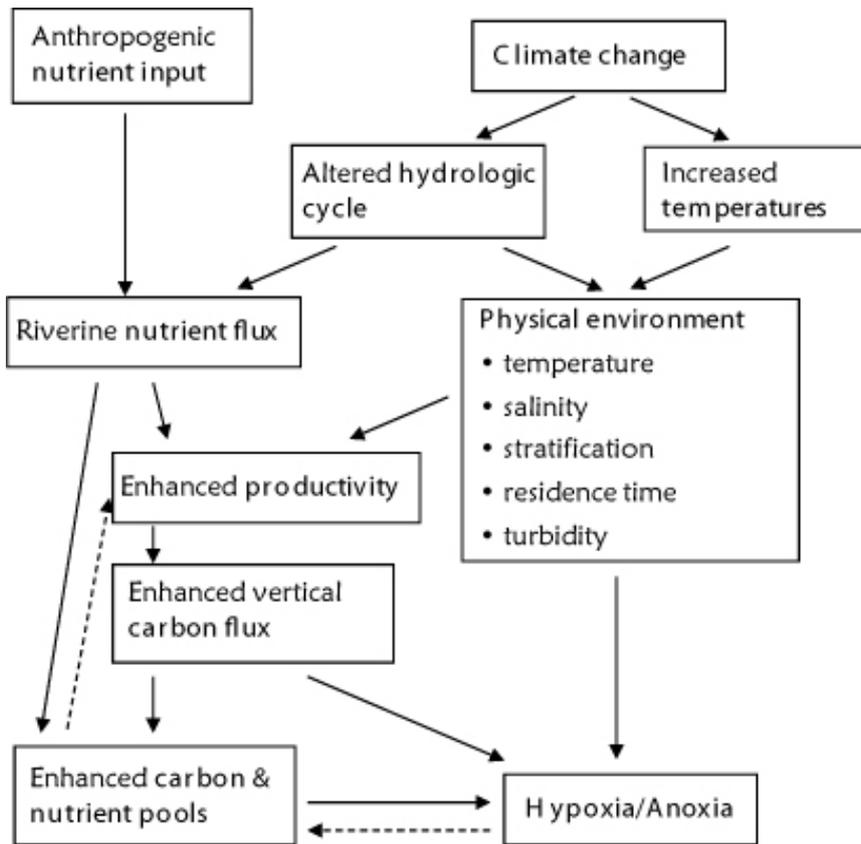


Figure 4. Coupling between climate variables and eutrophication. Possible pathways for the development of hypoxia and anoxia in shallow coastal areas. Broken arrows indicate feedback control (adapted from Justić et al., 2001; 2007).

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Oxygen depletion and benthic mortalities: the first in situ experimental approach to documenting an elusive phenomenon

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Abstract

Oxygen depletion events and anoxia are a key threat to shallow marine coastal seas worldwide. The mortalities they trigger, however, are difficult to document in full. We developed an underwater device to experimentally induce hypoxia and anoxia on the seafloor. The EAGU (Experimental Anoxia Generating Unit) combines a time-lapse camera and flashes with an array of sensors and a datalogger. The unit was successfully deployed in 24 m depth in the Northern Adriatic Sea for 3 to 5 d and yielded detailed information on the behavior and sequence of mortality of macrobenthic organisms – both epi- and infauna – under decreasing oxygen and increasing H₂S concentrations. This unit, designed as a chamber with an instrument lid, also can be deployed in an open configuration to document low dissolved oxygen (DO) events. The equipment can provide data for a catalog of behavioral patterns, define indicator species, help reconstruct past mortalities, and better gauge the stability and status of benthic communities.

“Dead zones” in the world’s oceans are at the top of the list of emerging environmental challenges (UNEP 2004). The nearly 150 such zones that have been identified (Diaz 2001; Diaz et al. 2004) are caused by oxygen deficiency in bottom-water layers. Waters with oxygen concentration below 2.0 mL L⁻¹ are termed hypoxic, with anoxia referring to oxygen-free condi-

tions (Diaz and Rosenberg 1995). The Northern Adriatic Sea is one such zone and is a case study for long-term decrease in dissolved oxygen concentration and associated benthic community changes and mortalities (Stachowitsch 1984; 1991; Justić et al. 1997). It combines many of the characteristics known to promote such events, such as relatively shallow waters, soft-bottoms, seasonal stratification and long water residence times (Ott 1992). Furthermore, it suffers from eutrophication, which is known to trigger and increase the severity of oxygen depletion events (Rabalais and Turner 2001; Grall and Chauvaud 2002; Gray et al. 2002). Finally, the Northern Adriatic has repeatedly experienced extensive and dense marine snow and so-called “mucilage” events, which are intimately related with eutrophication and benthic mortalities (Stachowitsch et al. 1990; Justić et al. 1993; Degobbis et al. 1999; Koenig 2000).

Benthic mortalities and marine snow events are known to have occurred here periodically for centuries (Crema et al. 1991). Even though the frequency and extension of benthic disturbances are increasing here and elsewhere (Justić 1991; Diaz and Rosenberg 1995; Diaz 2001; Wu 2002; Harley et al. 2006), such disturbances are still difficult to document. A first photographically documented mortality event was discovered by chance using an underwater-TV camera sled (Fedra et al. 1976). A subsequent, large-scale anoxia in 1977 was documented by Stefanon and Boldrin (1982). They relied on a large

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Oxygen depletion and benthic mortalities: the first *in situ* experimental approach to documenting an elusive phenomenon

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Running head: Continuous documentation of anoxia

Abstract

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Introduction

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Although seasonal anoxia in the northern hemisphere occurs mostly in late summer/fall (Pearson and Rosenberg 1978; Stachowitsch and Avcin 1988; Druon et al. 2004), its actual timing is related to local weather conditions. The onset and extent of such disturbances are difficult to predict and tend to elude investigation in the field. Finally, mortality events often run their course within a few days (Stachowitsch 1984), further hindering their full documentation.

Laboratory chamber/aquarium experiments on respiration and responses to decreasing oxygen concentrations typically involve individual specimens or species (Renaud 1986; de Zwaan 2001; Miller et al. 2002; Matozzo et al 2005; Shimps et al. 2005). Their results, while physiologically accurate, do not combine all the relevant information about actual behavioral responses, intra- and interspecific interactions, mortality sequences, and community-level processes in the natural environment.

We addressed this dilemma by developing a device that can create and fully document small-scale experimental anoxia, *in situ*, as well as document the sequence of benthic mortalities.

This instrument combines photo-documentation with detailed chemo-physical analyses and allows the behaviors and mortalities of benthic organisms to be analyzed during an oxygen depletion event from the onset. The focus is on the macrofauna because macroepi- and infauna are widely used to detect and monitor community responses to environmental change. Here, as in the past, we refer to the macrofauna as those organisms that are visible in situ to the naked eye and to the camera, although in certain other habitats, e.g., the deep-sea benthos, such organisms may be referred to as megafauna. Many benthic organisms are sedentary and long-lived, and the community structure therefore reflects environmental conditions integrated over extended periods (Bilyard 1987; Gray et al. 1988; Bourget et al. 2003; Ragua-Gil et al. 2004). Moreover, the benthos in the Northern Adriatic – via re-colonization and succession – can store information on prior disturbances over years or even decades and, therefore, can be regarded as a long-term memory of the overall system (Stachowitsch 1992).

Materials and Procedures

Design of the Experimental Anoxia Generating Unit (EAGU) – The EAGU (Fig. 1) creates anoxia by sealing a 50 × 50 × 50 cm volume of water off from the surrounding environment. The instrument lid is positioned atop two different bases. The first is the “open” configuration (hereafter referred to as “frame”), a 2 cm aluminum-profile frame, (L × W × H = 50 × 50 × 50 cm) that is positioned over selected benthic organisms on the sediment surface. This configuration permits full water exchange and does not disrupt normal bottom-water currents. We observed no sediment accumulation or scouring of the seabed adjacent to the frame. This configuration is used to document animal behavior under normoxic conditions (as a control before reconfiguring to generate anoxia) or to record oxygen depletion events.

The second, “closed” configuration (hereafter referred to as “chamber”) also consists of an aluminum-profile frame of the same size, but with 6-mm-thick plexiglass plates on its four vertical sides. This cube-like chamber is open above and below. The lower plexiglass edges are strengthened with sharpened aluminum elements. This chamber is pushed approximately 2 cm into the sediment to hinder water exchange through the substrate (Fig. 2). The watertight lid (simple rubber seal around upper edge of chamber) prevents exchange with the water column. This configuration is used to document behavioral responses to decreasing oxygen concentrations. The four lower corners of both configurations are equipped with removable 7-cm-long tapered metal tips that help stabilize the device in the sediment. The chamber is also

equipped with two 50-cm-long handles to facilitate transportation and manipulations.

The lid consists of a 12-mm-thick plexiglass plate measuring 51 × 70 cm and bears the equipment described below.

Camera equipment

A digital camera (Canon EOS 30D) with a zoom lens (Canon EFS 10-22mm, f/3.5-4.5 USM), mounted in an underwater carbon-fiber housing (Fig. 1) with a dome port (both Bruder). The camera's number of effective pixels is 8.2 MP. The time-lapse function is effected by a Canon Timer Remote Controller (TC-80N3), and a 1 GB flashcard is used. The lens and its setting (14 mm) were chosen to provide an optimal combination of distortion-free images, a view of the entire 50 × 50 cm sediment area along with a portion of the vertical plexiglass walls, and to position the camera as close to the bottom as possible. This provided clearer images in turbid conditions (frame) and reduced the water volume in the chamber.

Two underwater flashes ("midi analog," series 11897; Subtronic). The flashes are modified to be adjusted manually (we used the 1/16 setting) and are attached to the lid by PVC-swivel arms on two adjoining sides (Fig. 1).

Two external battery packs power both the camera and the flashes (akku-safe 9Ah Panasonic; Werner light power Unterwassertechnik).

The camera housing is positioned such that it lies centrally over the frame or chamber. The camera housing port fits snugly into an O-ring-equipped opening, with the dome projecting below the lid. The housing is further attached to the lid with an L-shaped aluminum bracket. The housing has four sockets: two for the flashes and two for the battery packs.

Available power is usually the limiting factor in stand-alone long-term measurements. A special electronic control circuit (Fig. 3) was developed in order to run the equipment for at least 72 h with sufficiently small and light external batteries in combination with a commercially available camera and flash.

The circuit was built on a small board (12 × 3 cm) using standard CMOS integrated circuits for logic functions and transistors for switching. The following functions were implemented: (1) A monitoring circuit (ICL7665 + Power Transistor) interrupts the 12 V supply power when the voltage falls below 10.2 V to prevent damage to batteries and electronics; (2) A stabilizing circuit (LM 317) provides a constant 7.5 V to the Canon camera. The camera automatically switches itself off 1 min after each shot; (3) A charging circuit (resistor +

diode) constantly recharges the internal batteries in the flashes. These batteries are needed to provide the high current necessary to charge the flashes within a few seconds. With good tuning of the charging current, the internal flash batteries need not be changed; only the main external battery packs are exchanged between deployments; (4) The logic circuit controls the sequence of operation, which is initiated by a pulse from the separate Canon timer to a series of monostable multivibrators. First, the flashes are switched on and the camera receives a wake-up pulse. When both flashes are fully charged after a few seconds, their ready signal triggers the camera via its remote trigger input. The camera, in turn, triggers the flashes via its synchronized output. About 0.1 s after the shot, the flashes are turned off to save energy. After the camera switches off (automatically after 1 min), the system is ready for the next cycle. An additional timer turns off the flashes after 20 s should a malfunction interrupt the above sequence.

The camera was timed to take one photograph every 6 min. The shot rate is set with the Canon timer. The 6-min interval was a compromise between available energy supply, desired experiment length, and flash card capacity. It allowed the full anoxia event to be documented; the overall duration of the experiment, 3–5 d, corresponded well with the rapid course of an earlier mortality here (Stachowitsch 1991). A lengthier interval would make it more difficult to reconstruct and attribute the movements of most mobile organisms to one and the same individual. We would also have missed many intra- and interspecific interactions.

Sensor equipment

Two oxygen microsensors (sensor type: OX-100, outside tip diameter 90–110 μm), one hydrogen sulfide microsensor (sensor type: H2S-50, outside tip diameter 40–60 μm), and one temperature microsensor (sensor type: TP-200, outside tip diameter 180–220 μm) (Unisense).

A two-channel datalogger unit (PA3000UD, Unisense) with one compartment containing four amplifier circuits with displays and datalogger, and one compartment containing the battery and communication cable (Fig. 1). Each of the two-channel loggers in the PA3000UD has a memory capacity of 4000 samples per channel and each was programmed to log sensor data every minute.

The sensors are positioned in plexiglass tubes (15 mm diameter; 40 cm length), and the tips are protected by factory-installed metal protector caps. The plexiglass tubes with the sensors

are pushed through O-ring-equipped sensor ports in the four corners of the lid during deployment (Fig. 1). For transport, all four sensors are pulled out and placed in a single plastic sheath on the lid (Fig. 2). The tips of the two oxygen sensors are positioned in different heights above the sediment (approx. 2 and 20 cm) in order to detect potential oxygen stratification in the water. The hydrogen sulfide sensor is positioned about 2 cm above the sediment, the temperature sensor at 20 cm. The datalogger is strapped to the part of the plexiglass-lid extending beyond the underlying frame or chamber (Fig. 1,2).

An additional pH-sensor (WTW, TA 197-pH) was inserted into the chamber through an opening (2 cm diameter; sealed with a plastic stopper during regular operation) in one plexiglass panel of the closed configuration once a day during daily control dives (Fig. 5D). This sensor is connected to a datalogger (WTW, Multi 197i) on the dive boat by a 60 m cable. Temperature was initially recorded with a Unisense, TP-200 sensor, but values were subsequently taken from the adjoining oceanographic buoy.

EAGU deployment—In a full deployment, the system is initially positioned in its open configuration over an aggregation of benthic organisms on the sediment for 24 h (Fig. 4A,5A). Aggregations were selected based on the presence of a wide range of representative organisms.

Minimizing diver disturbance during deployment is important, and appropriate precautions were taken. One diver positions the frame, while a second diver, positioned several meters above the bottom, suspends the fully equipped lid in the water. The second diver then carefully sets the lid down on the open frame and fastens it. The flashes are flipped down and fastened at the appropriate angle, the sensors removed from the sheath and inserted through the appropriate sensor ports to the predetermined depth, and the datalogger initiated with a magnetic trigger stick. Two U-shaped metal brackets on each side of the camera (Fig. 2) simplify handling and transport in the water. The brackets are connected with a sliding crossbar that bears a ring. This allows a short (ca. 1.5 m) holding rope to be attached with a carabiner: the diver swims a short distance above the lid and does not disturb the sediment. No diver ever needs to touch the bottom. We also attached three ca. 15-cm diameter floats to the crossbar: with standard SCUBA gear (appropriately inflated buoyancy compensator); this provides neutral buoyancy during manipulations (Fig. 2).

After 24 h, the EAGU is switched to its closed configuration over the same assemblage for another 2–3 d. This requires removing the sensors and flipping up the flashes.

One diver then unfastens and briefly lifts the lid, while the second exchanges the frame for the chamber. The sensors and flashes are then repositioned as above. The course of oxygen depletion is not manipulated: oxygen is not stripped and no H₂S is added.

After each experiment, the lid is transported to the boat and serviced. Datalogger values and photos are downloaded, all batteries exchanged, and sensors recalibrated. Camera and flashes are reactivated on board, and the datalogger is reactivated underwater.

Deployment site—The EAGU was deployed on a soft-bottom (poorly sorted silty sands) in 24 m depth in the Gulf of Trieste, Northern Adriatic Sea. We chose a site adjoining the oceanographic buoy of the Marine Biology Station, Piran, about 2 km offshore (45°32'55.6"N, 13°33'1.89"E), to avoid damage by commercial fisheries. The bottom here is dominated by macroepibenthic communities consisting largely of sponges, ascidians, and brittle stars. It has been described as the *Ophiothrix-Reniera-Microcosmus* (ORM) community based on the three dominant genera (Fedra et al. 1976). The organisms here are typically aggregated in so-called multi-species clumps – bioherms – consisting of a shelly base overgrown by sessile organisms, which in turn serve as a substrate for many mobile and semi-sessile species (Zuschin et al. 1999). Our first experiments also revealed a diverse infauna community with well-defined reactions to anoxia.

Assessment

EAGU-deployment—The EAGU system was deployed in September 2005 and from July–October 2006 for a total of 13 experiments. The frame and chamber were deposited on the bottom, exchanged as outlined above, and retrieved at the end of the experimental series. The lid was brought aboard and serviced after every experiment. Servicing the equipment on the lid and downloading the data took about 1 h, enabling us to immediately redeploy the system and obtain an uninterrupted series of experiments.

The system always induced anoxia within 1–2 d. Oxygen depletion reflected natural respiration rates, and the device itself prevented any substantial re-supply of oxygen from outside. For example, the sediment and water along the lower inner walls of the chamber apparently were not better oxygenated than elsewhere inside because both the upper and lower oxygen sensors showed virtually the same values. Moreover, some infauna organisms emerged directly along the plexiglass wall.

Each experiment was continued for an additional 1–2 d to document the reactions and mortalities of the enclosed epifauna and infauna. The generation of anoxia demonstrates that settling the lower edges of the chamber only 1–2 cm in the soft sediment was sufficient to

effectively seal off the fauna and hinder exchange with ambient water. An advantage of the chamber is that all the suspended particles sank to the bottom within a few hours, yielding clearer photographs than in the open configuration. The 1–2 d period was long enough to document the mortality of most, but not all species. The experiments were terminated when most organisms had died based on their body position and initial signs of decomposition, but usually before decomposition was too advanced to prohibit collection and identification of the organisms. At this point, the sediment has turned black and the enclosed water became more turbid (Fig. 4B,5C,D).

After each experiment, the instrument lid was removed and as many organisms as possible collected. This was done by hand, initially using a wide-mouthed syringe and small sieve. Soft-bodied or decomposing and fragile organisms were placed separately in small plexiglass boxes. All collected organisms then were transferred to the laboratory and placed in shallow plastic trays or small aquaria with seawater. These were carefully observed to separate living from dead organisms.

Images and image evaluation—Depending on deployment length, the experiments yielded between 636 (63 h 30 min deployment) and 1333 images (133 h, 12 min). They can be viewed individually, successively scrolled with a computer mouse, or processed into time-lapse sequences (MPG files) using the Adobe Premiere 6.5 program. The unedited time-lapse sequence lasted between 14 and 40 s, but was extended by about 35% for better viewing using Adobe Premiere.

The image sequence was analyzed image by image and the behavior of the organisms was recorded on data sheets containing numerous categories describing reactions to decreasing oxygen and increasing hydrogen sulfide concentrations. Such categories included emergence from the sediment (for infauna), locomotion and body movements, interactions, and mortality. General categories for all mobile species, for example, included major and minor movements (both horizontal and vertical), whereas species-specific categories included emergence from the shell (hermit crabs), arm-tipping (brittle stars), or detachment from the substrate (certain anemones). The camera set-up accurately depicted all these events. It also clearly documented color changes of the organisms and the sediment. Certain organisms (e.g., large gastropods) tended to climb up the plexiglass walls and were not visible for lengthy periods (the climbing activity itself, however, was a useful oxygen-related behavioral response). After a certain period of anoxia, these individuals fell from the lid and their subsequent behavior was documented.

The 6-min interval between images was retained for all experiments because it enabled the successive movements of the organisms to be traced in detail. In more rapidly moving animals (e.g., larger hermit crab, large gastropods), tracks on the soft sediment surface usually enabled the paths taken to be documented. This interval, however, cannot individually resolve rapid or rapidly repeated movements (e.g., body contractions of anemones and ascidians), but clearly captured key aspects of such movements. Moreover, lengthier phases of particular activities could be distinguished from inactive phases.

Datalogger results—The datalogger provided a dense set of data at the 1 min interval. The oxygen sensors demonstrated the success of the concept: oxygen values began to fall immediately after the frame to chamber switch and every experiment yielded anoxic conditions (Fig. 6); anoxia always remained until the end of the experiment. The sensor values changed in parallel, confirming the overall process. Moreover, the values of the lower sensor tended to be somewhat lower than those of the upper one (Fig. 6), demonstrating gradients in the water, and helping to explain why many benthic organisms in our chamber (and during field oxygen depletion events) tried to reach higher substrates as oxygen values fell.

The hydrogen sulfide electrode added a complementary set of information. In every case, hydrogen sulfide first appeared after about half a day of anoxia and values then tended to increase rapidly (Fig. 6).

Discussion

The EAGU system documented the detailed behavioral responses of benthic organisms to decreasing oxygen concentrations, from the onset of behavior modification to moribund states or mortality. Moreover, it allowed modified behavior to be defined by initially showing normal, pre-hypoxic behavior in exactly the same species and individuals (at least the sessile and hemi-sessile forms) subsequently subjected to experimentally reduced oxygen concentrations.

The image analyses revealed individual behavior as well as interactions and community-level responses. Our initial evaluation confirms previously described basic responses such as the emergence of infauna from the sediment (Fig. 4B,5B:”sc”), and the upward movement of many species to higher levels on available substrates (Fig. 5:”cr”). This confirms that the EAGU recreated conditions experienced during low DO events.

EAGU also provided previously unknown detail. For example, the emergence pattern of infauna individuals became visible, as did the relative emergence times of different species. Moreover, the speed and distance of post-emergence movement can be quantified. The system also captured species (e.g., infaunal shrimp) whose in situ behavior has never been documented before, either under normoxic or anoxic conditions. On the community level, the complete range of parallel and successive responses is combined in a single experiment. Interspecific interactions become apparent, for example, unexpected predation of brittle stars by anemones (Riedel et al. pers. comm.). Anemones, less sensitive to anoxia, preyed on more sensitive brittle stars, providing insight into potential benefits of anoxia for certain species and yielding new interpretations of post-anoxia community structure.

Finally, all these observations are directly correlated to dissolved oxygen and H₂S levels, allowing biological and chemical analyses in the same experiment. EAGU therefore provides insight into the course and effect of oxygen depletion events in the Northern Adriatic. It also will provide a catalog of behavioral modifications, distinguish between sensitive and tolerant species, and help interpret post-anoxia community composition.

The relatively small size and weight (40 kg) of our instrument – allowing deployment without larger vessels – as well as overall cost (ca. 30,000 €) and composition of readily available components and instrumentation should facilitate its deployment elsewhere.

Comments and recommendations

As in all newly developed underwater instruments, unexpected situations arose, setbacks were encountered, and modifications had to be made. For example, in some experiments the water was so (naturally) turbid that the open configuration would not have yielded useful images: in such cases, the experiments were started with the closed configuration (chamber), where the water quickly clears up (Fig. 5A,B). This particle settlement means that the EAGU chamber can be deployed even in very turbid waters. The sensors used in EAGU are basically designed for laboratory use and are the most sensitive components of the system. They are exposed to damage above and below water and have a limited life (even if only stored). Their cost (ca 400 € apiece) requires liberal replacement costs to be calculated for lengthier deployments.

The post-experiment collection of the enclosed organisms entails a compromise between advanced decomposition and a duration in which even the most resistant species are moribund or dead. This critical interval can be missed if weather conditions delay termination of the experiment and lid retrieval.

If collection and identification of more fragile organisms are an issue, then removal before the end of the experiment is recommended. This would require a sealable port in one of the plexiglass side walls large enough either to insert a long forceps, gripper, syringe, or to manually remove the dead organisms. It should minimize excessive entry of oxygenated water during manipulations.

A range of options exists for future deployments. One approach would be to place the EAGU over lebensspuren such as burrow openings of infaunal species or on undisturbed sediment rather than over visible macroepibenthic organisms. The device elicited clear and reproducible reactions in infaunal species, and the types of habitats in which they occur are often those affected by oxygen depletion in the Northern Adriatic Sea. Targeting the infauna would expand the range of species-specific behaviors already documented; it also would require taking sediment samples after the experiment to determine which species are resistant.

Finally, the EAGU's instrument package could be enlarged to include sensors that provide data on sediment chemistry. Such information would help interpret the anoxia process, in particular with regard to infaunal species. Ultimately, the meiofauna and microbial processes could be incorporated.

Another option is to add a third phase to the current two-phase approach (open followed by closed configuration). Redeploying EAGU in the open configuration directly over the formerly enclosed quadrat after an experiment (but without collecting the organisms) would document post-mortality events. Such an additional 4–5 d deployment would yield information on the fate of dead organisms and decaying organic matter, the potential recovery of moribund or still active individuals, and on the return to the normal sediment coloration.

Future deployments will deepen our understanding of sensitive coastal ecosystems and provide additional information for coastal managers and decision-makers.

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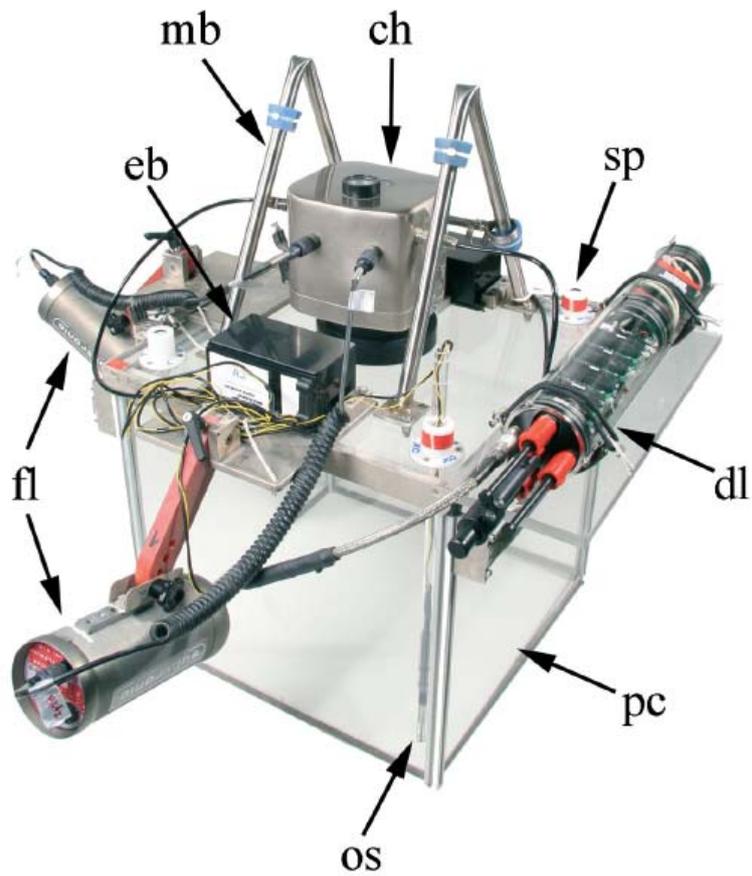


Fig. 1. Experimental Anoxia Generating Unit (EAGU) with instrument lid positioned on top of plexiglass chamber. Here, only one sensor is connected to the datalogger and inserted through a sensor port. ch: camera housing, dl: datalogger, eb: external battery, fl: flashes, mb: metal brackets, os: oxygen sensor, pc: plexiglass chamber, sp: sensor port.

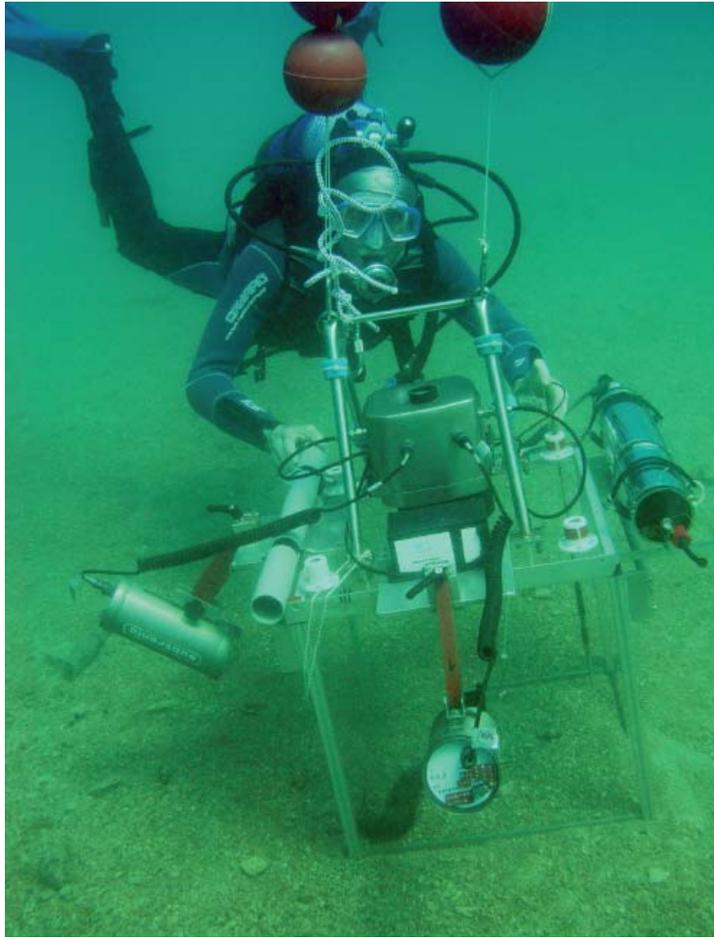


Fig. 2. Test deployment of EAGU in shallow (12 m) depth. Crossbar (top center) between the two brackets serves to lift and transport the lid and as an attachment site for buoys to provide neutral buoyancy during manipulations. Tube on left serves as a sheath for sensors during transport.

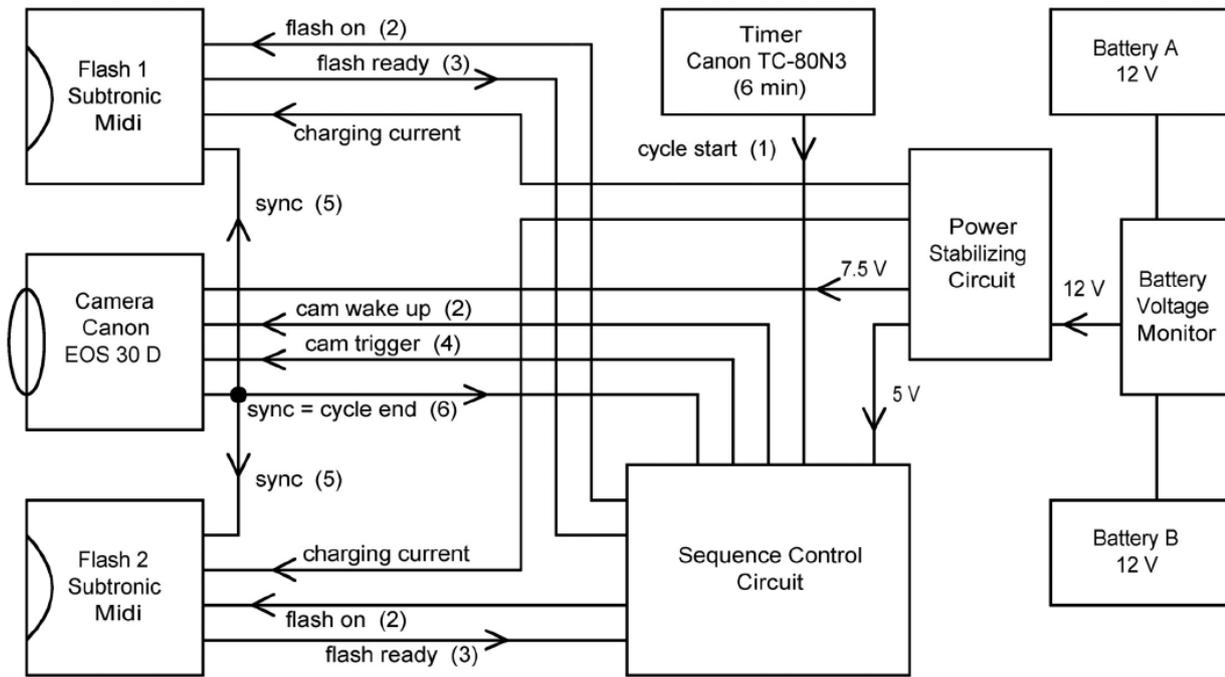


Fig. 3. Block diagram of control circuit built into camera housing. Numbers indicate sequence within cycle. Arrows show signal direction. For details see text.

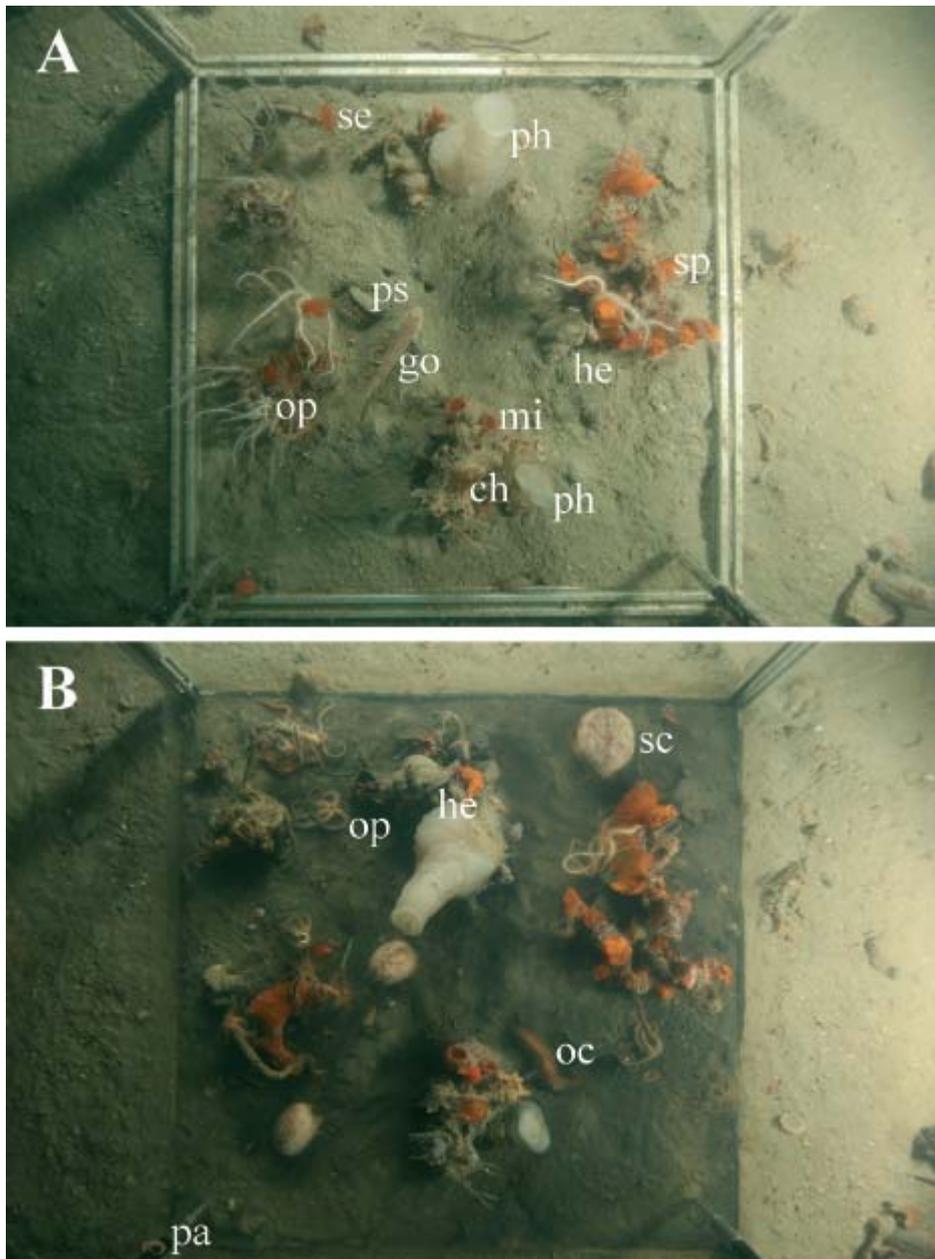


Fig. 4. Experiment 12. (A) open configuration (frame): 50 × 50 cm area contains the ascidians *Phallusia mammilata* (ph) and *Microcosmus* sp. (mi), the gastropod *Hexaplex trunculus* (he), brittle stars *Ophiothrix quinquemaculata* (op), serpulid tubeworms (se), sponges (sp), the sea urchin *Psammechinus microtuberculatus* (ps), the bivalve *Chlamys varia* (ch), and the fish *Gobius niger* (go). H2S- and lower oxygen sensor visible in lower left- and right-hand corner. (B) final image 72 h 54 min after switch to closed configuration (chamber). Note dark color of enclosed versus outside sediment. Emerged infauna includes three irregular sea urchins (*Schizaster canaliferus*: sc) as well as polychaetes and a sipunculan worm. Note dead and overturned brittle stars, broadly gaping *C. varia*, retracted tube worms, and the sea cucumber *Ocnus planci* (oc) exposed on sediment surface. A hermit crab (*Paguristes eremita*: pa), which has emerged from its shell, is crawling up the lower left sensor.

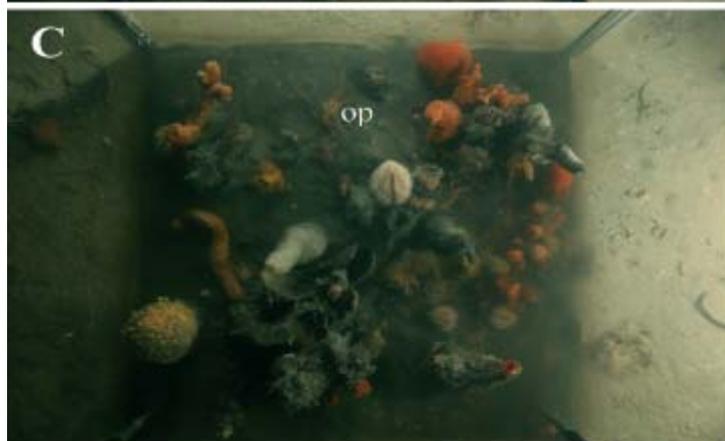
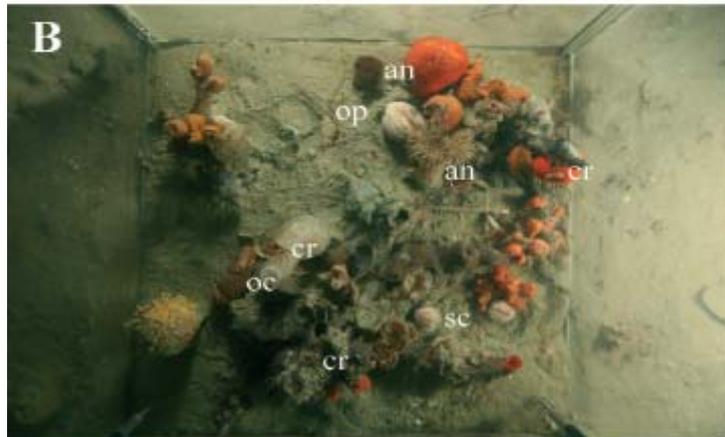
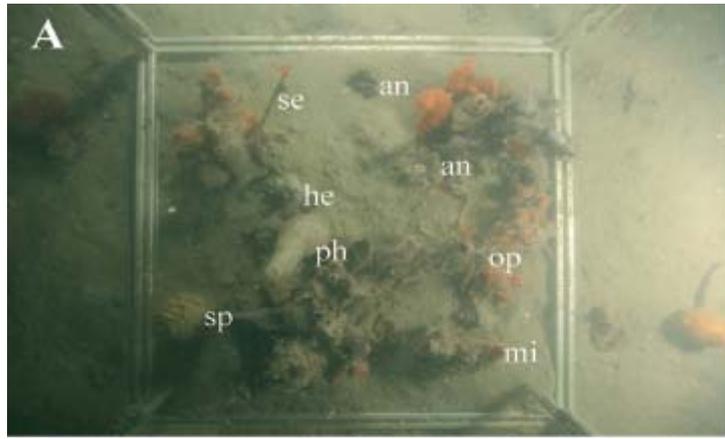


Fig. 5. Experiment 11. (A) open configuration (frame). Note relatively turbid water as well as particles and mucus (marine snow) on sensors. Anemones (an). For other abbreviations see Fig. 4. (B) 30 h 12 min after switch to closed configuration. The water rapidly becomes clear under chamber. Emerged infauna includes three *S. canaliferus*. Brittle stars overturned and dead, anemones extended upward, *O. planci* exposed on sediment surface, and three species of decapod crabs (cr) moving to elevated substrates. (C) 58 h 12 min after switch to closed configuration. Most organisms dead and decomposing. Exceptions: anemones, *O. planci*, *Microcosmus* sp., *H. trunculus*, and emerged *P. eremita* on *Microcosmus* opening (bottom right). Sediment has turned black and lowermost water layer becomes murky. (D) Final image 95 h 36 min after switch to closed configuration. pH-sensor inserted. Sediment and decaying organic matter have further darkened, the water has become even murkier. Accurate image analysis is no longer possible, but viewing the film in time-lapse mode reveals that certain organisms (e.g., anemones) are still alive.

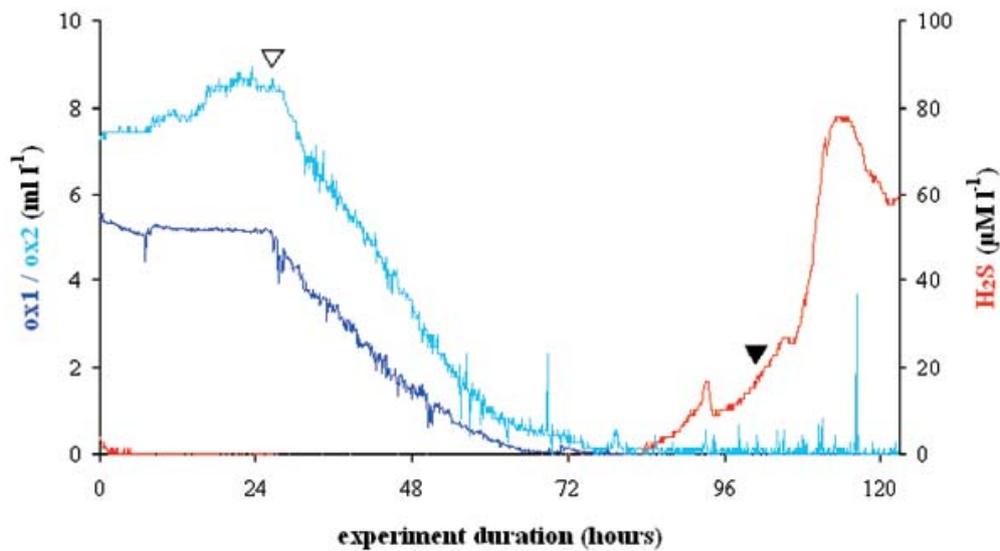


Fig. 6. Sensor data from experiment 12 (10–14 Oct 2006). Every sixth value entered (corresponding to image intervals). Open arrow = switch from frame to chamber, closed arrow = final photographic image. Note consistently higher values of upper oxygen sensor (ox2), immediate and continuous drop in oxygen values after switch, and steadily rising H₂S-values approximately half a day after attainment of anoxia. Separate internal batteries allow datalogger to continue to record after depleted battery packs shut camera system down.



Oxygen depletion under glass: Behavioural responses of benthic macrofauna to induced anoxia in the Northern Adriatic

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ABSTRACT

The Northern Adriatic Sea experiences seasonal low dissolved oxygen (DO) events and mortality of benthic organisms. The onset and extent of such disturbances, however, are difficult to forecast and study in the field. We address this problem by deploying a device that artificially induces and documents small-scale anoxia on soft-bottom in 24 m depth. Behaviour changes and mortality sequences of the soft-bottom macroepi- and infauna over a 5.5-day deployment were documented. An intervening oxygen peak created two phases of declining oxygen values. All initiated behaviours were interrupted during the reoxygenation and predictably re-appeared in the second phase, demonstrating the direct relationship between oxygen levels and behaviour. Beginning hypoxia ($\leq 2.0 \text{ ml l}^{-1}$ DO) elicited escape patterns such as increased horizontal and vertical locomotion. Moderate hypoxia ($\leq 1.0 \text{ ml l}^{-1}$ DO) triggered species-specific sublethal effects such as arm-tipping in ophiuroids or extension from the sediment in sea anemones. At severe hypoxia ($\leq 0.5 \text{ ml l}^{-1}$ DO) infaunal organisms began to emerge and first mortalities occurred. The crustacean *Macropodia* sp., the sea urchin *Psammechinus microtuberculatus* and the brittle star *Ophiothrix quinque maculata* were among the first to die; the sea anemone *Cereus pedunculatus* and the gastropod *Hexaplex trunculus* survived. Hydrogen sulphide (H_2S) increased until the end of the deployment to $163 \mu\text{M}$; temperature in the chamber remained nearly constant at 18.5°C . This design provides insight into species and community-level processes and is an important step in identifying potential indicator species for low DO events in the Northern Adriatic and elsewhere.

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1. Introduction

1.1. Northern Adriatic hypoxia

The Northern Adriatic Sea combines many features associated with the development of low dissolved oxygen (DO): semi-enclosed water body, soft substrates, a high riverine input (mainly from the Po River), high productivity and long water residence times (Stachowitsch, 1991; Ott, 1992). During late summer, hypoxia (defined here as DO levels $\leq 2 \text{ ml l}^{-1}$) and anoxia can develop through a combination of meteorological and hydrological conditions such as calm weather and water stratification (Franco and Michelato, 1992; Malej and Malačič, 1995). Low DO events and benthic mortalities have been noted here periodically for centuries (Crema *et al.*, 1991), but their frequency and severity have markedly increased during recent decades. High anthropogenic input of nutrients into the Northern Adriatic (Justić *et al.*, 1995; Danovam, 2003; Druon *et al.*, 2004) has led to a higher production and deposition of organic matter than there is oxygen supply to allow its decomposition (Rabalais and Turner, 2001; Bishop *et al.*, 2006). The average long-term decrease in water body transparency here over the 20th century, accompanied by

decreasing bottom oxygen concentrations since the 1950s, has been convincingly outlined by Justić (Justić *et al.*, 1987; Justić, 1988). Since the 1980s, severe oxygen deficiencies have been reported nearly every year (Fedra *et al.*, 1976; Stachowitsch, 1984; Hrs-Brenko *et al.*, 1994; Penna *et al.*, 2004). Most such events impacted rather restricted zones, but in 1983, for example, the affected area measured approx. 250 km^2 (Faganelli *et al.*, 1985), leading to large-scale loss of biodiversity with very slow recovery (Stachowitsch, 1991; Stachowitsch and Fuchs, 1995). The Northern Adriatic is therefore a case study for long-term eutrophication and shows profound effects on the species to community level (Šimunović *et al.*, 1999; Barmawidjaja *et al.*, 1995; Benović *et al.*, 2000; Kollmann and Stachowitsch, 2001).

1.2. Study species and objectives

Macroepifauna communities are widely distributed in the Northern Adriatic (Fedra, 1978; Zuschin *et al.*, 1999) and largely consist of decimetre-scale, interspecific, high-biomass aggregations termed multi-species clumps (Fedra *et al.*, 1976) or bioherms: one or more shelly hard substrates provide the base for sessile, suspension-feeding colonizers (mostly sponges, ascidians, anemones or bivalves), which in turn serve as an elevated substrate for additional vagile and hemi-sessile organisms (mostly brittle stars and crabs) (Zuschin and Pervesler, 1996). The

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Oxygen depletion under glass: Behavioural responses of benthic macrofauna to induced anoxia in the Northern Adriatic

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Abstract

The Northern Adriatic Sea experiences seasonal low dissolved oxygen (DO) events and mortality of benthic organisms. The onset and extent of such disturbances, however, are difficult to forecast and study in the field. We address this problem by deploying a device that artificially induces and documents small-scale anoxia on soft-bottom in 24 m depth. Behaviour changes and mortality sequences of the soft-bottom macroepi- and infauna over a 5.5-day deployment were documented. An intervening oxygen peak created two phases of declining oxygen values. All initiated behaviours were interrupted during the reoxygenation and predictably re-appeared in the second phase, demonstrating the direct relationship between oxygen levels and behaviour. Beginning hypoxia (≤ 2.0 ml l⁻¹ DO) elicited escape patterns such as increased horizontal and vertical locomotion. Moderate hypoxia (≤ 1.0 ml l⁻¹ DO) triggered species-specific sublethal effects such as arm-tipping in ophiuroids or extension from the sediment in sea anemones. At severe hypoxia (≤ 0.5 ml l⁻¹ DO) infaunal organisms began to emerge and first mortalities occurred. The crustacean *Macropodia* sp., the sea urchin *Psammechinus microtuberculatus* and the brittle star *Ophiothrix quinquemaculata* were among the first to die; the sea anemone *Cereus pedunculatus* and the gastropod *Hexaplex trunculus* survived. Hydrogen sulphide (H₂S) increased until the end of the deployment to 163 μ M; temperature in the chamber remained nearly constant at 18.5 °C. This design provides insight into species and community-level processes and is an important step in identifying potential indicator species for low DO events in the Northern Adriatic and elsewhere.

Keywords: Benthos; Epifauna; Hypoxia, Indicator; Infauna; Mediterranean

1. Introduction

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vagile and hemi-sessile organisms (mostly brittle stars and crabs) (Zuschin and Pervesler, 1996). The presence of a well-developed macroinfauna is expressed in the early designations (Schizaster chiajei-community) of the benthic communities here by Vatova (1949) and later authors (Gamulin-Brida, 1967; Orel and Mennea, 1969; Orel et al., 1987; Occhipinti-Ambrogi et al., 2002). Repeated low DO events, coupled with commercial fishing activities during recent decades, have severely and persistently changed the macroepifaunal community structure in the study area (Stachowitsch and Fuchs, 1995; Kollmann and Stachowitsch, 2001).

Even though the frequency and extension of hypoxic and anoxic zones are increasing worldwide (Diaz, 2001; Wu, 2002; Diaz and Rosenberg, 2008), their onset and extent remain difficult to predict. The quick course – within a few days – of benthic mortalities (Stachowitsch, 1984) further hinders full documentation. We therefore developed an underwater-chamber (EAGU, Experimental Anoxia Generating Unit) equipped with camera, flashes and a sensor array (Stachowitsch et al., 2007) to induce small-scale experimental anoxia in situ. This approach fully documents the complex processes and interactions expected in a community-level setting in the natural environment. This includes behavioural reactions on natural substrates, intra- and interspecific interactions (e.g. Riedel et al., 2008), and mortality sequences, all related to specific DO-thresholds.

During the experiment an intervening reoxygenation peak due to water intrusion created a two-phase oxygen decline. This enabled us to evaluate faunal responses to two successive hypoxia events. The hypothesis is that specific responses are interrupted during reoxygenation and re-appear upon renewed oxygen decline. This approach is a key step forward in compiling a catalogue of sublethal behavior patterns corresponding to the severity of hypoxia/anoxia as well as in identifying indicator species for oxygen deficiencies here and elsewhere.

2. Materials and methods

2.1. Study site

The experiment was conducted on a soft-bottom (poorly sorted silty sand) in 24 m depth in the Gulf of Trieste, Northern Adriatic Sea. The salinity was 38 ‰ and bottom water temperature averaged 18.5 °C. The deployment took place near the oceanographic buoy of the

Marine Biology Station, Piran (Slovenia), about 2 km offshore (45° 32' 55.68" N, 13° 33' 1.89" E), to avoid damage by commercial fisheries.

2.2. Experimental set-up and sampling

The EAGU (Experimental Anoxia Generating Unit) is a specially developed 0.5 m³ underwater device to artificially induce and document small-scale anoxia on the seafloor (see Stachowitsch et al., 2007 for detailed description). In short, the unit consists of two interchangeable bases (an open-sided aluminum frame and a plexiglass-chamber, both 50×50×50 cm) and a separate lid that houses a time-lapse camera, two flashes and a sensor array (DO, H₂S and temperature; Unisense®). Initially, the system documents behaviour during normoxic conditions: it is positioned for 24 h in its “open” configuration (aluminum frame plus instrument lid) over a macrobenthic assemblage (Appendix A, Fig. 2 a). In a second step, the frame is exchanged for a plexiglass chamber (“closed” configuration) and repositioned over the same assemblage for another 48-72 h. Here, the behavioural patterns of the animals at decreasing oxygen concentrations are documented.

Oxygen and H₂S sensors are Clark-type microsensors (outside tip diameter DO: 90-110 µm; H₂S: 40-60 µm) with a built-in reference and an internal guard cathode and anode, respectively. The temperature microsensor (outside tip diameter 180-220 µm) consists of a thermocouple within a tapered glass capillary. Oxygen and the temperature are measured approx. 20 cm above the sediment, H₂S at 2 cm. Images are produced at 6-min intervals, sensor data are logged every minute.

The EAGU was deployed for 5.5 days from 17-22 September 2005 over a benthic assemblage selected based on the presence of a wide range of representative organisms. Due to turbid water conditions only the closed configuration (plexiglass chamber) was used. DO fell continuously due the macrobenthic respiration, with an unexpected water intrusion leading to a temporary reoxygenation.

After the experiment, all intact organisms (living and dead) and biogenic structures on or embedded in the soft bottom were collected by hand and preserved in a 4 % formaldehyde:seawater solution. Due to the lengthier deployment (poor weather prevented retrieval), many more fragile organisms (e.g. brittle stars) had decomposed and could not be recovered.

2.3. Behavioural analysis

The experiment yielded a total of 1332 images (overall documentation time ca.133 h). We analysed image by image and further processed them into time-lapse sequences (MPG files) using the Adobe Premiere 6.5 program (film material available at <http://www.marine-hypoxia.com>). Fifteen species were selected for analysis based on the following criteria: visibility in situ to the camera (epi- and infauna), wide distribution in the Adriatic Sea and representative spectrum of taxonomic groups and ecological categories (mobility, feeding types) (Table 1).

We recorded whether the animals were visible/not visible (exposed, i.e. not hidden under/in a bioherm or in the sediment) and noted behaviours such as locomotion and body movements, interactions, and mortality. Species-specific categories included tentacle crown orientation (sea cucumbers), arm-tipping (brittle stars), presence of camouflage (regular echinoids) or body rotation (sea anemones).

Some behaviours were further subdivided into different states: horizontal and vertical displacements (locomotion) or minor and major extensions from the sediment (anemones). The recordings were evaluated as long as the animal was in view and clearly identifiable, i.e. until decomposition created poor visibility or until mortality or predation. Mortality was taken as the last locomotion or body movement observed, in some species (Table 2) plus two hours to account for a potential moribund phase.

Although we present only one deployment here, the initial evaluation of several other experiments confirms and strengthens the observation and conclusions made here. Moreover, we were able to observe several individuals of most species, the reoxygenation peak allowed us to observe virtually every behaviour twice, and the large number of photographs of each particular behaviour underlines the validity of the observed patterns.

2.4. Data analysis

Data were recorded as categories and analysed using the SPSS software package (version 11.5). Data were assigned to five DO categories: normoxia (>2.0 ml O₂ l⁻¹), beginning hypoxia (≤ 2.0 -1.01 ml O₂ l⁻¹), moderate (1.0-0.51 ml O₂ l⁻¹) and severe hypoxia (0.5-0.01 ml O₂ l⁻¹) and anoxia. Normal behaviours were those observed during normoxia or known from long-term observations in the study area. The Kruskal-Wallis test was used to determine if oxygen concentrations significantly changed behaviour. The Mann-Whitney-Wilcoxon test was chosen to compare the behavioural reactions between all oxygen categories.

3. Results

3.1 Sensor data

Immediately after deployment, DO concentrations continuously dropped from an initial 3.8 ml l⁻¹ – a value that is low but not unusual for the season – and reached beginning hypoxia within 8 h (Fig. 1). Moderate hypoxia occurred ~15 h, severe hypoxia ~19 h after deployment. Oxygen values then unexpectedly increased, quickly reaching normoxic conditions (2.6 ml l⁻¹ DO). Based on the suddenly turbid conditions inside the chamber, our explanation for this intervening peak is intruding water from the activity of a strongly ventilating burrowing organism (e.g. burrow restructuring by a thalassinidean shrimp). The oxygen values fell again until anoxic conditions were attained 51 h after deployment. In the following, we refer to the first phase of oxygen decline as “decline 1”, the second phase as “decline 2”. Once anoxia occurred, the H₂S values increased for about 9.5 h from 0 to 0.6 μM then rose steadily up until the end of the deployment to 163.0 μM after 5.5 days. The temperature in the chamber remained nearly constant at 18.5 °C.

3.2 Macrofauna responses

Declining oxygen values elicited significant behavioural responses in every case (Kruskal-Wallis test, Table 3). This included stress patterns such as initiation of movement or increased mobility, often directed towards higher substrates. The reactions to beginning and moderate hypoxia, interrupted during reoxygenation, re-appeared during the second decline (Fig. 1).

Note that the values in the normoxic category (Fig. 3 a'-m') may be artificially elevated because they include behaviours recorded during the reoxygenation (i.e. residual responses from decline 1 and re-initiated responses during decline 2).

Severe hypoxia and anoxia were reached in decline 2, with the first mortalities. After 9.5 h of anoxia, the sediment began to darken (Fig. 2), accompanied by increasing H₂S. Some taxa, such as anemones or gastropods, survived until the end of the experiment.

3.2. 1. Sessile epifauna

Calliactis parasitica (Couch, 1842). These symbiotic sea anemones (Fig. 2, “ca”) inhabit hermit crab-occupied gastropod shells. Of five species-specific behaviours (Table 1), three –

tentacle crown habitus, orientation and body rotation – are presented here. Under normoxia the tentacle crowns typically faced down, sweeping the sediment as the crab walks about. At beginning hypoxia, this orientation changed: the number of upwardly facing crowns observed per hour began to increase until, at severe hypoxia (decline 1), they faced upward in half the observations (Fig. 3 a). During the intermediate oxygen peak the crowns returned to their downward position until DO fell again during decline 2. The renewed reaction was identical to that at decline 1. This upward-orientation was maintained until the animals became moribund at h 77 (~26 h after the onset of anoxia; H₂S 51 µM), when they drooped to the side or faced downward. Between normoxia and severe hypoxia, the tentacle crowns were mostly open in all three *C. parasitica* (70-90% of observations; Fig. 3 a'). At anoxia this behaviour (<30% of observations) decreased significantly (Appendix A). The anemones also began to rotate (Fig. 3 b'), starting at moderate hypoxia and significantly increasing to 25% of observations at severe hypoxia and 30% at anoxia. One individual detached from the shell and fell to the sediment. All showed minor body movements until they died (see Table 2 and arrows in Fig. 3 a).

Cereus pedunculatus (Pennant, 1777). Two of these anemones (Fig. 2, “ce”) were in the sediment with the tentacle crown on the sediment surface. Body contractions (Fig. 3 b) were observed throughout the experiment, but increased at moderate hypoxia during the initial decline. At the reoxygenation peak, values dropped again to normal levels. During decline 2, contractions temporarily ceased. The type of contraction differed in the 2 phases: the initial type involved retraction into the sediment, those after the peak severe constriction or inflation of column diameter. Constriction or inflation increased strongly, remaining at high levels from h 69 (H₂S 18 µM) until the end. Tentacle crown closure (Fig. 3 c) paralleled that of contractions: it began at beginning hypoxia, ceased entirely at the intervening peak and reached higher values at anoxia. Rotation closely paralleled that of *C. parasitica*: it began at moderate hypoxia and increased significantly at severe hypoxia and anoxia (Fig. 3 b'). At the onset of hypoxia, *Cereus pedunculatus* extended from the sediment (Fig. 3 c'), beginning with minor extensions. As oxygen values dropped, minor, followed by major, extensions significantly increased. Both individuals survived.

Microcosmus sulcatus (Coquebert, 1797). This ascidian, part of a multispecies clump consisting mainly of sponges, sea anemones and bivalves (Fig. 2, “mi”), began to contract at beginning hypoxia (decline 1). This behaviour ceased during the reoxygenation and re-appeared during severe hypoxia at decline 2 (Fig. 3 d). It dropped off after anoxia and ceased when the animal became moribund at h 101 (H₂S 142 µM). During normoxia the siphons

were either closed (nearly 40% in 168 observations; Fig. 3 d') or half opened. With beginning hypoxia, siphons opened (almost 80%; Fig. 3 d'), accompanied by occasional siphon extension. At moderate and severe hypoxia, siphons began to close again (~50% open at severe hypoxia and anoxia). 128 h after deployment, the siphons drooped, which was interpreted as mortality (Table 2).

Phallusia mammilata (Cuvier, 1815). This ascidian (Fig. 2, “ph”), also part of the multi-species clump, started contracting at severe hypoxia. This activity abruptly ceased at the reoxygenation peak and began again at corresponding oxygen level during decline 2 (Fig. 3 e). At anoxia, contractions ended. The siphons were largely open under normoxia and beginning hypoxia (65 and 75%, respectively; Fig. 3 e'), and values dropped markedly at moderate hypoxia (50%; $P < 0.01$; Appendix A) and severe hypoxia, reaching 30% at anoxia. 79 h after deployment (H_2S 56 μM) the siphons closed. At h 83, *P. mammilata* drooped and died (Table 2).

3.2.2. Hemi-sessile epifauna

Chlamys varia (Linnaeus, 1758). The mantle tissue of the two adjoining pectinid bivalves (on same bioherm as above, Fig. 2, “ch”) was visible from normoxia to moderate hypoxia and remained unchanged on the first oxygen decline and reoxygenation. At severe hypoxia (one individual) and anoxia (second individual) this tissue began to swell. At h 63 it was retracted and remained in this state until the individuals were no longer visible (Fig. 3 f). Both *C. varia* showed a normal (“open”) gape under normoxia and beginning hypoxia. Normal gapes significantly decreased at moderate hypoxia and again at anoxia (Appendix A; Fig. 3 f'). The sustained wide gape was interpreted as mortality at h 74 and 92 (Table 2).

Ocnus planci (Brandt, 1835). This sea cucumber was attached to the lower side of the multi-species clump (Fig. 2, “oc”). It began to actively elongate at hypoxia (decline 1). This behaviour abruptly ceased and then re-appeared during moderate hypoxia in decline 2 and briefly at anoxia (h 59 and 60; Fig. 3 g). During normoxia, *O. planci* usually remained in its sideward (horizontal) position with the tentacle crown half-closed or closed. With beginning hypoxia, upward-orientated crown positions increased markedly from ~30 to 75% ($P < 0.01$; Appendix A), peaking at moderate hypoxia (Fig. 3 g'). Thereafter, this position changed dramatically and, at anoxia, the crown faced downward in nearly 90% of observations. On day 4 the animal became moribund and died after 79 h (Table 2); the animal was considerably more elongated than at the onset.

3.2.3. Mobile epifauna

Hexaplex trunculus (Linnaeus, 1758). Locomotion and location of three individuals changed as oxygen values fell. Vertical locomotion to the chamber lid began at hypoxia (decline 1; Fig. 3 h), and horizontal movements on the sediment significantly decreased (Appendix A; Fig. 3 h'). Two *H. trunculus* fell from the lid during decline 1, but immediately moved to the top again. During the reoxygenation, two individuals were again visible on the sediment, but retreated upward at hypoxia in decline 2. At severe hypoxia (declines 1 and 2) all three individuals were out of view on top of the chamber (Fig. 3 h'). Finally, at anoxia (H_2S 41 μM), two individuals again fell down: one remained moribund on the sediment, the second disappeared from view. All three gastropods survived.

Paguristes eremita (Linnaeus, 1767). One of the three hermit crabs evaluated here was inside the symbiotic sponge *Suberites domuncula*, the remaining two inhabited *Hexaplex* spp. shells with either one or two *C. parasitica* as epibionts (Fig. 2, “pa”). Extension from the shell started at beginning hypoxia and intensified at the end of decline 1 (Fig. 3 i). This ceased during the reoxygenation and rapidly increased again during decline 2, peaking at anoxia (~80% in 777 observations; Fig. 3 i'). At h 85 (H_2S 94 μM), one *P. eremita* left its shell, moved horizontally and disappeared from view (a fourth, smaller *P. eremite* was also detected outside its shell towards the end of the experiment). The other two remained extended until they became moribund and died (Table 2).

Psammechinus microtuberculatus (Blainville, 1825). Until beginning hypoxia, all three sea urchins (Fig. 2, “ps”) were hidden under sponges. Two emerged during decline 1, the third shortly after the reoxygenation. Once emerged, all animals tended to keep moving – except during the intervening normoxia – until the onset of anoxia (Fig. 3 j). During the reoxygenation the two visible individuals hid again. Camouflaging behaviour, i.e. shell debris placed onto their upper, aboral surface, also changed: all urchins lost their camouflage, starting at moderate hypoxia and with significant decreases from moderate to severe hypoxia, and from severe hypoxia to anoxia ($P < 0.01$; Fig. 3 j'). All *P. microtuberculatus* died on day 3 at severe hypoxia and anoxia (Table 2).

Ophiothrix quinque maculata (Delle Chiaje, 1828). Initially, all brittle stars (Fig. 2, “op”) in the chamber were positioned on multi-species clumps and mostly stationary. Typical suspension-feeding behavior was rare (i.e. arms were on rather than elevated above the substrate). Beginning hypoxia elicited a change in arm-posture: the arms were increasingly directed upward. Moderate hypoxia, in contrast, triggered conspicuous arm-tipping in the four

evaluated individuals (Fig. 3 k; ~40% in 280 observations, Fig. 3 k'). This stress behaviour nearly ceased during the reoxygenation and started again during decline 2 but was less frequent (Fig. 3 k). At severe hypoxia, the brittle stars gradually became motionless, clinging moribund to the substrate or lying on the sediment. All individuals showed these sequences synchronously. They died at the onset of anoxia (Table 2), whereby one specimen was predated by an anemone (right-most arrow in Fig. 3 k; for details see Riedel et al., 2008).

3.2.4. *Mobile cryptic epifauna*

Macropodia sp. This decorator crab emerged from hiding at beginning hypoxia (Fig. 3 l) and actively moved (decline 1): it scrambled over the epibenthic faunal assemblage, contacting numerous ophiuroids and crustaceans. This activity markedly dropped during the reoxygenation, where the animal remained nearly immobile on the sediment next to a bioherm. Beginning hypoxia (decline 2) caused a second burst of activity, which ceased when *Macropodia* sp. became moribund at severe hypoxia. Fig. 3 l' shows an initial increase in locomotion, peaking at moderate hypoxia (~70% in 71 observations), followed by a gradual decrease, stopping entirely at anoxia. At h 46 the crab showed final leg movements and died one hour later (Table 2). This was the first mortality in the experiment.

Pilumnus spinifer (H. Milne-Edwards, 1834). During normoxia this predatory crab mostly hid inside the above bioherm (Fig. 3 m, m'). It reacted to the oxygen decline by emerging during beginning hypoxia and moved mostly vertically on the ascidian *P. mammilata* (Fig. 3 m). During reoxygenation the crab hid again and re-appeared at beginning hypoxia in decline 2. The animal died shortly thereafter (h 54, Table 2).

3.2.5. *Mobile infauna*

Glycera sp. This infaunal polychaete (Fig. 2, "gl") first became visible (head emerged) at h 64 ($H_2S \sim 0.5 \mu M$; Fig. 3 n). For nearly 1 h its anterior end ploughed through the sediment surface in different directions before it fully emerged. The worm then remained coiled at the same position for nearly 8 h, but continued to show body movements. It died at h 73 (Table 2).

Schizaster canaliferus (Lamarck, 1816). This infaunal sea urchin (Fig. 2, "sc") emerged from the sediment at h 50. It then moved horizontally across the sediment (Fig. 3 o) and, 12 h later, turned on its back (initial H_2S increase). Weak body and spine movements were observed until mortality (Table 2).

Ophiura spp. Three brittle stars emerged from the sediment. The first emerged at beginning hypoxia (decline 1), moved at irregular intervals, and was ingested by the anemone *C. parasitica* (arrow; Fig. 3 p). The second *Ophiura* spp. emerged at severe hypoxia during decline 2 and remained largely immobile on the sediment for ca. 9 h before it hid under a bioherm (h 53; anoxia, $H_2S \sim 0.6 \mu M$). It then, along with a third individual, briefly re-appeared before both disappeared from view (h 61).

4. Discussion

Oxygen-depleted dead zones are the most severe manifestation of ecosystem degradation in shallow coastal waters. Our knowledge about the behavioural responses and mortalities of macroepi- and infaunal organisms to oxygen depletion is not commensurate with the crucial role (e.g. filter- and suspension feeding, bioturbation) this fauna plays or with the impact of biodiversity lost. Our experimental approach provided results on the sequence of *in situ* reactions at both the species and community levels and related them to distinct oxygen concentrations.

4.1. Critical oxygen levels and behavioural reactions

Four types of reactions occurred: (1) increase or decrease in normal activities, (2) initiation of atypical behaviours, (3) emergence of cryptic and infaunal organisms, and (4) mortalities. These sequences reflect increasing response to declining oxygen values and/or to increasing duration of anoxia/ H_2S .

The intermediate oxygen peak underscored and supported our hypothesis that responses are related to particular oxygen levels and that they would be interrupted during temporary re-oxygenation. Virtually all organisms and behaviours showed a nearly identical symmetrical pattern of activity around the reoxygenation peak (compare Fig. 3 a-p). The responses were related to specific DO concentrations during both declines (Fig. 3 a'-m').

4.1.1. Beginning hypoxia ($\leq 2 \text{ ml l}^{-1}$ DO).

The initiation of responses at 2 ml l^{-1} DO is in line with recognized thresholds for macrobenthic fauna (reviewed in Diaz and Rosenberg, 1995). Thus, bioherm-associated crustaceans such as *Pilumnus spinifer* and *Macropodia* sp. emerged from their hiding places at 1.5 and 1.7 ml l^{-1} DO, respectively. The infaunal *Ophiura* spp. appeared on the sediment surface at 1.5 ml l^{-1} DO. Hypoxia triggered increased activity: the ascidians *Microcosmus*

sulcatus and *Phallusia mammilata* both showed a maximum frequency of widely opened siphons, accompanied by siphon elongation in *M. sulcatus*. Atypical postures included the upwardly orientated tentacle crowns in *Calliactis parasitica*. All these responses can be interpreted as avoidance patterns aimed at optimizing oxygen consumption.

The early emergence of bioherm-associated crustaceans and *Ophiura* spp. closely resembles that observed in the 1983 mortality event: the crabs *Pilumnus spinifer* or *Pisidia longicornis*, and *Ophiura texturata* individuals, first became visible on day 1 of that event (Stachowitsch, 1984). Elsewhere, Johansson (1997) observed increased swimming activity in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as oxygen concentrations fell. In crustaceans, a second factor might trigger emergence: death in bioherm components (e.g. sponges) no doubt accelerates stress behaviour of the associated fauna (Stachowitsch, 1991). Brittle star behaviour – initially increased mobility quickly followed by inactivity and a moribund state – parallels laboratory observations by Vistisen and Vismann (1997) for *Ophiura albida*. Our in situ community-level approach, however, demonstrated another lethal aspect of this behaviour: the compromised animal was predated by the anemone *Calliactis parasitica*. This sea anemone meets its oxygen requirements directly by uptake from the enteric water (vs. ectodermal uptake, with subsequent diffusion through the mesogloea; Brafield and Chapman, 1983). Consequently, upright crowns (vs. normal “sweeping” position) provide access to the better oxygenated water further above the sediment (Diaz and Rosenberg, 1995; Hagerman, 1998). The same holds true for siphon elongation in *Microcosmus sulcatus*, paralleling observations on *Molgula manhattensis* by Sagasti et al. (2001).

4.1.2. Moderate hypoxia ($\leq 1 \text{ ml l}^{-1}$ DO).

Chlamys varia first modified its behaviour at moderate hypoxia (alternatingly open and closed valves). Bivalves are generally resistant to hypoxia because they can reduce their metabolic activity and energy utilization (Widdows, 1987). Valve closure and lowered metabolism are initial strategies (Theede, 1973; Heinonen et al., 1997; Storey and Storey, 2004) allowing certain species such as *Corbula gibba* or *Arctica islandica* to survive in repeatedly hypoxia-stressed areas (Rosenberg and Nilsson, 2005; Hrs-Brenko, 2006). *C. varia* tended to open rather than close its valves, potentially reflecting pectinid adaptations to brief functional hypoxia, for example during swimming or jumping movements (Grieshaber and Gäde, 1977; Grieshaber et al., 1994). This behaviour, however, might be insufficient to survive prolonged environmental hypoxia (Carroll and Wells, 1995). This would support

Nicholson and Morton's (2000) statement about “scallop being frequently absent from regions subjected to hypoxia”. As in *Pilumnus spinifer*, the death of key bioherm components will impact *C. varia*.

Other behaviours initiated at moderate hypoxia include minor/major extension in *Cereus pedunculatus*, arm-tipping in *Ophiothrix quinquemaculata*, discarding of camouflage in *Psammechinus microtuberculatus* and body contraction in *Phallusia mammilata*. The body elongation and upward tentacle extension in *C. pedunculatus* parallels that observed for the same species in the 1983 mortality (Stachowitsch, 1984) as well as that for *Diadumene leucolea* in laboratory studies (Sagasti et al., 2001). Column and tentacle extension may maximize the surface area to volume ratio while minimizing diffusion distances within the tissues, improving oxygen delivery and consumption (Sassaman and Mangum, 1972; Shick, 1991). In the community framework, this behaviour also resulted in anemone predation of ophiuroids.

In the ophiuroid, arm-tipping and the accompanying uplifted disc are interpreted as an attempt to reach higher oxygen concentrations. Such humped postures have been recorded elsewhere during hypoxia in *Ophiura texturata* (Dethlefsen and von Westernhagen, 1983; Dries and Theede, 1974), *O. albida* (Baden et al., 1990), or *Amphiura chiajei* and *A. filiformis* (Rosenberg et al., 1991; Vistisen and Vismann, 1997).

Camouflage behaviour protects echinoids from predation, desiccation or UV-radiation (Verling et al., 2004; Dumont et al., 2007). It may reduce the sea urchin's ability to move, creating a trade-off between “additional ballast” and reduced environmental stress (Dumont et al., 2007). The fact that *P. microtuberculatus* discarded its camouflage suggests that the cost of holding the material by its tube feet at moderate hypoxia is too cost-intensive. The two contraction phases of the ascidian *P. mammilata* at this concentration range have two potential explanations. *Pyura praeputialis* (Evans and Huntington, 1992) and *P. mammilata* (Fiala-Medioni, 1979) are known to increase ventilation by mantle wall contractions. By squirting, the ascidians expel hypoxic water from the branchial cavity and refill it with normoxic water (Evans and Huntington, 1992). In our experiment, contraction also coincided with a migration of dozens of bioherm associated crustaceans to the highest point (oral siphon) of the ascidian. This might also trigger contraction. Future evaluations of other *P. mammilata* individuals will provide more insight.

4.1.3. Severe hypoxia ($\leq 0.5 \text{ ml l}^{-1} \text{ DO}$) and anoxia.

Some behaviours began at severe hypoxia, others at anoxia. *Calliactis parasitica* rotated much more at severe hypoxia, after a slight increase at moderate values (Fig. 3 b'). *Cereus pedunculatus* showed one peak of contraction activity at severe hypoxia during decline 1, but the second peak occurred after ~ 8 h following decline 2 after anoxia. In this species, the cause and the type of contraction differed: those prior to the reoxygenation (retraction into the sediment) reflected responses to passing mobile organisms, those after the peak (severe constriction or inflation) were anoxia induced.

The infaunal *Schizaster canaliferus* emerged directly before anoxia, *Glycera* sp. 14 h later. *Chlamys varia* retracted its mantle tissue 12 h after anoxia. Infauna emergence and behaviour agree with previous studies from various estuarine and coastal areas (e.g. Jørgensen, 1980; Pihl et al., 1992; Nestlerode and Diaz, 1998). In laboratory studies (Nilsson and Rosenberg, 1994), the echinoid *Echinocardium cordatum* emerged above 0.7 ml l^{-1} . *Echinocardium* spp. are particularly sensitive to reduced oxygen concentrations (Baden et al., 1990). Whether the emergence of *S. canaliferus* at lower DO values ($\leq 0.5 \text{ ml l}^{-1}$) reflects a species-specific difference or the more natural habitat conditions will be determined based on additional deployments: these show bulging sediment prior to emergence, indicating an earlier onset of stress reactions. Post-emergence behaviour of the two genera is very similar: they crawl across the sediment and turn over on their backs before they die (Nilsson and Rosenberg, 1994).

Polychaetes are among the taxa considered to be tolerant. Llansò (1991), for example, reports that *Streblospio benedicti* can survive severe hypoxia for at least 2 weeks but dies within 55 h of anoxia. *Malacoceros fuliginosus* is also known to extend from the burrows and begin undulatory body movements as oxygen values drop below about 0.5 ml/l (Tyson and Pearson, 1991). Similar to the above species, *Glycera* sp. also showed undulatory movements (but fully emerged on the sediment) before activity ceased abruptly. It died 22 h after anoxia, but this may also reflect increasing H_2S values at this point. The presence of sulphide shortens tolerance due to its toxic effects and varies from species to species (Hagerman, 1998). In laboratory experiments, Vismann (1990) exposed the polychaetes *Nereis diversicolor* and *N. virens* to either hypoxia or hypoxia combined with sulphide. In the latter combination, both species left the sediment in higher numbers and showed a higher mortality.

Regardless of the onset of an atypical behaviour, its frequency tended to increase during the experiment. For example, hermit crabs increasingly extended from their shells. Major

extensions by *Cereus pedunculatus* increased steadily from moderate hypoxia to anoxia, as did the locomotion of *Macropodia* sp. from normoxia to moderate hypoxia. Such reactions decreased abruptly at some point, typically corresponding to a moribund state, to mortality, or to a new atypical behaviour. The latter includes *Paguristes eremita*, which left its shell and moved about fully exposed rather than merely extending out further. Another example is detachment from the hermit-crab shell by *Calliactis parasitica*.

4.2. Mortality

Atypical behaviours almost always ended in a moribund state or mortality, which, like behavioural responses, occurred at various stages. The first mortality was not a direct response to oxygen deficiency: one *Ophiura* spp. was predated by a *Calliactis parasitica* on emerging from the sediment (decline 1; 0.6 ml l⁻¹ DO). This reflected a differential response to hypoxia by predator and prey. The same holds true for five *Ophiothrix quinquemaculata* individuals that were consumed by either *C. parasitica* or *C. pedunculatus* (Riedel et al., 2008).

The first organisms died at severe hypoxia: *Macropodia* sp. at h 47, the first *Psammechinus microtuberculatus* and *Ophiothrix quinquemaculata* individuals at h 49. At this point, H₂S values had not yet begun to rise so that mortality can definitively be attributed to hypoxia. Under anoxia (beginning at h 51), the successive mortalities were: *Pilumnus spinifer* (h 54), *Glycera* sp. (h 73), *Schizaster canaliferus* (h 77), *Ocnus planci* (h 79), *Phallusia mammilata* (h 83), *Calliactis parasitica* (h 79 - 86), *Chlamys varia* (h 74 and 92), *Paguristes eremita* (h 99 and 104) and *Microcosmus sulcatus* (h 128). H₂S values had begun to increase steadily and therefore mortality cannot be attributed to oxygen values alone. Not all individuals of a particular species died at the same time: the first *C. varia* died at h 74, the second at h 92. The three *P. microtuberculatus* died at h 49, 54 and 58 – a 9 h time difference. Additional deployments will shed more light on this phenomenon.

Anoxic conditions were present for the last 83 h and H₂S had reached 163 µM by the end of the 5.5-day experiment. Two species survived to the end: the anemone *Cereus pedunculatus* and the gastropod *Hexaplex trunculus*. *C. pedunculatus* was still very active at the end and probably had emerged from the sediment entirely with its pedal disc. One *H. trunculus* was still on the lid, the remaining two fell to the sediment: one continued to extend its foot and/or head and tentacles until the end, the other presumably retreated again to the lid.

Previous field and laboratory studies identify anemones as particularly tolerant to hypoxia. In Limfjorden (Denmark), for example, they were the most resistant species (Jørgensen, 1980). Wahl (1984) demonstrated the high tolerance of *Metridium senile* in laboratory experiments: 50% of the individuals tested survived up to 3 weeks of anoxia, and some were still alive after 40 days. In the 1983 mortality in the Adriatic, one week after the onset of the event, isolated anthozoan survivors included *Ragactis pulchra*, *Cerianthus membranaceus* and *Epizoanthus erinaceus* (Stachowitsch, 1984). Interestingly, all *C. parasitica* had died at this point, which corresponds with our present results. Sea anemones exhibit a number of adaptations for extended survival under hypoxia (Shick, 1991). This includes the use of anaerobic pathways and mechanisms for metabolic depression (Rutherford and Thuesen, 2005). Finally, behaviours such as elongation and peristalsis may increase exposure to the sea water both through the body wall and the coelenteron (Sassaman and Mangum, 1972; Shick, 1991). The lower survival rate of *Calliactis parasitica* versus *Cereus pedunculatus* might reflect different anaerobic pathways or the stiffer peripheral column mesogloea of the former, which may hinder oxygen diffusion (Brafeld and Chapman, 1983; Shick, 1991). We attribute the survival of *Hexaplex trunculus* to its tolerance to low DO (e.g. field observations of surviving individuals in the 1983 event; Stachowitsch, 1984) and to its ability to crawl up to the plexiglass lid and take advantage of the potentially somewhat higher oxygen values. Biochemical pathways to sustain energy production anaerobically are well developed in molluscs (Gäde, 1983; Brooks et al., 1991). In contrast, Wu (1982) found a decrease in dominance and abundance of predatory gastropods (*Murex trapa*, *Nassarius crematus*, *N. signinjorensis*, *Turricula nelliae*) along a hypoxic gradient in Tolo Harbour, Hong Kong, but this may reflect more stable, long-term gradients.

Overall, this experiment demonstrated the success of the EAGU concept on 4 levels: (1) The sensor data showed rapidly declining oxygen values followed by increasing H₂S values. (2) The behavioural response patterns of both benthic infauna and epifauna corresponded with previous field observations during a 1983 mortality event here, demonstrating that the experimental approach mimics more extensive oxygen crises (Stachowitsch, 1984, 1992). (3) The induced mortality was rapid, reflecting the course of the above event, in which N90% of the macroepifaunal biomass was lost within only 4 days. (4) The unexpected reoxygenation demonstrated that the observed behaviours – both within and among species - were related to specific, decreasing oxygen concentrations: all initiated behaviours were interrupted and then reappeared. The next step is to synthesize the results of this and additional deployments into a community-based scenario. This will ultimately allow us to draw conclusions on ambiental

oxygen conditions from behaviours observed in situ and will help to better determine the status of benthic systems exposed to hypoxia.

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Table 1

Selected behaviours evaluated during oxygen depletion.

Class/Genus	N	Life habit	Mobility	Feeding	Species-specific behaviours evaluated
Anthozoa					
<i>Calliactis</i>	3	epi	sessile	susp	crown habitus ¹ & orientation ² , body contraction & rotation, pharynx protrusion
<i>Cereus</i>	2	epi	sessile	susp	crown habitus ¹ , body contraction & rotation, pharynx protrusion, extension/sediment ³
Gastropoda					
<i>Hexaplex</i>	3	epi	mobile	carni	locomotion ⁴ , location ⁵ , foot habitus ⁶
Bivalvia					
<i>Chlamys</i>	2	epi	hemi	susp	valve gape ⁷ , mantle state ⁸
Polychaeta					
<i>Glycera</i>	1	in	mobile	carni	locomotion ⁴
Crustacea					
<i>Paguristes</i>	3	epi	mobile	omni	locomotion ⁴ , extension/shell ⁹
<i>Macropodia</i>	1	epi/cry	mobile	omni	locomotion ⁴ , fragmentation
<i>Pilumnus</i>	3	epi/cry	mobile	omni	locomotion ⁴
Holothuroidea					
<i>Ocnus</i>	1	epi	hemi	susp	crown habitus ¹ & orientation ² , body contraction & elongation, drooping
Echinoidea					
<i>Psammechinus</i>	3	epi	mobile	omni	locomotion ⁴ , spine droop, camouflage
<i>Schizaster</i>	1	in	mobile	depo	locomotion ⁴ , spine droop
Ophiuroidea					
<i>Ophiothrix</i>	4	epi	mobile	susp	locomotion ⁴ , fragmentation, arm-tipping
<i>Ophiura</i>	3	epi	mobile	omni	locomotion ⁴ , fragmentation, arm-tipping
Ascidacea					
<i>Microcosmus</i>	1	epi	sessile	susp	siphon habitus ¹ , body contraction, siphon extension, drooping
<i>Phallusia</i>	1	epi	sessile	susp	siphon habitus ¹ , body contraction, drooping

Life habit: epi = epifauna, in = infauna, cry = cryptic fauna. Feeding: susp = suspension feeder, carni = carnivore, omni = omnivore, depo = deposit feeder. Behaviour subdivisions: ¹open, half open, closed tentacle crown; ²up-, side-, downward orientated tentacle crown; ³minor, major extension from sediment, ⁴horizontal, vertical locomotion; ⁵bottom (sediment), top (on top of chamber); ⁶retracted, extended foot; ⁷closed, half open, normal, widely gaping valves, ⁸normal, swollen, retracted mantle tissue; ⁹normal, extended, out from shell. No superscript: absent/present.

Table 2

Criteria and time of death (surviving taxa omitted)* predated individual.

Class/Genus	N	Final activity	Time of death (h) no. individuals	DO (ml l ⁻¹)	H ₂ S (μM)
Sessile epifauna					
<i>Calliactis</i>	3	contraction/crown move.	79/81/86	0/0/0	57/69/96
<i>Microcosmus</i>	1	drooping	128	0	153
<i>Phallusia</i>	1	drooping	83	0	87
Hemi-sessile epifauna					
<i>Chlamys</i>	2	sustained wide gape+2 h	74/92	0/0	41/113
<i>Ocnus</i>	1	drooping	79	0	57
Mobile epifauna					
<i>Paguristes</i>	3	body movement+2 h	99/104/-	0/0	122/137
<i>Psammechinus</i>	3	locomotion+2 h	49/54/58	0.3/0/0	0/0.6/0.6
<i>Ophiothrix</i>	4	arm movement	49/51/53/55*	0.3/0.1/0/0	0/0/0.6/0.6
Mobile cryptic epifauna					
<i>Macropodia</i>	1	leg movement	47	0.2	0
<i>Pilumnus</i>	1	leg movement	54	0	0.6
Mobile infauna					
<i>Glycera</i>	1	body movment	73	0	33
<i>Schizaster</i>	1	locomotion+2 h	77	0	43
<i>Ophiura</i>	3	-	22* - -	1.8	0

- mortality not visible.

Table 3

Kruskal-Wallis test for significant differences in the species-specific behaviours shown in Fig. 3 during oxygen depletion.

Taxon	Variable	<i>N</i>	Chi-square	<i>df</i>	<i>P-value</i>
<i>Calliactis</i>	crown open	2366	783.745	4	< 0.001
	crown half open	2366	573.959	4	< 0.001
	body rotation	2366	266.081	4	< 0.001
<i>Cereus</i>	body rotation	2662	219.822	4	< 0.001
	extension minor	2662	877.735	4	< 0.001
	extension major	2662	2296.818	4	< 0.001
<i>Microcosmus</i>	siphon open	1202	68.740	4	< 0.001
	siphon closed	1202	130.109	4	< 0.001
<i>Phallusia</i>	siphon open	1311	168.412	4	< 0.001
<i>Chlamys</i>	valves gape normal	2138	1212.888	4	< 0.001
	valves widely gaping	2138	984.112	4	< 0.001
<i>Ocnus</i>	crown orientation up	1330	346.574	4	< 0.001
	crown orientation down	1330	936.120	4	< 0.001
<i>Hexaplex</i>	location top	2976	246.142	4	< 0.001
	locomotion horizontal	2976	207.373	4	< 0.001
<i>Paguristes</i>	extension from shell	1453	351.090	4	< 0.001
	out from shell	1453	89.368	4	< 0.001
<i>Psammechinus</i>	camouflage	2807	2388.686	4	< 0.001
<i>Ophiothrix</i>	arm-tipping	3135	614.716	4	< 0.001
<i>Macropodia</i>	locomotion (hori./verti.)	1329	185.149	4	< 0.001
<i>Pilumnus</i>	exposure (hidden)	1329	934.130	4	< 0.001

Bold numbers indicate statistically highly significant differences ($P < 0.01$).

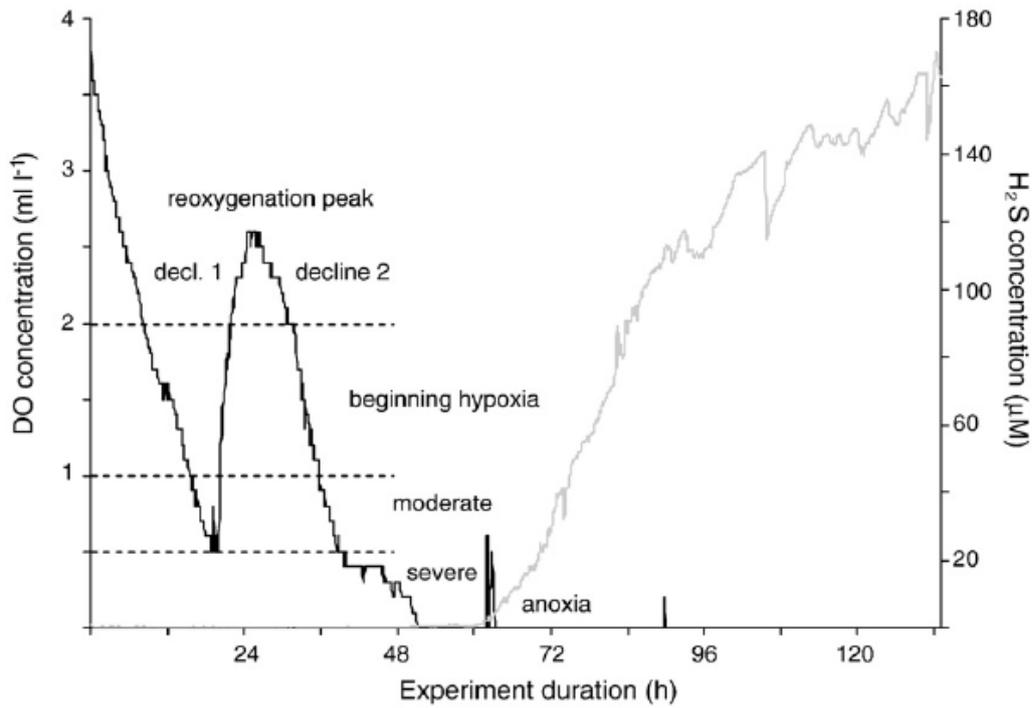


Fig. 1. Dissolved oxygen- (black line) and hydrogen sulphide concentrations (grey line) during the experiment (17 to 22 September 2005). Temperature remained nearly constant at about 18.5 °C (data not shown). Dashed lines: selected threshold values.

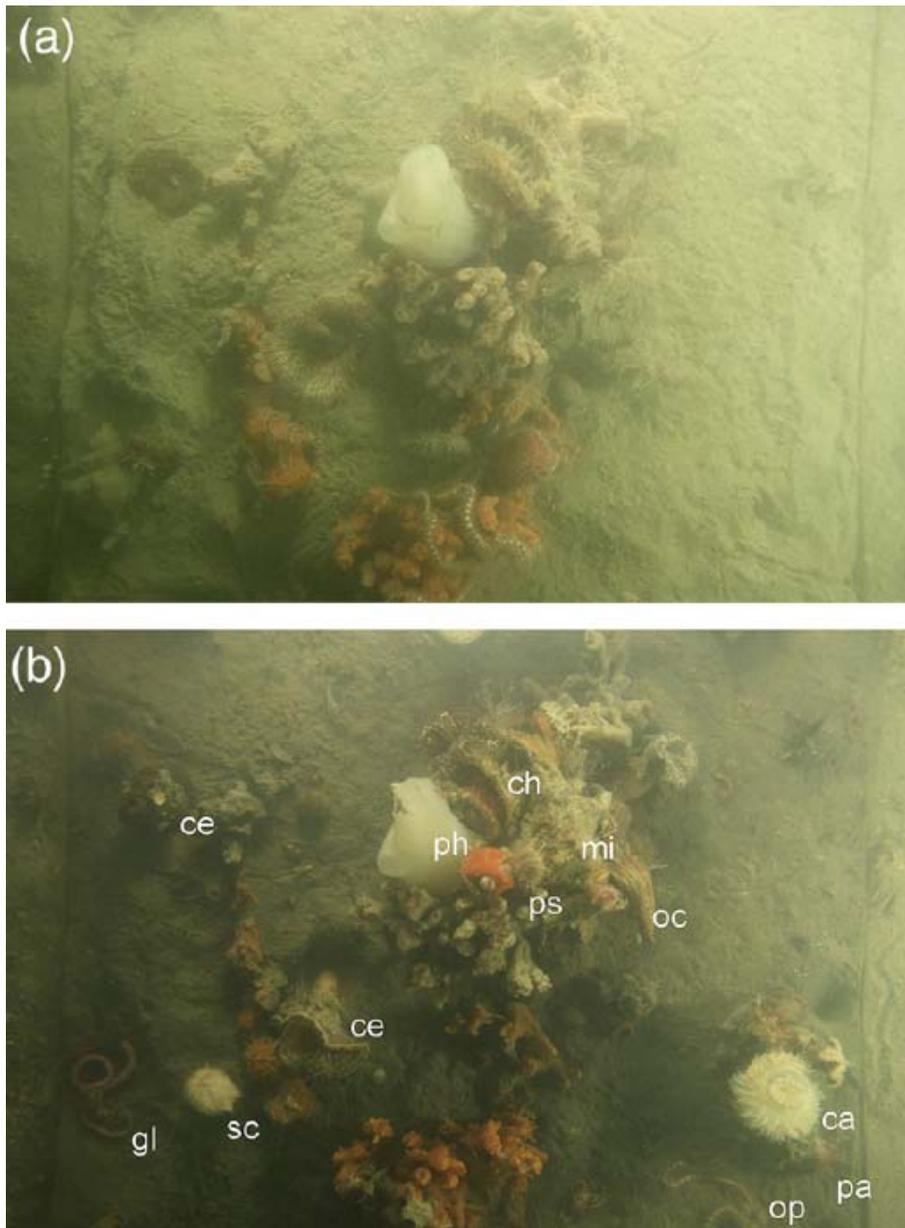
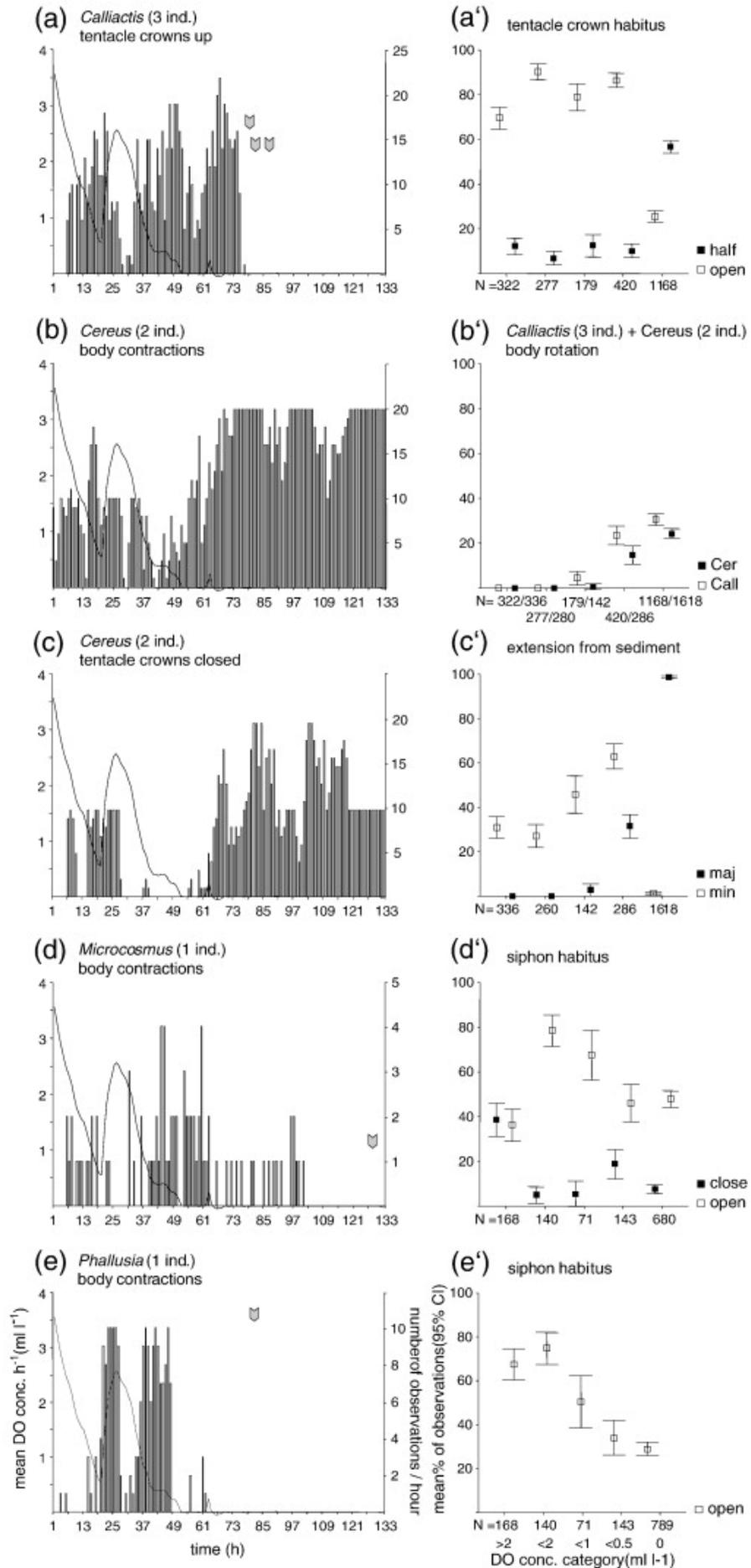
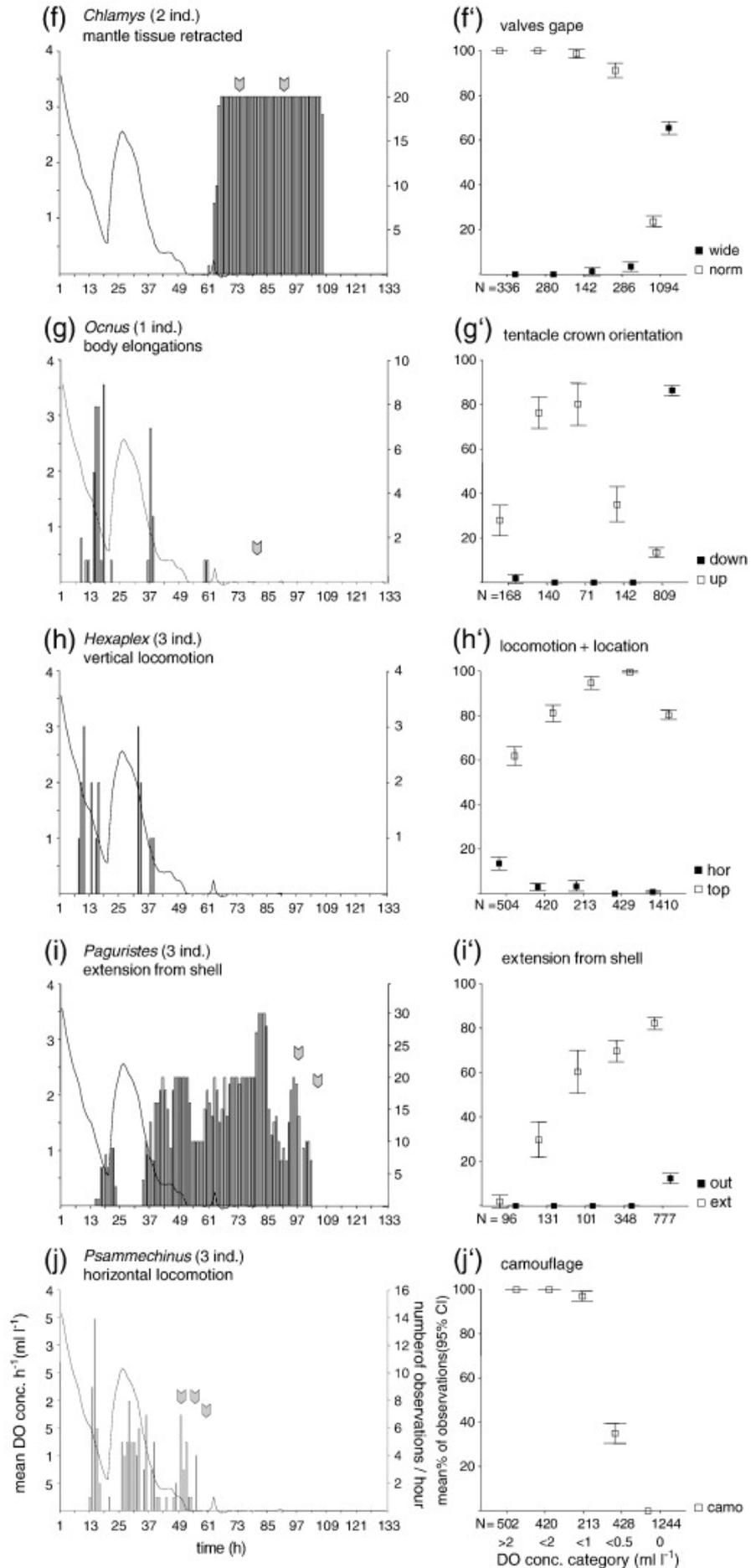
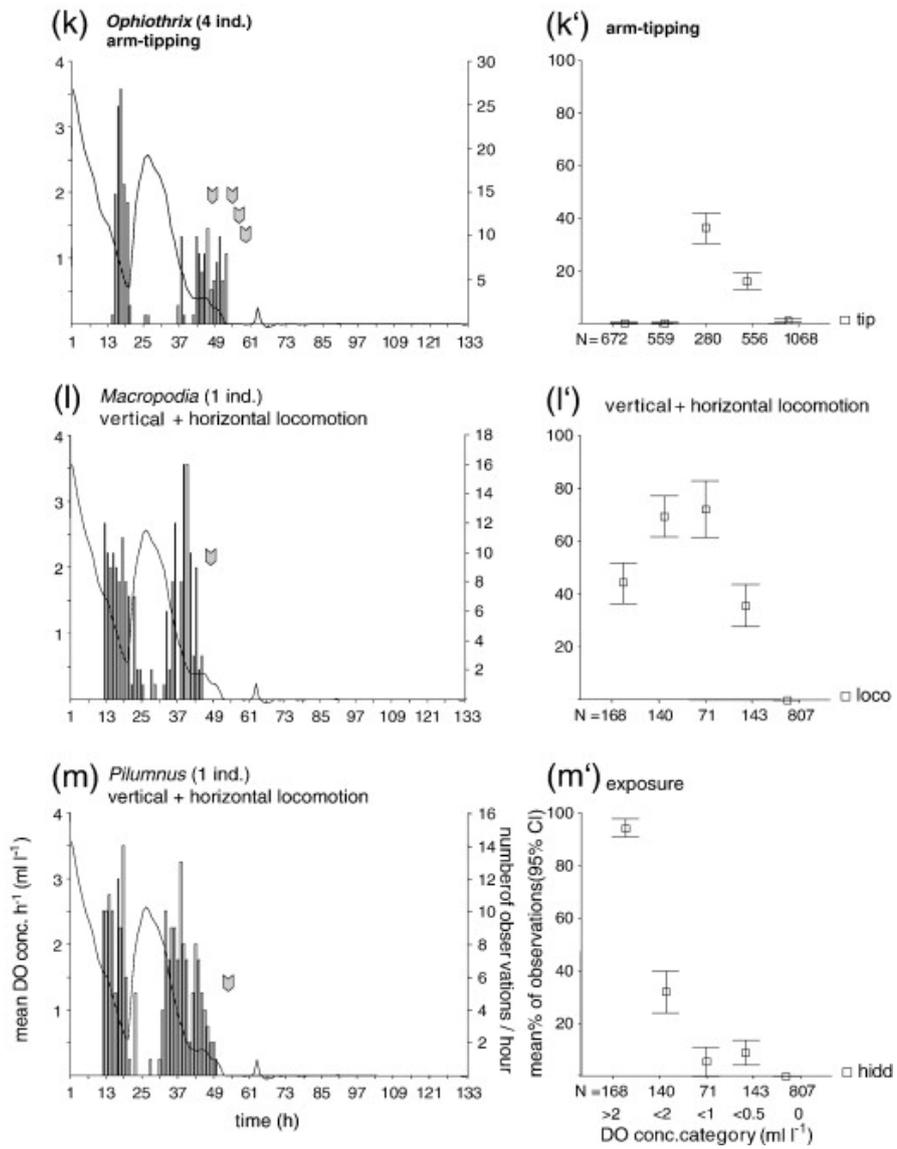


Fig. 2. Images taken on (a) day 1 of deployment (DO 2.6 ml l⁻¹) and (b) day 3 (anoxia, H₂S 29 μM) of the 5.5-day experiment. (ca) sea anemone *Calliactis parasitica* with upward-orientated tentacle crown, (ce) two anemones *Cereus pedunculatus* extended above sediment; (ch) two adjoining bivalves *Chlamys varia* widely gaped and with retracted mantle tissue; (gl) dead emerged polychaete *Glycera* spp. on sediment surface; (mi) ascidian *Microcosmus sulcatus* with extended siphons; (oc) holothurians *Ocnus planci* initially elongated then collapsed; (pa) hermit crab *Paguristes eremita* extended from shell, dead; (ph) ascidian *Phallusia mammilata*; (ps) echinoid *Psammechinus microtuberculatus* without camouflage; (op) brittle star *Ophiothrix quinquemaculata* and (sc) emerged irregular echinoid *Schizaster canaliferus* on sediment surface.







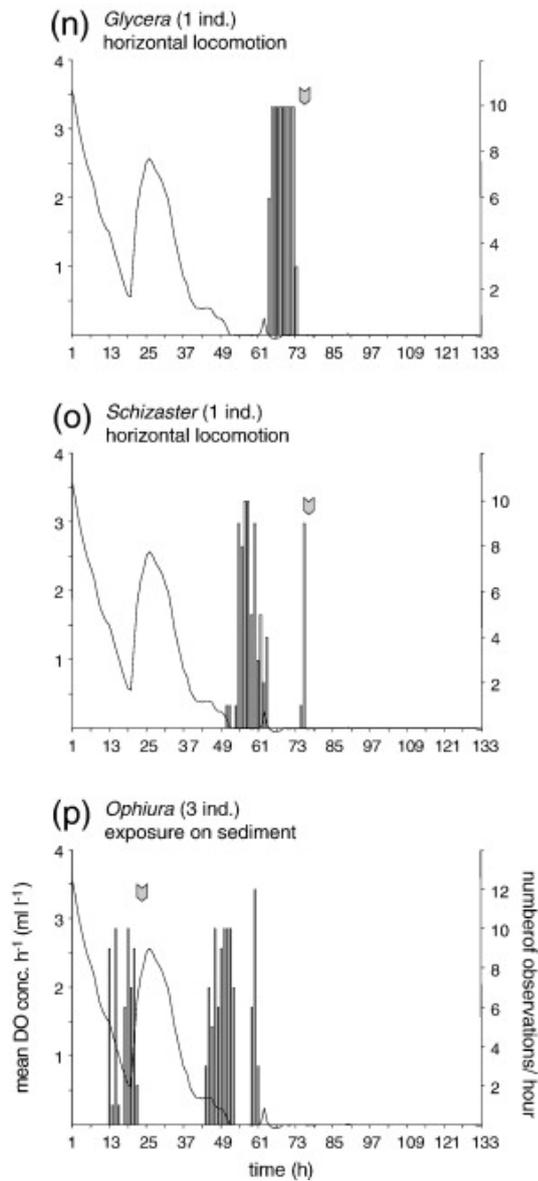


Fig. 3. Changes in species-specific behavioural responses during induced hypoxia and anoxia. Histograms (a-p) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour); note different scales on second y-axis. Arrow(s): time of death. Error bar diagrams (a'-m') show course of selected behaviours during five oxygen categories; N = the number of photographs evaluated per oxygen category. Cer = *Cereus*, Call = *Calliactis*, maj = major, min = minor, norm = normal, hor = horizontal, ext = extended, camo = camouflaged, tip = arm-tipping, loco = locomotion, hidd = hidden. The proportions of behaviours per oxygen category do not necessarily total 100% because not all behavioural states are included (see Table 1). For details see text.

Appendix A. Supplementary data

Mann-Whitney U test for significant differences in species-specific behaviour (Fig. 3) between the five oxygen categories. Numbers 1-5 under “comparison” refer to DO categories: normoxia, beginning (≤ 2.0 ml l⁻¹ DO), moderate (≤ 1.0 ml l⁻¹ DO) and severe hypoxia (≤ 0.5 ml l⁻¹ DO), and anoxia, respectively. Bold numbers: highly significant ($P < 0.01$); underlined numbers: significant ($P < 0.05$) differences.

Taxon	Variable	Comparison	Mann-Whitney <i>U</i>	Wilcoxon <i>W</i>	<i>Z</i>	<i>P</i> -value	
<i>Calliactis</i>	crown open	1 vs. 2	35348.5	87351.5	-6.259	<0.001	
		1 vs. 3	26094.5	78097.5	-2.289	<u>0.022</u>	
		1 vs. 4	56266.0	108269.0	-5.599	<0.001	
		1 vs. 5	105109.0	787805.0	-14.657	<0.001	
		2 vs. 3	21994.0	38104.0	-3.406	0.001	
		2 vs. 4	55874.0	144284.0	-1.582	0.114	
		2 vs. 5	56872.5	739568.5	-19.962	<0.001	
		3 vs. 4	34832.0	50942.0	-2.268	<u>0.023</u>	
		3 vs. 5	48547.5	731243.5	-14.198	<0.001	
		4 vs. 5	95914.0	778610.0	-21.688	<0.001	
		crown half open	1 vs. 2	42277.0	80780.0	-2.113	<u>0.035</u>
			1 vs. 3	28696.0	80699.0	-0.138	0.890
	1 vs. 4		66304.0	154714.0	-0.829	0.407	
	1 vs. 5		104182.0	156185.0	-14.186	<0.001	
	2 vs. 3		23396.0	61899.0	-2.006	<u>0.045</u>	
	2 vs. 4		56276.0	94779.0	-1.456	0.145	
	2 vs. 5		81207.0	119710.0	-14.917	<0.001	
	3 vs. 4		36698.0	125108.0	-0.843	0.399	
	3 vs. 5		58361.0	74471.0	-11.005	<0.001	
	4 vs. 5		131116.0	219526.0	-16.448	<0.001	
	body rotation		1 vs. 2	44597.0	83100.0	0.000	1.000
			1 vs. 3	27531.0	79534.0	-3.820	<0.001
		1 vs. 4	51681.0	103684.0	-9.352	<0.001	
		1 vs. 5	130410.0	182413.0	-11.394	<0.001	
		2 vs. 3	23683.5	62186.5	-3.546	<0.001	
		2 vs. 4	44458.5	82961.5	-8.717	<0.001	
		2 vs. 5	112185.0	150688.0	-10.620	<0.001	
		3 vs. 4	30409.5	46519.5	-5.582	<0.001	
3 vs. 5		77167.0	93277.0	-7.330	<0.001		
4 vs. 5		227916.0	316326.0	-2.747	<0.01		
<i>Cereus</i>		body rotation	1 vs. 2	47040.0	86380.0	0.000	1.000
			1 vs. 3	23688.0	80304.0	-1.538	0.124
	1 vs. 4		40992.0	97608.0	-7.268	<0.001	
	1 vs. 5		205968.0	262584.0	-10.089	<0.001	
	2 vs. 3		19740.0	59080.0	-1.404	0.160	
	2 vs. 4		34160.0	73500.0	-6.659	<0.001	
	2 vs. 5		171640.0	210980.0	-9.244	<0.001	
	3 vs. 4		17467.0	27620.0	-4.525	<0.001	
	3 vs. 5		87855.0	98008.0	-6.452	<0.001	
4 vs. 5	209296.0	250337.0	-3.545	<0.001			

extension minor	1 vs. 2	45248.0	84588.0	-1.034	0.301	
	1 vs. 3	20320.0	76936.0	-3.094	<0.01	
	1 vs. 4	32680.0	89296.0	-7.975	<0.001	
	1 vs. 5	190880.0	1500651.0	-20.446	<0.001	
	2 vs. 3	16176.0	55516.0	-3.830	<0.001	
	2 vs. 4	25708.0	65048.0	-8.547	<0.001	
	2 vs. 5	167696.0	1477467.0	-18.395	<0.001	
	3 vs. 4	16821.0	26974.0	-3.375	0.001	
	3 vs. 5	63642.0	1373413.0	-23.896	<0.001	
	4 vs. 5	88471.0	1398242.0	-31.465	<0.001	
	extension major	1 vs. 2	47040.0	86380.0	0.000	1.000
		1 vs. 3	23184.0	79800.0	-3.086	<0.01
		1 vs. 4	32928.0	89544.0	-11.110	<0.001
		1 vs. 5	3192.0	59808.0	-42.741	<0.001
		2 vs. 3	19320.0	58660.0	-2.818	<0.01
		2 vs. 4	27440.0	66780.0	-10.227	<0.001
2 vs. 5		2660.0	42000.0	-41.900	<0.001	
3 vs. 4		14488.0	24641.0	-6.734	<0.001	
3 vs. 5		4585.0	14738.0	-38.473	<0.001	
4 vs. 5		75527.0	116568.0	-33.170	<0.001	
<i>Microcosmus</i> siphon open	1 vs. 2	7798.0	17668.0	-6.9	<0.001	
	1 vs. 3	3992.5	6548.5	-5.1	<0.001	
	1 vs. 4	9632.5	19928.5	-3.8	<0.001	
	1 vs. 5	39472.0	271012.0	-10.4	<0.001	
	2 vs. 3	4938.5	14808.5	-0.2	0.845	
	2 vs. 4	8620.5	18490.5	-3.6	<0.001	
	2 vs. 5	46270.0	56140.0	-1.2	0.248	
	3 vs. 4	4404.0	6960.0	-2.6	0.010	
	3 vs. 5	23618.5	26174.5	-0.7	0.513	
	4 vs. 5	43229.5	274769.5	-4.1	<0.001	
	siphon closed	1 vs. 2	6790.0	20986.0	-7.4	<0.001
		1 vs. 3	4097.5	18293.5	-4.4	<0.001
		1 vs. 4	10829.5	25025.5	-1.8	0.079
		1 vs. 5	50476.0	64672.0	-2.7	<0.01
		2 vs. 3	4425.0	6981.0	-1.731	0.083
		2 vs. 4	6765.0	17061.0	-5.6	<0.001
2 vs. 5		33020.0	264560.0	-6.6	<0.001	
3 vs. 4		3987.5	14283.5	-3.0	<0.01	
3 vs. 5		19393.0	250933.0	-3.1513	<0.01	
4 vs. 5		47751.0	58047.0	-0.389	0.697	
<i>Phallusia</i> siphon open	1 vs. 2	10920.0	25116.0	-1.375	0.169	
	1 vs. 3	4941.0	7497.0	-2.501	<u>0.012</u>	
	1 vs. 4	7977.0	18273.0	-5.902	<0.001	
	1 vs. 5	40623.0	352278.0	-9.488	<0.001	
	2 vs. 3	3762.5	6318.5	-3.533	<0.001	
	2 vs. 4	5932.5	16228.5	-6.867	<0.001	
	2 vs. 5	29907.5	341562.5	-10.406	<0.001	
	3 vs. 4	4242.0	14538.0	-2.309	<u>0.021</u>	
	3 vs. 5	21972.5	333627.5	-3.761	<0.001	
	4 vs. 5	53528.0	365183.0	-1.228	0.219	
<i>Chlamys</i> valves gape normal	1 vs. 2	47040.0	86380.0	0.000	1.000	
	1 vs. 3	23520.0	33673.0	-2.178	<u>0.029</u>	
	1 vs. 4	43848.0	84889.0	-5.527	<0.001	
	1 vs. 5	43512.0	642477.0	-24.818	<0.001	
	2 vs. 3	19600.0	29753.0	-1.988	<u>0.047</u>	
	2 vs. 4	36540.0	77581.0	-5.056	<0.001	
	2 vs. 5	36260.0	635225.0	-23.332	<0.001	
	3 vs. 4	18817.0	59858.0	-2.935	<0.01	
	3 vs. 5	19483.0	618448.0	-17.956	<0.001	
	4 vs. 5	50712.0	649677.0	-20.993	<0.001	

	valves widely gaping	1 vs. 2	47040.0	86380.0	0.000	1.000
		1 vs. 3	23520.0	80136.0	-2.178	<u>0.029</u>
		1 vs. 4	46368.0	102984.0	-3.453	0.001
		1 vs. 5	63504.0	120120.0	-20.979	<0.001
		2 vs. 3	19600.0	58940.0	-1.988	<u>0.047</u>
		2 vs. 4	38640.0	77980.0	-3.154	<0.01
		2 vs. 5	52920.0	92260.0	-19.555	<0.001
		3 vs. 4	19882.0	30035.0	-1.231	0.218
		3 vs. 5	27932.0	38085.0	-14.545	<0.001
		4 vs. 5	59524.0	100565.0	-18.675	<0.001
<i>Ocnus</i>	crown orientation up	1 vs. 2	6062.0	20258.0	-8.454	<0.001
		1 vs. 3	2844.5	17040.5	-7.438	<0.001
		1 vs. 4	11065.0	25261.0	-1.367	0.172
		1 vs. 5	58184.5	385829.5	-4.616	<0.001
		2 vs. 3	4778.5	14648.5	-0.634	0.526
		2 vs. 4	5843.0	15996.0	-6.954	<0.001
		2 vs. 5	21048.5	348693.5	-16.336	<0.001
		3 vs. 4	2769.0	12922.0	-6.187	<0.001
		3 vs. 5	9568.0	337213.0	-13.732	<0.001
		4 vs. 5	45024.0	372669.0	-6.347	<0.001
	crown orientation down	1 vs. 2	11550.0	21420.0	-1.586	0.113
		1 vs. 3	5857.5	8413.5	-1.131	0.258
		1 vs. 4	11715.0	21868.0	-1.598	0.110
		1 vs. 5	10453.5	24649.5	-22.181	<0.001
		2 vs. 3	4970.0	7526.0	0.000	1.000
		2 vs. 4	9940.0	20093.0	0.000	1.000
		2 vs. 5	7700.0	17570.0	-21.417	<0.001
		3 vs. 4	5041.0	15194.0	0.000	1.000
		3 vs. 5	3905.0	6461.0	-17.260	<0.001
		4 vs. 5	7810.0	17963.0	-21.507	<0.001
<i>Hexaplex</i>	location top	1 vs. 2	85428.0	212688.0	-6.408	<0.001
		1 vs. 3	36000.0	163260.0	-8.938	<0.001
		1 vs. 4	67176.0	194436.0	-14.222	<0.001
		1 vs. 5	289764.0	417024.0	-8.263	<0.001
		2 vs. 3	38626.5	127036.5	-4.641	<0.001
		2 vs. 4	73354.5	161764.5	-9.258	<0.001
		2 vs. 5	293625.0	1288380.0	-0.380	0.704
		3 vs. 4	43435.5	66226.5	-4.341	<0.001
		3 vs. 5	128419.5	1123174.5	-5.155	<0.001
		4 vs. 5	243733.5	1238488.5	-9.826	<0.001
	locomotion horizontal	1 vs. 2	94584.0	182994.0	-5.721	<0.001
		1 vs. 3	48198.0	70989.0	-4.078	<0.001
		1 vs. 4	93522.0	185757.0	-7.897	<0.001
		1 vs. 5	310152.0	1304907.0	-12.310	<0.001
		2 vs. 3	44538.0	132948.0	-0.299	0.765
		2 vs. 4	87516.0	179751.0	-3.524	<0.001
		2 vs. 5	289950.0	1284705.0	-3.353	0.001
		3 vs. 4	44187.0	136422.0	-3.773	<0.001
		3 vs. 5	146401.5	1141156.5	-3.254	0.001
		4 vs. 5	300085.5	392320.5	-1.834	0.067

<i>Paguristes</i>	extension from shell	1 vs. 2	4547.0	9203.0	-5.345	<0.001
		1 vs. 3	2021.0	6677.0	-8.749	<0.001
		1 vs. 4	5436.0	10092.0	-11.747	<0.001
		1 vs. 5	7449.0	12105.0	-16.714	<0.001
		2 vs. 3	4589.5	13235.5	-4.660	<0.001
		2 vs. 4	13729.0	22375.0	-7.870	<0.001
		2 vs. 5	24256.0	32902.0	-12.717	<0.001
		3 vs. 4	15967.0	21118.0	-1.725	0.084
	3 vs. 5	30718.0	35869.0	-5.093	<0.001	
	4 vs. 5	118203.0	178929.0	-4.720	<0.001	
	out from shell	1 vs. 2	6288.0	14934.0	0.000	1.000
		1 vs. 3	4848.0	9999.0	0.000	1.000
		1 vs. 4	16656.0	21312.0	-0.5	0.599
		1 vs. 5	32688.0	37344.0	-3.6	<0.001
		2 vs. 3	6615.5	11766.5	0.0	1.000
		2 vs. 4	22728.5	31374.5	-0.6	0.540
2 vs. 5		44605.5	53251.5	-4.3	<0.001	
3 vs. 4		17523.5	22674.5	-0.5	0.590	
3 vs. 5	34390.5	39541.5	-3.7	<0.001		
4 vs. 5	118882.5	179608.5	-6.662	<0.001		
<i>Psammechinus</i>	camouflage	1 vs. 2	105420.0	193830.0	0.000	1.000
		1 vs. 3	51957.0	74748.0	-3.774	<0.001
		1 vs. 4	37901.0	129707.0	-21.499	<0.001
		1 vs. 5	0.0	774390.0	-41.773	<0.001
		2 vs. 3	43470.0	66261.0	-3.453	0.001
		2 vs. 4	31710.0	123516.0	-20.080	<0.001
		2 vs. 5	0.0	774390.0	-40.780	<0.001
		3 vs. 4	17365.5	109171.5	-14.855	<0.001
		3 vs. 5	3732.0	778122.0	-37.526	<0.001
		4 vs. 5	172294.0	946684.0	-21.958	<0.001
<i>Ophiothrix</i>	arm-tipping	1 vs. 2	187601.0	344121.0	-0.420	0.674
		1 vs. 3	60088.0	286216.0	-16.275	<0.001
		1 vs. 4	157132.0	383260.0	-10.524	<0.001
		1 vs. 5	355548.0	581676.0	-2.020	<u>0.043</u>
		2 vs. 3	49891.0	206411.0	-15.078	<0.001
		2 vs. 4	130525.0	287045.0	-9.758	<0.001
		2 vs. 5	295406.5	451926.5	-2.153	<u>0.031</u>
		3 vs. 4	62084.0	216930.0	-6.563	<0.001
		3 vs. 5	96872.0	667718.0	-18.767	<0.001
		4 vs. 5	252458.0	823304.0	-11.741	<0.001
<i>Macropodia</i>	locomotion (horizontal + vertical)	1 vs. 2	8792.0	22988.0	-4.431	<0.001
		1 vs. 3	4307.0	18503.0	-3.921	<0.001
		1 vs. 4	11005.0	21301.0	-1.500	0.134
		1 vs. 5	37929.0	363957.0	-19.603	<0.001
		2 vs. 3	4843.5	14713.5	-0.381	0.703
		2 vs. 4	6644.5	16940.5	-5.652	<0.001
		2 vs. 5	17350.5	343378.5	-24.946	<0.001
		3 vs. 4	3240.5	13536.5	-4.976	<0.001
		3 vs. 5	8070.0	334098.0	-24.794	<0.001
		4 vs. 5	37122.0	363150.0	-17.430	<0.001

<i>Pilumnus</i>	exposure (hidden)	1 vs. 2	5698.0	19894.0	-10.061	<0.001
		1 vs. 3	1088.5	15284.5	-12.773	<0.001
		1 vs. 4	5708.5	19904.5	-10.223	<0.001
		1 vs. 5	65215.5	391243.5	-4.439	<0.001
		2 vs. 3	3469.0	13339.0	-4.362	<0.001
		2 vs. 4	9917.0	19787.0	-0.157	0.875
		2 vs. 5	25227.0	351255.0	-21.602	<0.001
		3 vs. 4	3590.5	13886.5	-4.257	<0.001
		3 vs. 5	4141.5	330169.5	-26.568	<0.001
		4 vs. 5	25231.5	351259.5	-21.834	<0.001

Sea anemones and brittle stars: unexpected predatory interactions during induced in situ oxygen crises

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Abstract During oxygen crises, benthic faunas exhibit a series of behavioural patterns that reflect the duration and severity of the event. During artificially induced oxygen deficiencies at 24 m depth in the Northern Adriatic Sea, we photographically documented predation by the sea anemones *Cereus pedunculatus* (Pennant, 1777) and *Calliactis parasitica* (Couch, 1842) on the brittle star *Ophiothrix quinquemaculata* (Delle Chiaje, 1828). Five predatory events were recorded with four anemones during nine deployments totalling 817 h of observation. Under near-anoxic conditions, individuals of both actinians made contact with, pulled in and consumed the brittle stars. The duration of each predatory event was 1.5–7.5 h. In three of the five events, brittle star remains were regurgitated after an additional 2.0–12.5 h of digestion by the anemones. Our time-lapse sequences demonstrate that oxygen deficiency, beyond eliciting a series of specific behaviours in members of each species, also promotes previously unobserved interspecific interactions. Our results show that sea anemones are not only highly resistant to anoxia, but may also benefit by taking advantage of prey that are more vulnerable to anoxic conditions.

Introduction

The level of dissolved oxygen is a crucial environmental variable that has changed drastically in shallow coastal marine ecosystems worldwide (Diaz and Rosenberg 1995). “Dead zones”, caused by hypoxia (oxygen concentrations $<2.0 \text{ ml l}^{-1}$) and anoxia (no oxygen) in bottom-water layers, top the list of emerging environmental challenges (UNEP 2004), and the problem is likely to become worse in the coming years (Wu 2002).

The point at which benthic animals are affected by low oxygen concentrations varies, but first indications of stress generally begin to appear when oxygen drops below $2.0\text{--}3.0 \text{ mg l}^{-1}$ ($1.4\text{--}2.1 \text{ ml l}^{-1}$; Rabalais and Turner 2001). Direct effects of exposure to hypoxia such as migration, physical inactivity and mass mortalities are well documented (Stachowitsch 1984; Buzzelli *et al.* 2002; Montagna and Ritter 2006). Reproduction and growth may also be affected (Breitburg 1992; Miller *et al.* 2002; Stierhoff *et al.* 2006). Beyond these direct effects, there is increasing evidence for indirect effects (Eby *et al.* 2005) such as changes in competition and predation (Brante and Hughes 2001; Sagasti *et al.* 2001; Decker *et al.* 2004). Although mobile benthos are able to migrate out of the affected area, the less mobile fauna—unable to escape or avoid hypoxic waters—exhibit a series of behavioural patterns in response to decreasing oxygen concentrations (Mistri 2004). Infauna, for example, emerge from the sediment surface. Epifaunal organisms attempt to raise themselves above the hypoxic bottom layer, either by moving onto higher substrates (Stachowitsch 1991) or raising their bodies (i.e. arm-tipping brittle stars, siphon-stretching bivalves or tiptoeing crustaceans; reviewed by Diaz and Rosenberg 1995). These hypoxia-induced behaviours, however, may render the animals more vulnerable to predation (Wu 2002). They may

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The Northern Adriatic, a shallow (mean depth 35 m), semi-enclosed sea, has a long history of seasonal hypoxia and anoxia due to water column stratification (Justic 1987). Anthropogenically induced long-term eutrophication (Marchetti et al. 1989; Barmawidjaja et al. 1995) has increased the frequency, duration and severity of oxygen stress during recent

decades (Danovaro 2003; Lotze et al. 2006). Today, the Northern Adriatic is a case study for eutrophication with the full range of typical symptoms such as oxygen depletion, altered behaviour and mortality of benthic organisms, as well as marine snow events. Macroepifauna communities are widely distributed in the Gulf of Trieste (Fedra 1978; Zuschin et al. 1999). The community on the fine-sediment substrates at ~24 m depth largely consists of interspecific aggregations termed multi-species clumps (Fedra et al. 1976) or bioherms. The brittle star *Ophiothrix quinquemaculata* is one of the three designating species in this benthic community (*Ophiothrix-Reniera-Microcosmus* community) and is found almost exclusively on sponges and ascidians, with its arms extending upward in a suspension-feeding position. The sea anemones *Cereus pedunculatus* and *Calliactis parasitica* also are prominent members of this community. *Cereus pedunculatus* lives partially buried in the sediment with the tentacle crown on the sediment surface, although some are “integrated” into epifaunal aggregations. *Calliactis parasitica* shows a different life habit as a common mutualistic symbiont on gastropod shells occupied by hermit crabs, and as an epibiont on living gastropods (Stachowitsch 1980; Caruso et al. 2003). Little information is available on the prey composition of these two particularly abundant anemone species in the Mediterranean. Chintiroglou and Koukouras (1991, 1992), who studied the diet of both anemones, showed that *Calliactis parasitica* is a non-selective omnivorous suspension feeder, but also can remove food particles from the sediment through tactile tentacle motion. It preys upon a wide spectrum of organisms, particularly gastropods and crustaceans (Chintiroglou and Koukouras 1991). *Cereus pedunculatus* is an opportunistic omnivorous suspension feeder; due to its relatively short tentacles; this anemone cannot actively search for prey. It feeds almost exclusively on organic detritus and on motile prey that blunder into its tentacles. *Cereus* also feeds almost exclusively on crustaceans, mainly amphipods and decapods (Chintiroglou and Koukouras 1992); echinoderms have never been identified as prey items. Conversely, studies on predators of ophiuroids in shallow-water habitats have focused on demersal fish (Witman and Sebens 1992; Rosenberg and Selander 2000), various crabs and shrimps (Wurzian 1977; Aronson 1987; Makra and Keegan 1999), the Norwegian lobster (Baden et al. 1990a) and asteroids (Sloan 1980; Gaymer et al. 2002), but have never included actinians.

During 2005 and 2006, we artificially induced small-scale oxygen deficiencies in situ in the North Adriatic to study the effects of hypoxia and anoxia on the structure and behaviour of the macrobenthic fauna. This study, involving time-lapse photography and sensor measurements, is the first to document a unique predatory interaction between sea anemones and brittle stars.

Materials and methods

The fieldwork was carried out from mid-September to mid-October 2005, and July to mid-October 2006, using SCUBA diving techniques. The study site was approximately 2 km off Piran (Slovenia, Northern Adriatic Sea; 45°32.69`N 13°34.94`E) on muddy sand bottom at a depth of 24 m. Both years of fieldwork yielded a total of 10 complete deployments. All were conducted with the Experimental Anoxia Generating Unit (EAGU), a specially designed and constructed state-of-the-art underwater benthic chamber which artificially induces oxygen deficiencies in situ (Stachowitsch et al. 2007).

Experimental set-up

The EAGU was deployed for up to 4 days at a time, in two different configurations, to autonomously generate oxygen crises and quantify benthic responses. Initially the system was positioned for 24 h in its “open” configuration (50 × 50 × 50 cm open-sided aluminum frame plus instrument lid) over a selected macrobenthic assemblage to document behaviour during normoxic conditions. Assemblages were selected based on the presence of a wide range of representative organisms. In a second step, the aluminum frame was exchanged for a plexiglass chamber (“closed” configuration), open on the bottom and top and of the same size as the frame and repositioned over the same assemblage for another 48–72 h. Here, the behavioural patterns of the animals at decreasing oxygen concentrations were documented. The lid housed a digital underwater camera with time-lapse function and two flashes. It also accommodated two oxygen-, one hydrogen sulphide- and one temperature sensor, and the datalogger. The two oxygen sensors were mounted at different heights (2 and 20 cm, respectively) above the sediment in order to detect potential oxygen gradients in the enclosed water body. The hydrogen sulphide sensor was fixed at 2 cm height, and the temperature sensor at 20 cm height above the sediment. The camera was adjusted to produce a series of images at 6-min intervals, whereas the sensor values were logged every minute (Stachowitsch et al. 2007).

After each deployment, as many organisms and biogenic structures as possible on or embedded in the soft bottom were collected by hand. In many deployments, however, the decomposition was advanced, and/or the animals were no longer visible because of an amorphous black organic layer (see Fig. 5D in Stachowitsch et al. 2007); smaller or more fragile organisms such as brittle stars were often missed. In the case of the sea anemones,

some retracted deep into the sediment and could not be retrieved. The collected samples were carefully examined for surviving organisms in the laboratory and then were preserved in a 4% formalin: seawater solution.

Both sea anemones and brittle stars were common faunal elements enclosed by EAGU. The 10 deployments contained a total of 38 sea anemones (no individuals in deployment 9), of which we collected 29 individuals (Fig. 1a). They also contained at least 381 brittle stars (211 individuals were collected; Fig. 1b). The maximum density of the former was seven individuals/0.25 m² (surface enclosed by EAGU), the latter 65 individuals/0.25 m². We measured the largest column diameter, height and wet weight of all preserved (contracted) sea anemones. We also measured the expanded tentacle crown diameters of each individual in the films. The size (disc diameter and arm-tip to arm-tip length) and wet weights of the brittle stars were measured based on 40 individuals collected in deployment no. 7, which was ended before they started to decompose. We used these values for a representative size frequency distribution. Because fewer individuals were always collected than those visible in the images, we also counted all brittle stars based on individual frames of each film. Three randomly chosen frames were evaluated in the closed configuration. At least one of the evaluated frames was from the hypoxic phase, when more individuals were typically visible on the surface of sponges and other organisms. The highest number counted was chosen. Finally, we measured the disc and overall diameters of the five predated ophiuroids based on the images.

Data analyses

Sea anemones were present in 9 out of 10 deployments conducted in 2005 and 2006. The total documentation time in these 9 deployments was 817 h 6 min. Of a total of 8,290 images, 1,115 images were taken during the open, and 7,175 images during the closed EAGU configuration.

We viewed all time-lapse sequences to detect predatory events and then examined every image from the start to finish of such interactions. The predatory interactions were interpreted in the framework of the oxygen and hydrogen sulphide concentrations provided by the sensors. Dissolved oxygen (DO) concentrations during open EAGU configuration generally varied from 3.0–5.0 ml l⁻¹ (with occasional peaks after storm conditions). In all deployments, the closed chamber created hypoxic conditions within 24 h, and anoxic conditions in about 2 days. Dead organisms were not removed during the deployment; this simulated natural

mortality events and avoided disturbing the experimental set-up. It also promoted hydrogen sulphide (H₂S) formation in the chamber within 1 day of anoxic conditions. Temperature remained steady from start to finish in the early deployments (17–19°C), and thus was not measured in subsequent deployments. Time-lapse movies were produced from the still image series using the Adobe Premier 6.5 program. Statistical analyses were performed using the software package SPSS 15.

Anemone feeding behaviour

Anemone behaviour was recorded in categories that described reactions to decreasing oxygen and increasing hydrogen sulphide concentrations. For *Cereus*, behavioural categories included:

- tentacle crown habitus—tentacle crown either open (all tentacles fully expanded) or closed (animal retracted so that no tentacles visible).
- Extension—tentacle crown, normally expanded on the sediment surface, is elevated to different degrees above the surface (minor: slightly above the sediment; major: far above the sediment, with part of column clearly visible).
- body contraction—column diameter of extended animals severely constricted or strongly inflated at some level above the sediment.
- body rotation—rotating or swaying movement of highly extended individual.
- mouth and pharynx protrusion—mouth “puckered” or part of pharynx protruded (after McFarlane 1975).

For *Calliactis*, the above categories were modified: extension was deleted and tentacle crown orientation (i.e. away from or towards the sediment), as well as detachment from the substratum (a hermit-crab-occupied snail shell in the predation event recorded here), was added. Predation involved a clear sequence of events: feeding, digestion and regurgitation. The feeding response involved contact, pulling in of the prey, transfer to the mouth and ingestion itself. After contact was made, the captured food was held on the tentacles and moved to the mouth by ingestive movements, i.e. the tentacles shortened and bent, and the tentacle crown margin folded over toward the mouth. Feeding was completed when the last remnants of the brittle star arms were no longer visible and the anemones’ mouth was closed. Digestion (McFarlane 1975) or retention (Shick 1991) comprised the time between mouth

closure and the beginning of regurgitation. Regurgitation was complete when the remains of the brittle stars were ejected fully and the mouth closed again.

Results

Behaviour during normal oxygen concentrations

The percent areal coverage by individual anemone crowns as measured from the images ranged from 0.11 to 3.14% (Fig. 2a). This corresponds to an average crown diameter/anemone of 67.0 mm (range: 19–100 mm). The wet weights of the collected individuals ranged from 0.14 g in deployment 3 (one individual) to 24.73 g in deployment 5 (three individuals).

In all open EAGU configurations, we observed that the sea anemones *Cereus pedunculatus* or *Calliactis parasitica* contacted potential prey items such as small crabs. Anemones also contacted *Ophiothrix* individuals under normal oxygen conditions, but this never developed into predatory interactions. When *Cereus pedunculatus* contacted crabs or brittle stars, the tentacle crown mostly remained open, and the touched part sometimes slightly undulated. If the anemones retracted into the sediment, this lasted for only a few minutes. Contact with large, moving organisms such as the gastropod *Hexaplex trunculus* or hermit crabs caused prompt retraction—sometimes completely and for several hours—into the sediment. *Calliactis parasitica* reacted quickly, although less sensitively, to contact with other organisms: the crown undulated or retracted slightly. Complete contraction was observed only when the hermit crab that carried the anemones forced its way between closely adjoining multi-species clumps. One scenario was visible in all images: brittle stars, clinging to sponges or ascidians, generally maintained a safety distance of at least 1.0–2.0 cm from nearby anemones. If contact was made with an anemone, the ophiuroids immediately retracted their arms and typically moved a few centimetres away.

Behaviour during decreasing oxygen concentrations

Declining oxygen concentrations after the chamber was sealed elicited a clear change in the behaviour of ophiuroids towards sea anemones: contact with either anemone species no longer caused the brittle stars to flee. Some extended their arms to within millimetres of the

anemones. If touched, however, the anemones never showed any feeding patterns in these situations, but kept their tentacle crowns wide opened.

As oxygen values approached near-anoxia, a total of five predatory interactions (13% of the 38 sea anemones recorded) were observed in 3 out of 9 deployments. Four took place between *Cereus pedunculatus* and *Ophiothrix quinquemaculata*, and one between *Calliactis parasitica* and the brittle star (Table 1). Except during predation event 1 (deployment 1), hydrogen sulphide was absent. Predated brittle stars were significantly larger than a representative ophiuroid assemblage of one of the deployments (Fig. 2b; Mann–Whitney U test, $Z = -2.132$, $P = 0.033$). The average

individual wet weight was 1.31 g, range 0.10–2.48 g.

Predation events also tended to involve the largest sea anemones (Fig. 2a).

At the onset of each predation event, the anemones showed typical behavioural responses to oxygen stress, such as extension and rotation (Fig. 3a, b), swaying, or “mouth puckering”. These behaviours increased significantly at near-anoxic conditions ($<0.5 \text{ ml l}^{-1}$) in both species (Fig. 3, Table 2).

Most brittle stars initially responded to decreasing oxygen concentrations by arm-tipping (i.e. elevating their discs above the substrate and standing only on their arm-tips), but had already become motionless and either clung lethargically to the substrate or lay moribund on the sediment surface at the time of the predatory events.

All anemones that fed on brittle stars survived until the end of each deployment, whereas all the brittle stars died. In two deployments, a total of four *Calliactis parasitica* died, three of them in the longest one (depl. 1; 133 h 6 min closed configuration, retrieval delayed due to bad weather). No *Cereus* mortality occurred.

Predation event 1—*Cereus pedunculatus* and *Ophiothrix quinquemaculata*

The anemone raised its tentacle crown and began rotating in response to anoxia (Fig. 4a). It first caught one, then a second arm of the brittle star, which resisted being pulled in by clinging to its sponge perch about 10 cm away (42 min; Fig. 4b). Its detachment (Fig. 4c) and transport to the mouth occurred from one image to the next (6 min; Fig. 4d, e). The complete feeding phase (7 h 42 min; Table 1) was the longest documented. This was followed 2 h 6 min later by regurgitation of the mucus-enclosed remains (Fig. 4f), which fell onto the sediment. The anemone, showing extreme pharynx protrusion, continued to move its tentacles

crown until the end of the deployment 2.5 days later, despite anoxia and increasing hydrogen sulphide concentrations.

Predation events 2 and 3—*Cereus pedunculatus* and two *Ophiothrix quinquemaculata*

Both brittle stars had made contact with the anemone prior to the actual predation event: one was pulled from a sponge and remained attached to the anemone column for 90 min before fleeing onto its original sponge. The latter individual (event 2) was pulled from another sponge, contact was then interrupted and it remained on the sediment. Almost 2 h after lying motionless, it made a slight move. In the next image, the anemone swayed its column, bent its tentacle crown down and pulled in the brittle star. Body rotations and tentacle movements transported the prey towards its mouth. Feeding took more than 2 h (Table 1).

About 42 min after this predation event, the same anemone recaptured the other brittle star (event 3), which in the meantime had left the sponge and lay moribund on the sediment surface. Feeding (1 h 42 min) involved the same patterns as in event 2. Moreover, the anemone briefly touched an arm of a third *Ophiothrix* while it devoured the second individual. The anemone moved until the end of this deployment (ca. 3 days later; $\sim 104 \mu\text{Mol l}^{-1} \text{H}_2\text{S}$), but no regurgitation was observed.

Predation event 4—*Calliactis parasitica* and *Ophiothrix quinquemaculata*

This sea anemone was attached to a shell inhabited by the hermit crab *Pagurus cuanensis*, which already was overturned and largely inactive. Thus, the anemone had a considerably reduced radius of activity compared to the large areas it “sweeps” when the crab normally walks across the bottom. The brittle star lay moribund on an adjoining ascidian about 8 cm away. It was missing one arm, perhaps because a few images earlier it briefly (2 images long, i.e. 12 min) touched another *Calliactis* specimen on the same shell.

Although initial contact was not visible, the anemone pulled the brittle star from the ascidian onto the sediment, where it landed upside-down, two arms already attached to the tentacle crown. The anemone then raised its crown and pulled the brittle star in. The ophiurid’s central disc was devoured in only 12 min; the remaining, projecting arms took another 4 h 42 min. Mucus-covered remains (disc and attached arm stubs) were regurgitated after 6 h. The anemone continued to move until the end of the deployment approximately 1.5 days later.

This event was concurrent with predation event 4. The extended anemone touched one arm of a moribund ophiuroid on the sediment. This 36 min contact elicited no visible predatory reaction: the anemone retracted partly into the sediment and undulated its crown. It then pulled the brittle star onto its tentacle crown and consumed it within 2 h. Half a day later, remnants were regurgitated in mucus and fell onto the sediment. The anemone moved until the end of the deployment (1.5 days later).

Discussion and conclusions

The sea anemones and brittle stars in our experimentally induced oxygen deficiencies repeatedly showed a series of distinctive, predictable sublethal and lethal responses that paralleled those observed in earlier benthic mortalities in the Northern Adriatic Sea (Stachowitsch 1984, 1991). All anemones responded similarly with elongation, expanded tentacle crowns, rotation, swaying and contraction. *Calliactis parasitica* additionally detached from their hermit-crab occupied shell. *Ophiothrix quinquemaculata* responded with arm-tipping and movement to higher substrates such as atop sponges and ascidians. Rising a few centimeters above the sediment surface has been observed in other studies (Baden et al. 1990b; Rosenberg et al. 1991; Sagasti et al. 2001) and is interpreted as an attempt to avoid low oxygen concentrations near the sediment surface.

With ongoing oxygen stress, however, the ophiuroids became less active and most ultimately left their perches. Most echinoderms are intolerant of low oxygen concentrations (Gray et al. 2002; Levin 2003), and our results confirm that *O. quinquemaculata* is a hypoxia-sensitive species in the Northern Adriatic Sea. All died long before the end of the deployments.

In contrast, all *C. pedunculatus* and six of ten *C. parasitica* individuals survived until the end of the deployments. This observation confirms in situ and laboratory studies that demonstrate that sea anemones are particularly tolerant to hypoxia and also can survive extended periods of anoxia (Jørgensen 1980; Wahl 1984; Stachowitsch 1991).

They can sustain their energy production when confronted with hypoxia by switching from aerobic to anaerobic pathways (e.g. opine and more efficient fermentations such as glucose-succinate or aspartate-succinate pathways; Shick 1991, p. 127). Moreover, sea anemones, like other cnidarians (scypho- and hydromedusae: Rutherford and Thuesen 2005), exhibit

metabolic depression—a down regulation of metabolic demand under hypoxic conditions. Finally, behaviours such as elongation and peristalsis may increase exposure to the sea water both through the body wall and the coelenteron (Shick 1991). The lower survival rate of the epifaunal *Calliactis parasitica* versus the infaunal *Cereus pedunculatus* could reflect a potentially different anaerobic pathway or the stiffer peripheral column mesoglea of the former, which may be a stronger barrier for oxygen diffusion (Shick 1991). Note that *C. pedunculatus* is zooxanthellate (Davy et al. 1997; Visram et al. 2006) and that symbiotic anemones even inhabit depths where irradiance is greatly reduced (Muller-Parker and Davy 2001). However, the potential role of this symbiosis in the eutrophic, 24 m depth here remains unknown.

While sublethal behavioural responses to declining oxygen concentrations are advantageous for surviving or avoiding hypoxic layers, they may be inappropriate for avoiding predation (Diaz and Rosenberg 1995). Large foragers can migrate into and out of hypoxic areas and thus increase their predation efficiency (Pihl et al. 1992). If, however, both predators and prey are affected, then relative tolerance will govern predation efficiency (Breitburg et al. 1994). Nestlerode and Diaz (1998) suggested that relative hypoxia tolerance might lead to selective predation on certain taxa, whereby the most intolerant prey will be exploited (Sandberg 1994).

Our results for *C. pedunculatus* and *C. parasitica* are in situ proof of this: by exploiting moribund ophiuroids, anemones switched to an alternative prey item apparently absent from their normal diet elsewhere in the Mediterranean (Greece: Chintiroglou and Koukouras 1991, 1992). This makes *O. quinquemaculata*—a highly abundant species in this community—an unexpected but potentially important secondary food source for *Cereus* and *Calliactis* during periods of oxygen stress. Our observations suggest that the anemones were able to take advantage of stressed ophiuroids only within a narrow range of low oxygen concentrations, and that the number of contacts under all other conditions was not indicative of successful predatory interactions. In the Northern Adriatic, we never observed predatory interactions between anemones and ophiuroids during normal oxygen conditions, neither in our current EAGU experiments nor in earlier 16 mm time-lapse camera films (Fedra 1974). Those early films revealed that brittle stars actively and successfully avoided potential mobile predators, such as the sea star *Astropecten aurantiacus* and the hermit crab *Paguristes eremita*, fleeing before contact and maintaining a constant distance (Stachowitsch 1979). The brittle stars did not flee from all larger mobile epifauna: they climbed atop holothurians and used them as transportation. Here, we show that brittle stars, under normoxic conditions, avoided

encounters with anemones as well and maintained a safe distance of at least 1–2 cm. If they did make contact, they either retracted their arms and/or moved out of reach. If contact was made under normoxia or in the early phases of hypoxia, predation never occurred: all predation events took place during nearanoxia (Table 1). The activity of the anemones coupled with the moribund brittle stars increased predatory feeding efficiency and reduced prey escape. In one case (event 3), a still-active brittle star initially was able to escape an anemone. In predation event 1 (Fig. 4), the anemone also required 48 min to pull in the living prey from its sponge perch. Moribund brittle stars, however, were pulled in without resistance: they were no longer able to use well-known post-contact avoidance strategies such as active flight or arm-autotomy (Wilkie 1978; Drolet et al. 2004). Our results showed that the anemones which successfully consumed brittle stars were the largest within the respective deployments (Fig. 2a), and that the predated brittle stars were typically in the larger size categories

(Fig. 2b). The combined reach of large, rotating anemones and the overall diameters of larger brittle stars is one explanation for this correlation. Otherwise, there was no apparent correlation between number of anemones and number of brittle stars. We attribute this in part to benthic topography. The location of the anemones on the sediment, combined with the position of the brittle stars on the multispecies clumps, will play a role in determining predation events. Nonetheless, the fact that brittle stars tended to leave aggregations during hypoxia means that the configuration and size of the multi-species clumps themselves (we tended to choose larger aggregations) are less important. Accordingly, our observations do not reflect a “worst case scenario”.

Beyond mechanical stimuli, chemical stimuli are also generally required to elicit a complete feeding response in most cnidarians (Nagai and Nagai 1973; Elliott and Cook 1989; McFarlane and Lawn 1991). Chemical cues, emitted from damaged or dead individuals, can cause marine predators to act as opportunistic scavengers in lieu of their normal predatory role (Brewer and Konar 2005). Our study provides the first direct proof of this strategy in sea anemones. The combination of stimuli needed for attack, along with the normal resistance ability of the prey, could explain the relatively narrow oxygen concentration window for successful predation. Moreover, an ‘aerobic shutdown’ before switching to anaerobic metabolism (Shick 1991) may explain why the anemones fed on the ophiuroids only after a lengthier severe hypoxia.

The duration and extent of many behaviours are altered by stress (Abrams 1982). During hypoxia, for example, behaviour related to respiration can increase (siphon activity of

bivalves; Rosenberg et al. 1991), and those not related to respiration can decrease (the duration of amphipod swimming activity; Johansson 1997). This also pertains to prey-handling times, which take longer under hypoxic conditions (shore crab *Carcinus maenas*; Brante and Hughes 2001). In the present study, prey handling and consumption also appeared to be prolonged by anoxia. The feeding time of the *Cereus pedunculatus* individual that experienced the highest H₂S-concentrations and the longest anoxia (Table 1: predation event 1) was much longer than in the other individuals. The regurgitation itself also was nearly as twice as long. In contrast, the period between ingestion and regurgitation under anoxia was several times more rapid than in anemones under normoxic conditions, i.e. 1–2 days (Nagai and Nagai 1973; McFarlane 1975). Finally, anoxia also alters optimal foraging strategies, allowing the consumption of prey that would normally require longer handling and digestion times (Beddingfield and McClintock 1993).

In conclusion, our in situ experimental approach revealed a previously unreported predator–prey interaction. Considering the frequency of oxygen crises in the Northern Adriatic Sea, this type of predation may be a common event. Such predation reflects the different tolerances and behaviours of the species experiencing oxygen deficiency: the prey’s moribund condition and the predator’s tolerance favour these events. This, combined with the anemones’ considerable extension and body rotation in all directions, increases their chances to contact prey. Even though the window of opportunity is relatively narrow (i.e. after brittle stars reduce their activity but before they begin to decompose), it is apparently long enough to afford the anemones an advantage in these unstable environmental conditions. This may help explain why anemones are often a dominant element in the composition of post-mass mortality benthic communities in the Northern Adriatic Sea.

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Table 1 Predatory interactions between the two anemone species and the brittle star *Ophiothrix quinquemaculata*

Predation event	<i>Cereus pedunculatus</i>				<i>Calliactis parasitica</i>
	1	2	3	5	4
Deployment	1	8	8	10	10
	17–23/09/05	5–10/10/06	5–10/10/06	17–21/10/06	17–21/10/06
Feeding	7 h 42 min	2 h 18 min	1 h 42 min	1 h 42 min	5 h 6 min
Date	19 Sept.	7 Oct.	7 Oct.	19 Oct.	19 Oct.
Time	14:02–21:44	9:01–11:19	12:01–13:43	01:43–03:25	01:01–6:07
DO (ml l ⁻¹)	0	0	0	0.2	0.2
H ₂ S (μMol l ⁻¹)	0.6	0	0	0	0
Digestion	2 h 6 min	–	–	12 h 36 min	6 h 36 min
Regurgitation	60 min	–	–	36 min	24 min
Time (start)	00:02	–	–	16:13	12:55
DO (ml l ⁻¹)	0	–	–	0.1	0.1
H ₂ S (μMol l ⁻¹)	3.0	–	–	0	0
Total elapsed time ^a	10 h 48 min	–	–	14 h 54 min	12 h 24 min

Predation events 2 and 3: same anemone individual. All times are based on 6-min time-lapse intervals. Top DO and H₂S-concentrations refer to values at onset of feeding, bottom concentrations to onset of regurgitation

Dash no regurgitation observed

^a Initial contact to complete regurgitation

Table 2 Results of the Mann–Whitney U test for differences in the number of major extensions (Fig. 3a) and body rotations (Fig. 3b) in the five oxygen categories in deployment 1 and 10

	Deployment	Comparison	Mann–Whitney U	Wilcoxon W	Z	P -value
Major extension (see Fig. 3a)	1	1 vs. 2	1176.0	21630.0	0.000	1.000
		1 vs. 3	5796.0	19992.0	-2.180	<u>0.029</u>
		1 vs. 4	6048.0	20244.0	-10.380	<0.001
		1 vs. 5	22428.0	36624.0	-15.891	<0.001
		2 vs. 3	4830.0	14700.0	-1.991	<u>0.047</u>
		2 vs. 4	5040.0	14910.0	-9.616	<0.001
		2 vs. 5	18690.0	28560.0	-14.781	<0.001
		3 vs. 4	2699.0	5255.0	-6.788	<0.001
	10	3 vs. 5	10287.5	12843.5	-10.667	<0.001
		4 vs. 5	47810.0	58106.0	-3.991	<0.001
		1 vs. 2	5940.0	19635.0	1.503	0.133
		1 vs. 3	5857.5	19552.5	-2.131	<u>0.033</u>
		1 vs. 4	8910.0	22605.0	-16.213	<0.001
		1 vs. 5	577.5	14272.5	-18.755	<0.001
		2 vs. 3	2628.0	5329.0	-0.581	0.561
		2 vs. 4	4152.5	6853.5	-11.948	<0.001
Body rotation (see Fig. 3b)	1	2 vs. 5	363.0	3064.0	-15.753	<0.001
		3 vs. 4	4363.0	7064.0	-11.738	<0.001
		3 vs. 5	470.5	3171.5	-15.589	<0.001
		4 vs. 5	35121.0	123952.0	-6.937	<0.001
		1 vs. 2	105840.0	194250.0	0.000	1.000
		1 vs. 3	51660.0	178920.0	-4.372	<0.001
		1 vs. 4	83916.0	211176.0	-11.206	<0.001
		1 vs. 5	527184.0	654444.0	-8.861	<0.001
	10	2 vs. 3	43050.0	131460.0	-3.994	<0.001
		2 vs. 4	69930.0	158340.0	-10.288	<0.001
		2 vs. 5	439320.0	527730.0	-8.104	<0.001
		3 vs. 4	37180.5	59971.5	-6.025	<0.001
		3 vs. 5	232506.0	255297.0	-4.181	<0.001
		4 vs. 5	475953.0	3422331.0	-4.573	<0.001
		1 vs. 2	53955.0	176715.0	-1.503	0.133
		1 vs. 3	52470.0	175230.0	-3.995	<0.001
10	1 vs. 4	251212.5	373972.5	-10.635	<0.001	
	1 vs. 5	136620.0	259380.0	-8.812	<0.001	
	2 vs. 3	23323.5	47413.5	-2.139	0.032	
	2 vs. 4	111774.0	135864.0	-7.006	<0.001	
	2 vs. 5	60766.5	84856.5	-5.730	<0.001	
	3 vs. 4	115563.0	139653.0	-5.948	<0.001	
	3 vs. 5	62701.5	86791.5	-4.483	<0.001	
	4 vs. 5	386067.0	594402.0	-2.813	0.005	

Numbers 1–5 under “comparison” refer to oxygen categories $>2.0\text{--}0\text{ ml l}^{-1}$ DO, respectively. Bold numbers indicate highly significant ($P < 0.01$); underlined numbers indicate significant ($P < 0.05$) differences

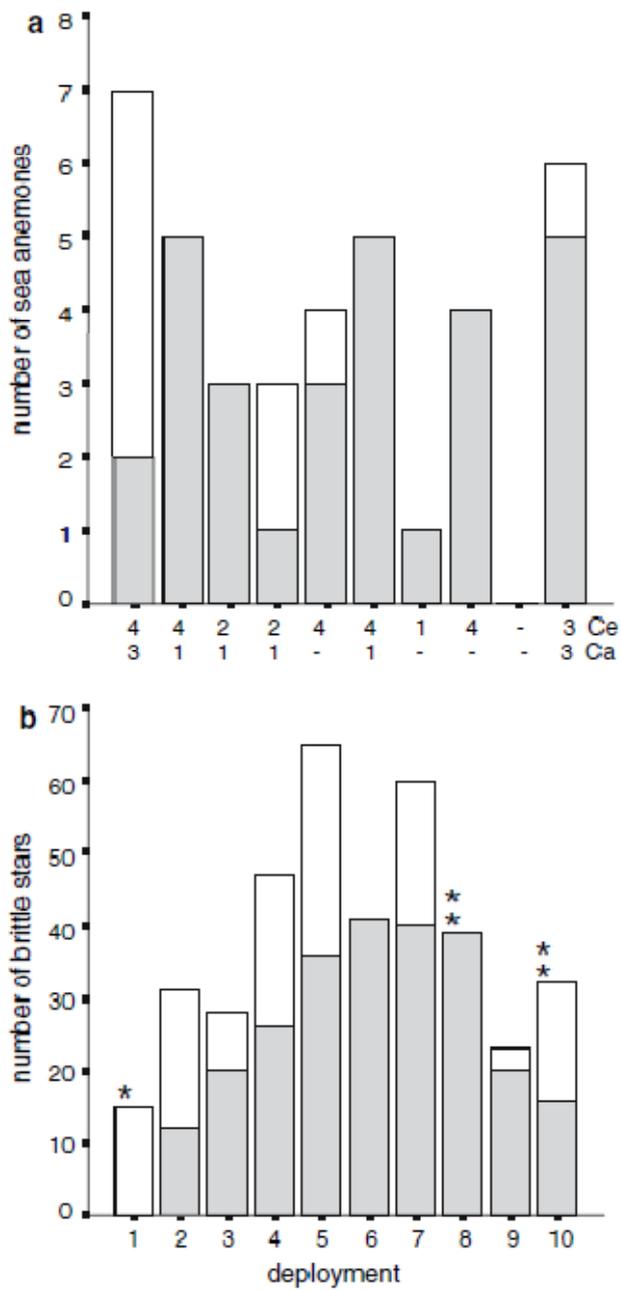


Fig. 1 Number of **a** sea anemones and **b** ophiuroids per deployment (area: 0.25 m²; Ce = *Cereus pedunculatus*, Ca = *Calliactis parasitica*). Shaded area represents number of collected specimens, open area total number of individuals visible in film. Asterisks indicate deployments with predation events

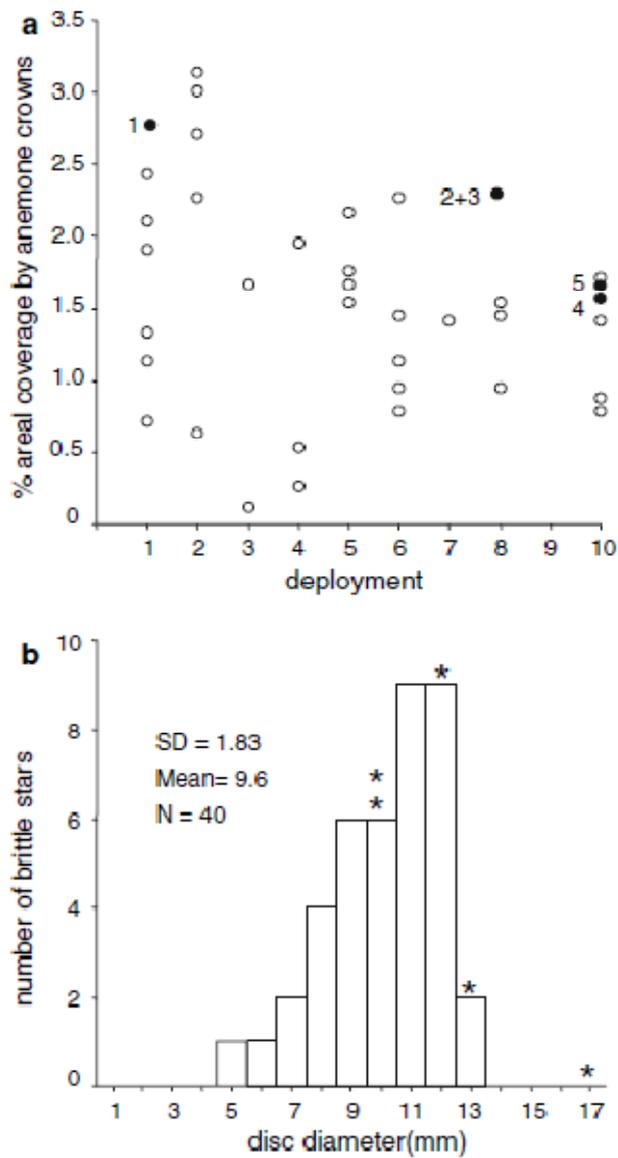


Fig. 2 a Percent areal coverage by crowns of all 38 sea anemones documented in the Wlms (tentacle crown diameter: average 67 mm, range: 19–100 mm). *Solid dots* represent predatory individuals, and *numbers* refer to predation events listed in Table 1. **b** Representative size–frequency distribution of adult *Ophiothrix quinquemaculata* based on 40 individuals collected in deployment 7. *Asterisks* indicate size class of the five predatory individuals in deployments 1, 8 and 10

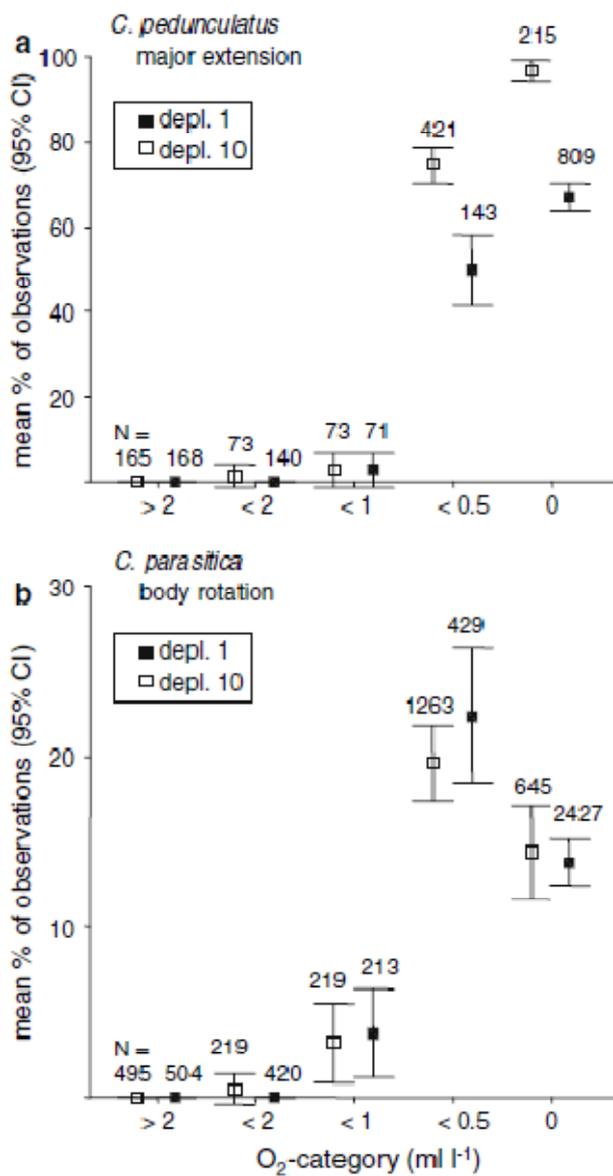


Fig. 3 Changes in sea anemone behaviour during the course of oxygen depletion in deployments 1 and 10 (the two evaluated entirely for all anemone behaviour). **a** Major extensions of four *Cereus pedunculatus* individuals from the sediment (two in each deployment). **b** Body rotations of six *Calliactis parasitica* individuals (three in each deployment). Numbers above error bars refer to number of photographs taken and evaluated per oxygen category

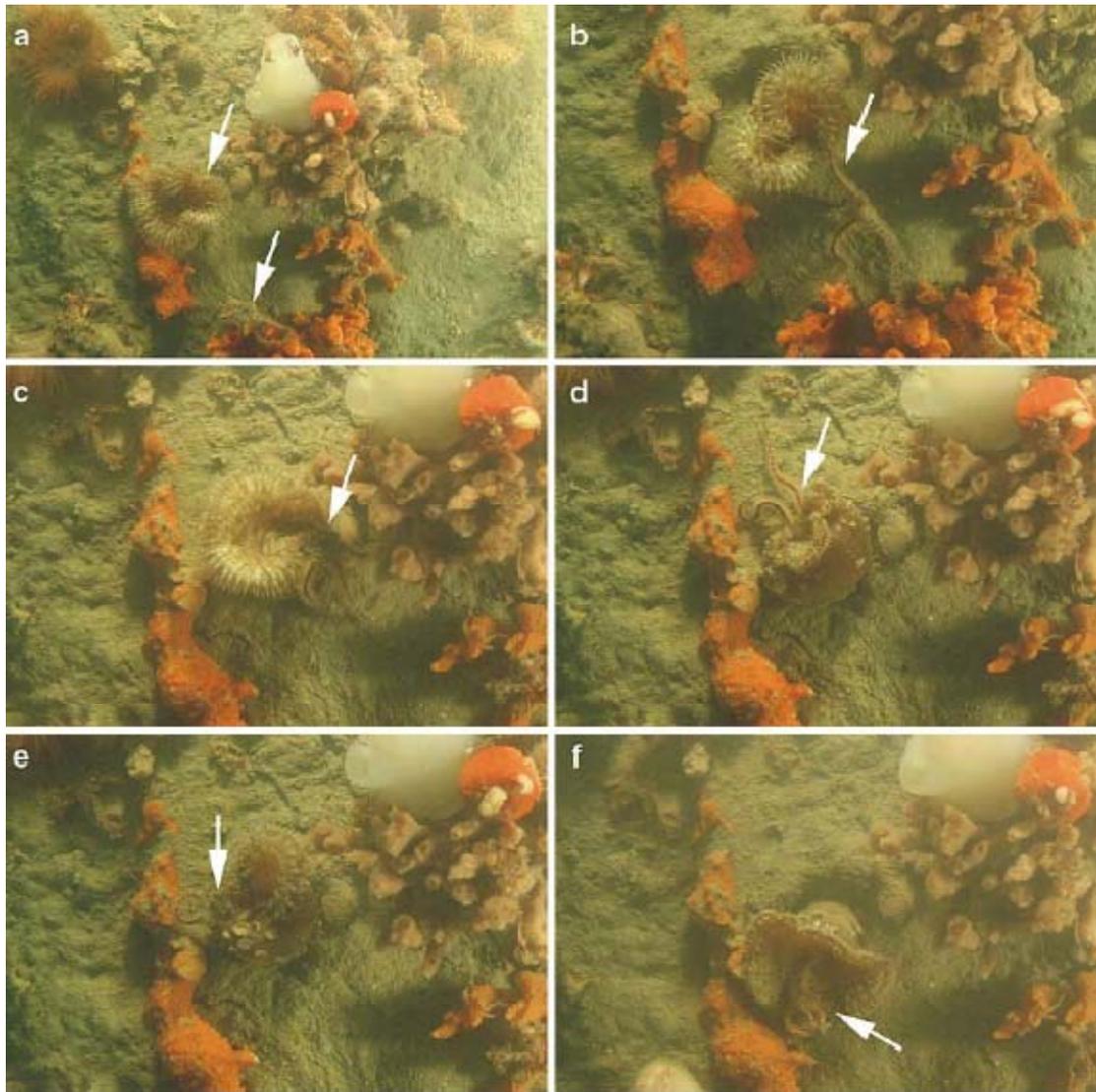


Fig. 4 Predation by *Cereus pedunculatus* on *Ophiothrix quinquemaculata* (predation event 1). **a** Overview of enclosed macroepifauna. *Cereus* (top arrow) with elevated tentacle crown and *Ophiothrix* (bottom arrow) on sponge about 10 cm away. **b** Anemone catches arm of brittle star, which holds onto sponge with one arm. **c** Brittle star pulled on to tentacle crown. **d** Anemone begins to ingest the prey. **e** End phase of feeding with arms still protruding from mouth. **f** Regurgitation of brittle star remains; note extension of anemone from sediment

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A scientist's work basically consists of questions and answers. In my thesis I tried to answer ecological questions, but the unanswered question is why I started it all...

My heart always "beat easy with the rhythm of the sea", although after my master thesis I shied away from a full scientific career. I searched for jobs but couldn't or maybe *didn't* want to find one. It was then that Michael "well well well" Stachowitsch with his fantastic story about a "cheese cover-like chamber, a state-of-the-art technique!", and Martin Zuschin with "these extremely interesting bioherms in the Northern Adriatic", planted the seed of doing research (while having a glass of tea in the old town of Aqaba). Their enthusiasm was enough to make me fall for university life straight away.

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[Le trio infernal]

Curriculum vitae – Mag. Bettina Riedel

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Private Details

Date of birth: 01/05/1974, Klagenfurt, Austria

Nationality: Austria

Education

- Since 07/2005 PhD, Title: “Oxygen crises in the Northern Adriatic: effect on the structure and behaviour of the macrobenthos”, Univ. of Vienna, Austria. Supervisors: Dr. M. Stachowitsch, Ao. Prof. Dr. M. Zuschin, Prof. Dr. J. Ott
- 08/2000-10/2001 MSc (Hons), major in marine ecology. Univ. of Vienna, Austria. Supervisors: Dr. M. Stachowitsch, Prof. Dr. J. Ott
- 1993-2000 Biology/ecology with special emphasis on marine ecology, Univ. of Vienna, Austria

Research Activities

- 10/2008 Project submission (P21542-B17), FWF/Austrian Science Fund
“Low dissolved oxygen events in the Northern Adriatic: *in situ* experimental insights into multi-level responses before, during and post-anoxia”; with Dr. M. Stachowitsch, Ao. Prof. Dr. M. Zuschin
- 01/2009-12/2010 ÖAD/Austrian Exchange Service
Bilateral project for “Scientific-technical Cooperation” between Slovenia and Austria (granted 11/2008)
- 03/2005-02/2009 Project (AP17655-B03), FWF/Austrian Science Fund
“Oxygen crises in the North Adriatic: effect on the structure and behavior of the macroepibenthos”; with Dr. M. Stachowitsch, Ao. Prof. D. M. Zuschin
Web: www.marine-hypoxia.com
- 09/2002 &
09/2000 Field research for the project “Reactions of a Northern Adriatic benthic community to repeated disturbances”; Marine Biology Station Piran (MBS), Slovenia; with Dr. M. Stachowitsch
- 06-10/1999 Laboratory work for the project “Marin Environmental Monitoring Program in the Persian Gulf 1999”; Texplor/Univ. of Vienna; with Dr. M. Stachowitsch, Dr. R. Kikinger

Teaching

Since 2007	Mentor of diploma students <ul style="list-style-type: none">• Dipl. Ing. A. Haselmair: “Experimentally induced anoxia in the Northern Adriatic Sea: behavioural responses and mortalities of benthic crustaceans”• A. Steckbauer: “Reactions of a macrobenthic community to anoxia: behavior and mortality sequences in a time-lapse camera experiment in the Gulf of Trieste”
WS 2008 & 2007	“Introduction to Marine Biology“ (selected lectures, with Dr. M. Stachowitsch & Dr. D. Abed-Navandi; 3 ECTS-Punkte)
SS 2008	“Marine Turtle Field Course“ (with Dr. M. Stachowitsch; 10 ECTS-Punkte)

Skills

Technical: DO/H₂S/pH/temp. microelectrode *in situ* measurements, CTD, water movement measurements (MR flowmeter, plaster dissolution, fluorescein), scientific photo- and film documentation, PADI-divemaster (since 2002), boat operator licence (since 2005)

Software-Applications: all MS-Office applications, webdesign, Endnote, Unix, statistics (SPSS, PRIMER, FISAT II), image processing (Adobe Photoshop, Adobe Premiere)

Languages

German: native

English: fluent

Italian: proficient

Conferences – Symposia - Workshops

2008

- ProMare, Univ. Vienna, Vienna, Austria, 27 Nov. (invited talk)
- World Conference on Marine Biodiversity (WCMB), Valencia, Spain, 11-15 Nov. (talk)
- Research meeting of Eurasia-Pacific-Uninet, Vienna, Austria, 5 Nov. (talk)
- 43rd European Marine Biology Symposium (EMBS), Ponta Delgada, Azores/Portugal, 8-12 Sept. (talk & poster)
- CIESM workshop (n°35; “Mediterranean biogeographic indicators of global warming“), Helgoland, Germany, 28-31 May (invited scientist, talk)
- Initialisierungsworkshop Plattform Biodiversität Forschung Austria (BDFA), Nationalpark Thayatal, Austria, 14-15 April

2007

- 15th meeting of the European Platform for Biodiversity Research Strategy (EPBRS), Porto, Portugal, 7-9 Nov. (invited Austrian scientist, poster)
- GSA Annual Meeting & Exposition, Denver, Colorado, USA, 28-31 Oct. (poster)
- The International Conference and the 97th Annual Meeting of the Geologische Vereinigung, Bremen, Germany, 1-5 Oct. (poster)
- 42nd European Marine Biology Symposium (EMBS), Kiel, Germany, 27-31 Aug. (2 posters)
- International Symposium on Integrated Coastal Zone Management, Arendal, Norway, 11-14 June (talk)

2006

- Marine Biology Institute, Piran, Slovenia, 2 Oct. (invited talk)
- 41st European Marine Biology Symposium (EMBS), Cork, Ireland, 3-10 Sept. (talk & 3 posters)
- 76. Jahrestagung der Paläontologischen Gesellschaft, Kiel, Germany, 28-30 Aug. (poster)

2005

- 40th European Marine Biology Symposium (EMBS), Vienna, Austria, 21-25 Aug. (2 posters)
- Congress assistance; 40th European Marine Biology Symposium (EMBS), Biocenter Althanstrasse 14; 1090 Vienna, 21-25 August 2005 (organised by Ott, Bright, Schiemer, Hohenegger & Zuschin)

Awards

2007 MARS-poster award at the 42nd EMBS (European Marine Biology Symposium), Kiel, Germany, 27-31 August 2007

Memberships

Since 2007 ProMare Advisor (Austrian non-profit organization to support marine science)
<http://www.promare.at>
Full member since 2002

Since 2007 Animal Behavior Society (ABS); <http://www.animalbehavior.org>

Further education

05-06/2008 Workshop “English in an European and International Context”, Personalentwicklung Univ. of Vienna, Austria

04-06/2006 Workshop “Presentations and Lectures in the University Context”, Personalentwicklung Univ. of Vienna, Austria

10/2004-09/2006 Master-level studies “Landscape design and architecture“, Univ. of Natural Resources and Applied Life Sciences, Vienna, Austria

Publications

Peer-reviewed journals

- Sawyer J., Zuschin M., Riedel B., Stachowitsch M. (in review). Predatory-prey interactions from *in situ* time-lapse observations of a subtidal mussel bed in the Gulf of Trieste (Northern Adriatic)
- Haselmair A., Stachowitsch M., Zuschin M., Riedel B. (in submission). Bioherm-associated crustacean response to low DO events in the Gulf of Trieste (Northern Adriatic Sea)
- Riedel B., Zuschin M., Haselmair A., Stachowitsch M. 2008. Oxygen depletion under glass: behavioural responses of benthic macrofauna to induced anoxia in the Northern Adriatic. *J. Exp. Mar. Biol. Ecol.* 367: 17-27; doi:10.1016/j.jembe.2008.08.007
- Riedel B., Stachowitsch M., Zuschin M. 2007. Sea anemones and brittle stars: unexpected predatory interactions during induced *in situ* oxygen crises. *Mar. Biol.* 153: 1075-1085; doi: 10.1007/s00227-007-0880-0
- Stachowitsch M., Riedel B., Zuschin M. Machan R. 2007. Oxygen depletion and benthic mortalities: the first *in situ* experimental approach to documenting an elusive phenomenon. *Limnol. Oceanogr.: Methods* 5: 344-352

Monographs

- Riedel B., Zuschin M., Stachowitsch M. (*CIESM Workshop Monographs*, no. 35; in press). Dead zones: a future worst-case scenario for Northern Adriatic biodiversity

Theses

- Riedel B., 2008. Oxygen crises in the North Adriatic: Effect on the structure and behaviour of the macroepibenthos. *Doctor thesis*, University of Vienna, Austria
- Riedel B., 2001. Selected macroepifauna as an indicator of disturbance in Northern Adriatic sublittoral soft bottoms. *Master thesis*, University Vienna, Austria

Public Relations (selection)

- 09/08/2008 Print medium: Die Presse; “Adria in Atemnot: Das Mittelmeer ringt nach Luft“
- 2007 Movie: “The 11th Hour“ (feature film documentary by L. DiCaprio)
- 15/12/2005 Television (Germany & Swiss): 3sat NANO; “Meeresboden unter Plexiglas untersucht Todeszonen“
- 12/11/2005 Print medium: Der Standard; “Der Adria geht langsam die Luft aus“
- 09/09/2005 Television (Austria): ORF Modern Times; “Meeresboden unter Glas“
- 26/07/2005 Print medium: Kurier; “Wenn dem Meer die Luft ausgeht“