MASTERARBEIT

Titel der Masterarbeit

A time-lapse camera experiment on benthic reactions to anoxia in the Northern Adriatic Sea

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

Project coordinators: Doz. Dr. Michael Stachowitsch & Ao. Prof. Dr. Martin Zuschin

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1. Introduction

Dissolved oxygen (DO) is among the most important environmental variables in coastal marine ecosystems. Oxygen is essential for animal life: in aquatic environments it is produced by plants, algae and cyanobacteria during photosynthesis, or dissolves from the atmosphere in the water, and it is used in respiration by all aerobic organisms. Once dissolved into the water, oxygen can mix down into bottom waters. Hypoxia/anoxia occurs if DO cannot reach the deeper layers or if the consumption rate in the bottom water is higher than the supply from the surface. Hypoxia is a condition of low dissolved oxygen that cannot sustain most animal life, and it is defined as DO values below 2 ml l^{-1} (Diaz, 2001). The susceptibility to oxygen depletion varies across benthic organisms, but generally from the point of hypoxia on they start to exhibit atypical behaviors. These include emergence of the infaunal species, migration, physical inactivity or unexpected inter- and intraspecific interactions (see Riedel et al., 2008a). If oxygen concentrations drop to 0 ml O_2 l^{-1} the conditions are designated as anoxic. In the absence of oxygen, microbes use other electron acceptors in their metabolism, such as NO_3, CO_2 or SO_4^{2-}. Hydrogen sulfide (H_2S) is the most important of the reduced compounds: it is toxic to metazoan life. This makes it difficult to separate its effect on marine fauna from the effect of oxygen deficiency. Most likely the two effects stress the organisms in an additive if not synergistic manner (Diaz & Rosenberg, 1995). According to Vaquer-Sunyer and Duarte (2010) the survival times in benthic communities under hypoxia are reduced by an average of 30% if they are exposed to H_2S.

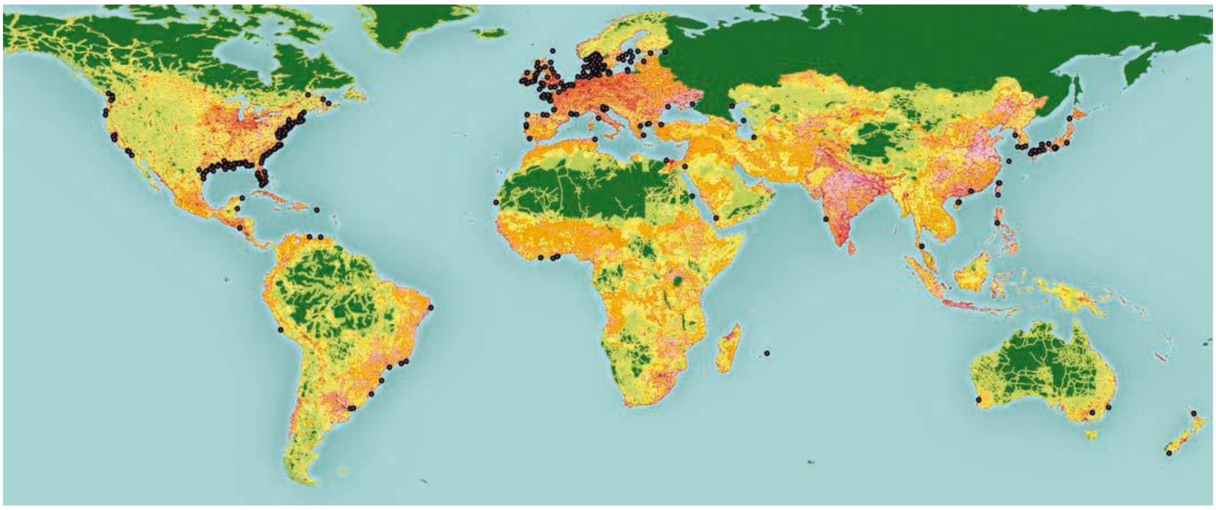


Fig. 1: Worldwide occurrence of eutrophication-associated dead zones (Diaz & Rosenberg, 2008)

Marine environments with low DO have existed throughout the history of the Earth, but their number is most likely increasing in shallow seas today (Diaz, 2001). Oxygen depletion, beyond triggering aberrant behavior, can lead to mortalities of benthic fauna. In severe cases this can lead to so-called dead zones. The extent of dead zones has increased exponentially globally since the mid-20th century, and at present a total area of more than 245,000 square kilometers, including more than 400 marine sites, are affected by this phenomenon (Fig. 1).

The causes of oxygen depletion (Fig. 2) include both physical processes, such as water column stratification which hinders the oxygenation of the bottom water layers, and excess nutrient input (Diaz, 2001). Stratification is a consequence of strong thermal or salinity gradients, including freshwater input from strong precipitation or flow-off from land. Nutrients are introduced either as a result of upwelling (a natural, wind-driven movement of deeper, cooler, nutrient-rich water towards the ocean surface) or as a result of anthropogenic activities. Eutrophication usually occurs where human populations or agricultural production are high (Levin et al., 2009). Increased nitrogen and phosphorus availability leads to enhanced production of dissolved and particulate organic matter, particularly in the pelagic subsystem in the form of plankton blooms (Stachowitsch, 1984). The sinking of planktonic material is followed by higher microbial respiration in bottom waters and thus reduces oxygen levels there (Gray at al., 2002). Climate changes may also affect the development of hypoxia (Conley et al., 2009): increased temperatures strengthen pycnoclines (a cline caused by a strong, vertical density gradient), enhance the respiratory oxygen demand of organisms and reduce oxygen solubility (Vaquer-Sunyer & Duarte, 2008). Considering the sources of hypoxia, the situation appears to be worsening in the recent decades (Wu, 2002; Rabalais et al., 2009). First, with the growing human population, a further increase of nutrient flux into coastal waters can be expected. A second phenomenon affecting the development of hypoxia is the global warming caused by greenhouse gases. The warming of the water leads to the expansion of thermoclines, and the increased air temperatures can be related to more intense freshwater input and to enhanced formation of haloclines. As a consequence, the extension and the number of the affected coastal sites is increasing (at an exponential growth rate of 5.54% year^{-1}, Vaquer-Sunyer & Duarte, 2008) worldwide.

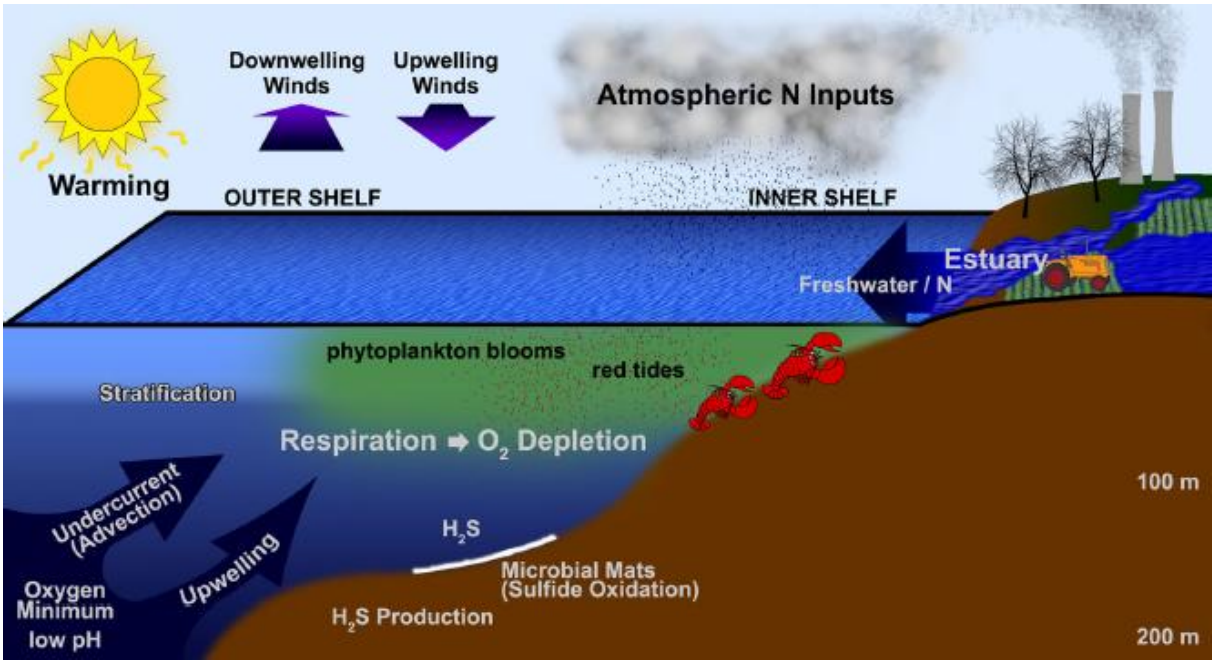


Fig. 2: Schematic representation of the source mechanisms of oxygen depletion (Levin et al., 2009).

The circulation pattern and geomorphology of certain marine environments can also facilitate the occurrence of hypoxic conditions. Our study site, the northern Adriatic Sea, combines many features associated with the development of low DO.

The Northern Adriatic Sea, one of the three main basins in the Adriatic Sea, extends from the Gulf of Trieste southward to the Ancona-Pula line. It is a shallow (mean depth about 30 m), semi-enclosed basin with seasonally changing hydrodynamic and biological conditions (Justić et al., 1987, Degobbis et al., 1995), and it can thus be classified as a sensitive ecosystem. The northern Adriatic Sea is the most eutrophic region of the Mediterranean Sea due to the high nutrient input, dominated by the Po River (Degobbis et al., 2000). The enhanced phosphorus loading (mainly originating from detergents and synthetic fertilizers) leads to increased primary production near the surface. The result is higher respiration in the bottom waters (Justić, 1987) due to the tight benthic-pelagic coupling in these shallow waters. The concentration of DO decreases significantly in the bottom layers, paralleling the intense nutrient inflow, especially when the water column stratification persists (Zavatarelli et al., 1998). Beyond the anthropogenic nutrient load, the freshwater flux of the Po changes the general circulation between the central and the northern part of the Adriatic and increases the water column stratification (Spillman et al., 2007). In the autumn and winter months the water circulation is mainly driven by thermohaline processes, modified by Bora or Sirocco winds (blowing northeasterly and southerly, respectively). The water body is separated into two parts: the western coastal waters are diluted by freshwater inflow, while the offshore waters are more dense and saline (Degobbis et al., 2000). The cold, low-salinity water flows along the western coast southwards (Western Adriatic Current), whereas the Eastern Adriatic Current transports warm, high-salinity water along the eastern coast northwards (Artegiani et al., 1997b). At this time, the water column is well mixed vertically, induced by the heat flux from the sea to the atmosphere. In spring, with the increasing surface temperatures, a thermocline starts to form. High freshwater injection increases the degree of stratification and the themohaline circulation becomes weaker, reducing the water exchange with the central basin (McKinney 2007). In summer the horizontal salinity gradients are less definite due to the negligible freshwater inflow (Degobbis et al., 2000) and the thermocline becomes stable due to the heat accumulation in the surface waters (Russo et al., 2005). The water column stratification hinders the exchange between the oxygen-rich surface water and the bottom layers, and hypoxia occurs. Hypoxic and anoxic events have been reported to occur in the northern Adriatic Sea periodically since the end of the 19th century, but in the recent decades they have increased in intensity, frequency and extension (Crema et al., 1991).

Three different types of hypoxia can be differentiated based on their occurrence and severity. In less than 20% of the hypoxic events worldwide, oxygen depletion is episodic, i.e. occurring less than once per year. Episodic oxygen deficiency is a first sign that a system has reached a critical point of eutrophication. Periodic oxygen depletion has been reported in around 25 % of the hypoxic/anoxic environments. In these cases, low DO conditions occur more than once per year, but they last a shorter period, from days to weeks. The most general form of eutrophication-induced hypoxia, responsible for about half the known dead zones, usually occurs once per year. This can begin in the summer after spring blooms and ends in autumn. During this part of the year the water temperature is the highest and the stratification the strongest (Diaz & Rosenberg, 2008). In the 1970s and 1980s in the northern Adriatic Sea, this seasonal oxygen deficiency was reported almost yearly, with mortality of benthic populations in various parts of the Gulf of Trieste in 1974, 1983 and 1988 (Stachowitsch, 1991). In 1983, during the most extensive event, more than 90% of macroepifauna biomass was lost within only four days (Stachowitsch, 1984). The mortality events are often coupled with large amounts of marine snow (small amorphous aggregates with a size ranging from a few millimeters to several meters, Danovaro et al., 2009) in the water column. Water column stratification under summer conditions supports the assemblage of small-sized aggregates into macroflocs, stringers and clouds in the water column along with creamy or gelatinous surface layers, which are collectively known as marine mucilage (Stachowitsch et al., 1990; Precali et al., 2005). A mucilage event was reported in the Adriatic Sea for the first time in 1729, and in the last three decades its frequency appears to be increasing. Mucus is released by many marine organisms, whereby the massive marine snow amounts primarily involve photosynthetic extracellular release (PER) by phytoplankton under stress (Herndl & Peduzzi, 1988; Degobbis et al., 1995). As stratification decreases, marine snow settles on the bottom, covering the sediment, sometimes episodically. The deposition of the aggregates on the sea floor and higher oxygen consumption due to the decomposition further deplete the DO and hypoxic/anoxic conditions can occur (Stachowitsch et al., 1990). Beyond the indirect effect of oxygen deficiency, the settled mucilaginous material can also directly suffocate the benthic fauna.

Another factor contributing to the rapid community destruction is the nature of the community occupying the area itself. The soft-bottom of the northern Adriatic Sea harbors well-developed benthic communities (Fedra, 1978). One of these is the *Ophiothrix-Reniera-Microcosmus* (O-R-M) community, inhabiting large parts of the Gulf of Trieste. The term stems from the biomass dominants of this macroepifaunal assemblage: the brittle star *Ophiothrix quinquemaculata*, the sponges *Reinera* spp. and the ascidians *Microcosmus* spp. (Fedra et al., 1976). It is characterized by high biomass aggregations (370 g average wet weight/m^2) of vagile and sessile invertebrates, mainly filter- and suspension-feeding organisms which are typically found in the form of so-called multi-species clumps or bioherms. These consists of biogenic structures (shells of bivalves and gastropods), which serve as a basis for sessile organisms like ascidians and sponges. These, in turn, present a substrate for mobile species. High biomass corresponds with high respiration, and the aggregation into multi-species clumps may accelerate mortalities even in more resistant taxa in a positive feedback loop of decay (Stachowitsch, 1986).

This community is capable of removing great amounts of pelagic biomass from the water column due to its high filtering efficiency and capacity under normal conditions (Ölscher & Fedra, 1977). Thus, it is considered to have a stabilizing function in the ecosystem (Ott & Fedra, 1977), and such communities are considered to be a natural eutrophication control (Officer et al., 1982). However, repeated disadvantageous conditions, such as oxygen deficiencies coupled with massive marine snow development, can overwhelm this stabilizing capacity. The result may be various degrees of degradation of community structure or defaunation of benthic populations (Stachowitsch, 1984).

The responses of the benthic community to oxygen depletion events are complex. Microbes, foraminiferans and metazoan meiofauna sustain high biomass during low DO events, unlike other groups. Bacteria aggregate at the sediment surface and are the only group capable of sustaining high abundance and diversity under such conditions. Meiofauna migrates upward within the sediment, as does the macroinfauna (Levin et al., 2009). Epifauna species initially show severe signs of stress such as attempting to reach more oxygenated water layers. This leads to aggregations on elevated sites (taller sessile epifauna or even vagile species) or upward body extension (less mobile species). Beyond the direct lethal effects of low DO concentration and the presence of H_2S, several indirect causes, such as increased susceptibility to predators, can cause mortality (Riedel et al., 2008a). After the onset of the oxygen depletion-induced behavioral modifications, the deterioration of the community proceeds very rapidly (Stachowitsch, 1992; Stachowitsch & Fuchs 1995).

This quick course of ecosystem collapse, and the difficulty to predict the onset/extent of such events, hinders the full documentation and investigation in the field. To solve this problem, an underwater device (EAGU – Experimental Anoxia Generating Unit) was developed by Stachowitsch et al. (2007). This chamber, equipped with camera, flashes and a sensor array, induces and fully documents small-scale experimental anoxia *in situ*. It provides insight into the course and effect of hypoxia/anoxia. The combination of time-lapse photo-documentation and the recording of physico-chemical data allows behavioral reactions to be correlated with DO and H_2S concentrations.

The present study focuses on the full range of complex processes within a selected EAGU deployment and includes interactions, sublethal behavior changes and mortality sequences within the ORM community (macroepi- and infauna). The behaviors and mortalities are correlated to specific DO thresholds proposed by Diaz and Rosenberg (1995). This work will help to compile a generally valid catalogue of reactions at both the species and community levels, and a list of sensitive and tolerant species. Combined with further experiments, it will ultimately help to better determine the status of benthic ecosystems exposed to oxygen depletion here and elsewhere.

2. Material and Methods

2.1 Study site

The study site is located on soft-bottom (poorly sorted silty sand) in 24m depth in the Gulf of Trieste, Northern Adriatic Sea (45° 32´ 55.68´´N, 13° 33´ 1.89´´ E) (Fig. 3). Between 2005 and 2006, a total of 13 experiments using the EAGU approach were conducted. The present thesis evaluates experiment Nr. 11. That deployment took place from 5-10 October.

Macroepifauna communities are widely distributed in the Northern Adriatic, largely aggregated in so-called multi-species clumps or bioherms: small hard substrates (e.g. bivalve shells) provide the base for epigrowth of sessile suspension-feeders (mostly sponges, ascidians, anemones and bivalves), which in turn save as a substrate for additional mobile and hemi-sessile organisms (mainly brittle stars and crustaceans). The most widespread community, found also at the study site, is the so-called O-R-M-community, based on the three dominating genera, the brittle star *Ophiothrix quinquemaculata*, the sponge *Reniera* spp. and the ascidian *Microcosmus* spp.

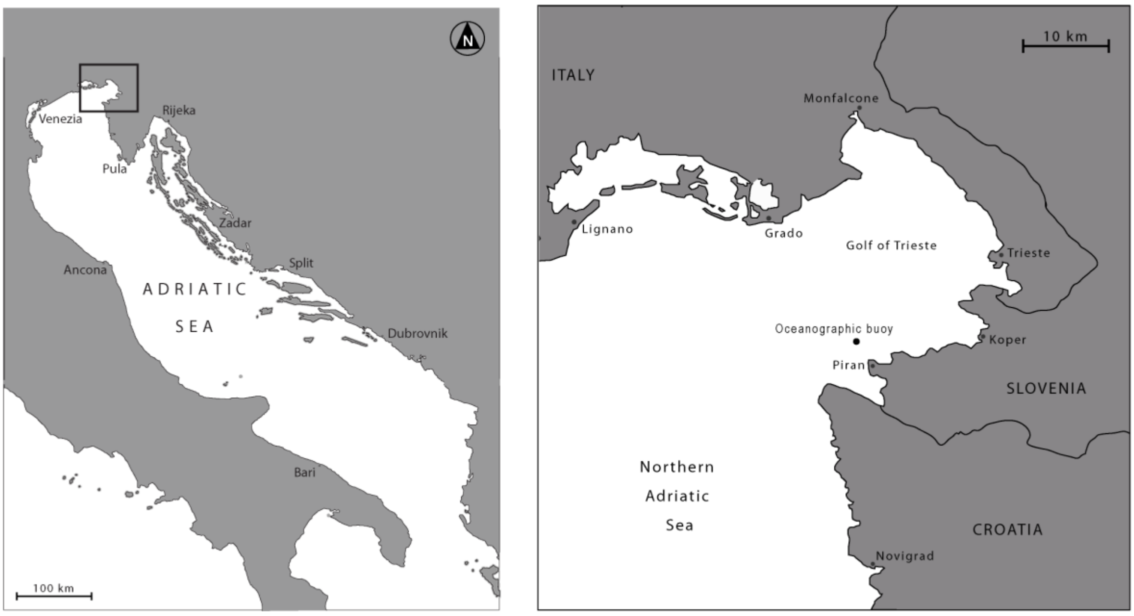


Fig. 3: Study site located about 2 km off coast of Piran near the oceanographic buoy of the Marine Biology Station Piran, Slovenia (Northern Adriatic Sea) (map taken from Haselmair, 2008).

2.2 Technical set-up

We used a specially designed underwater-device, the so-called EAGU (Experimental Anoxia Generating Unit, Fig. 4) to document the behavioral responses and mortality sequences of macrobenthic assemblages to artificially induced small-scale anoxia on the seafloor.

The system consists of two interchangeable bases, an open-sided aluminum frame and a plexiglass chamber (both 50x50x50cm). A separate lid (51x70cm) houses the time-lapse camera, two flashes, two main batteries, the datalogger and the sensor array.

- The digital camera (EOS 30D, EFS 10-22 mm, f/3.5-4.5 USM zoom lens, TC-80N3 Timer Remote Controller; all Canon®), located in a carbon fibre underwater housing (Bruder®), was programmed to take images in 6-min intervals. This rate is a compromise between available energy supply, desired experiment length, and flash card capacity (Stachowitsch et al., 2007). Both the camera and the flashes ("midi analog", series 11897; Subtronic®) are powered by 9Ah Panasonic external battery packs (Werner light power Unterwassertechnik®).
- Oxygen (OX-100, outside tip Ø 90-110µmol l^{-1}) and hydrogen sulfide (H_2S-50, outside tip Ø 40-60µmol l^{-1}) sensors are both Clark-type microsensors (sensors and PA3000UD datalogger: Unisense®), and are inserted through O-ring equipped sensor ports in the corners of the lid. To detect a potential oxygen gradient, the tips of the two oxygen sensors were positioned 2 cm (Ox1) and 20 cm (Ox2) above the sediment; the H_2S sensor was mounted in 2 cm height. Sensor data were logged in 1-min intervals. Oxygen microelectrodes were calibrated in fully oxygenated seawater and in seawater deoxygenated with sodium hydroxide. The H_2S microelectrode was calibrated in seawater and in a standard pH buffer, reduced with a stock solution of total sulfide (for more information see Unisense instruction manual). Temperature and pH in the plexiglass chamber were measured once a day for ca. 5 min separately by a SCUBA diver who inserted the sensor (TA 197-pH) through an opening in the chamber wall (closed with a plastic stopper during regular operation) (datalogger: 197i; all WTW®).

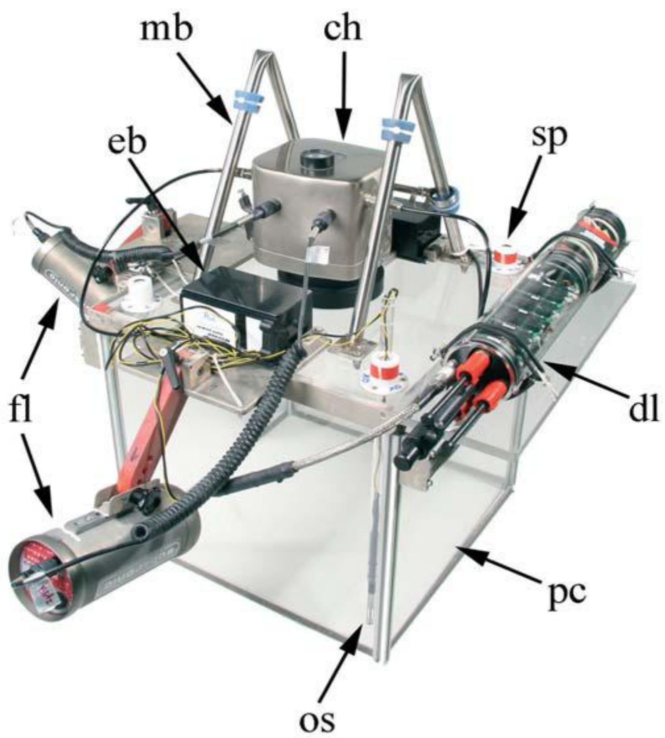


Fig. 4: Experimental Anoxia Generating Unit (EAGU): the instrument lid is positioned on top of the plexiglass chamber. Here, one sensor (bottom center) is connected to the datalogger and inserted through a sensor port in the lid. ch: camera housing, dl: datalogger, eb: external battery, fl: flashes, mb: metal brackets for lifting and handling lid, os: oxygen sensor, pc: plexiglass chamber, sp: sensor port. Photo: Gregor Eder.

2.3 Experimental procedure

The experiment consists of two phases: in a first step, the aluminum frame with the lid is positioned over a macrobenthic assemblage for ca. 1 day (“open” configuration). This set-up allows full water exchange and does not change the water currents. Here, the animal behavior under normoxic conditions is documented. In a second step, the frame is exchanged for the plexiglass chamber (“closed” configuration) and repositioned over the same assemblage for another 2-3 days. The device (pushed approximately 2 cm into the sediment) seals the water in the chamber off from the surrounding environment, so that the gradual oxygen reduction reflects the natural respiration rates of enclosed organisms. In this phase, the behavioral responses to decreasing oxygen and increasing H_2S concentrations are documented.

After the experiment, all organisms (living and dead) were collected by hand, transported to the laboratory, and preserved in a 4% formaldehyde:seawater solution.

2.4 Data analyses

Experiment Nr. 11 yielded a total of 1193 images. The overall documentation time was ca. 120 h (23.7 h open, 95.6 h closed configuration). The behavior of each individual was analyzed image by image and recorded on Excel data sheets containing numerous categories describing behavior patterns in hypoxia and anoxia (see below and Table 1). The images were further processed into time-lapse sequences using the program Adobe Premier 6.5 (film material available at http://www.marine-hypoxia.com). The behavioral analyses of bioherm-associated crustaceans and hermit crabs are the topic of two other master theses, and these groups/organisms are therefore excluded from this study.

2.4.1 Behavioral analysis

Fifteen species (epi- and infauna) were selected for behavioral analysis based on the following criteria: visibility *in situ* to the camera, wide distribution in the Adriatic Sea and high diversity in taxonomic groups and ecological categories (mobility, feeding types; see Riedel et al., 2008).

All individuals of a species were evaluated. *Ophiothrix quinquemaculata* is an exception: due to the brittle star’s high abundance and the difficulty in differentiating single individuals, only 4 conspicuous specimens were analyzed.

Generally valid categories evaluated included visibility (exposure, i.e. not hidden under/in a bioherm or in the sediment), body movements, locomotion, interactions and mortality. Species-specific categories included arm tipping (brittle stars), color change (anemones), spine movements (sea urchins) or body contractions (ascidians and anemones). Some behaviors in certain species were further subdivided into different states: locomotion of gastropods, for example, was divided into minor/major and horizontal/vertical displacement, or the crown habitus in the sea cucumber and the anemones into closed/half open/open states (Table 1). Images were analyzed as long as the animal was visible and clearly identifiable on the photo. When accurate image analysis was no longer possible due to poor overall visibility (e.g. darkened sediment), viewing the time-lapse movie helped to determine that certain organisms (e.g., anemones) were still alive.

Mortality was defined as the last locomotion or body movement observed, in some species plus two hours to account for a potential moribund phase (Table 2).

Table 1: Behavioral patterns evaluated. Life habit: epi=epifauna, in=infauna. Feeding: susp=suspension feeder, carn=carnivore, omni=omnivore, depo=deposit feeder. Behavior subdivisions: [1]original, trans, final color; [2]open, half open, closed tentacle crown; [3]retraction into the sediment, constrictions/inflations of column diameter; [4]minor, major extension from the sediment; [5]minor horizontal-, major horizontal locomotion, turns, minor upward /downward-, major upward/downward locomotion; [6]retracted, extended foot; [7]closed, half open, normal, widely gaping valves; [8]constant, opening gape, closing gape; [9]normal, swollen, retracted mantle tissue; [10]minor, major locomotion; [11]horizontal locomotion, squirming; [12]up-, side-, downward orientated tentacle crown; [13]minor, major body extension; [14]front end upright, back end upright; [15]horizontal, upward, downward locomotion, [16]lateral arm-posture, arm-tipping, clinging to clumps.

Class/Genus	**N**	**Life habit**	**Mobility**	**Feeding**	**Species-specific behaviors**
Anthozoa					
Cereus pedunculatus	4	epi	sessile	susp	color[1], crown habitus[2], body contraction[3] and rotation, pharynx protrusion, extension[4]
Gastropoda					
Hexaplex trunculus	6	epi	mobile	carn	locomotion[5], foot habitus[6]
Fusinus sp.	1	epi	mobile	carn	locomotion[5], foot habitus[6]
Bivalvia					
Chlamys varia	1	epi	hemi	susp	valve gape[7], valve gape process[8], mantle state[9]
Corbula gibba	12	in	mobile	susp	locomotion[10]
Abra alba	2	in	mobile	depo	locomotion[10], siphon visible
Polychaeta					
Serpula vermicularis	2	epi	sessile	susp	crown habitus[2]
Anopla (Nemertini)					
Tubulanus annulatus	1	epi	mobile	omni	locomotion[11]
Holothuroidea					
Ocnus planci	1	epi	hemi	susp	crown habitus[2] and orientation[12], body extension[13] and orientation[14], decomposition
Echinoidea					
Schizaster canaliferus	4	in	mobile	depo	locomotion, spine movements
Ophiuroidea					
Ophiothrix quinquemaculata	4	epi	mobile	susp	locomotion[15], arm position[16]
Ascidiacea					
Microcosmus sulcatus	3	epi	sessile	susp	siphon habitus[2], body contraction, drooping
Phallusia mammilata	1	epi	sessile	susp	siphon habitus[2], body contraction, drooping

Table 2: Criteria and time of death (omitted are surviving taxa and those individuals out of view at the end of the experiment)

Life habit/Genus	N	Final activity	T of death (h)	DO (ml l^{-1})	H_2S (µmol l^{-1})
Sessile epifauna					
Microcosmus	3	drooping	x/x/118	0	110
Phallusia	1	drooping	80	0	261
Serpula	2	crown retraction + 2h	84/84	0	250
Hemi-sessile epifauna					
Chlamys	1	sustained wide gape + 2h	75	0	257
Ocnus	1	body movement	90	0	244
Mobile epifauna					
Ophiothrix	4	arm movement	47/47/48/49	0	0.01/0.2/0.2
Mobile infauna					
Schizaster	4	locomotion + 2h	58/67/75/77	0	38/40/257/255

2.4.2 Statistical analysis

The behavioral patterns analyzed were related to the values of the lower oxygen sensor (Ox1). The data were assigned to five dissolved oxygen (DO) categories: normoxia (>2.0 ml O_2 l^{-1}), weak hypoxia (≤2.0- 1.01 ml O_2 l^{-1}), moderate (1.0-0.51 ml O_2 l^{-1}) and severe hypoxia (0.5- 0.01 ml O_2 l^{-1}) and anoxia. The nonparametric Kruskal-Wallis test was used to determine whether oxygen concentrations significantly changed behavior. To compare the behavioral responses between the oxygen categories, the Mann-Whitney-Wilcoxon test was chosen. Statistical analyses were performed using the software package SPSS 17.0.

3. Results

3.1 Sensor data

During the open configuration, the DO concentration of the higher oxygen sensor (Ox2) ranged between 2.5 and 3.2 ml l^{-1} and the lower (Ox1) between 2.6 to 3.9 ml l^{-1}. The two oxygen curves were parallel most of the time, but Ox2 followed a slightly increasing trend, and, thus, it intersected the Ox1 curve once at h 7 (Fig. 5). Immediately after switching to the closed configuration (red arrow, Fig. 5), DO values of both sensors rapidly decreased. The difference between the curves became smaller until both showed the same value (15 h after EAGU closure). Ox1 reached weak hypoxia within ~7 h. Moderate hypoxia occurred after ~10 h, severe hypoxia ~13 h, and anoxia ~19 h after chamber deployment. Once anoxia occurred, hydrogen sulfide (H_2S) increased, peaking at hour 78 (304 µmol l^{-1}). Thereafter, H_2S concentrations strongly fluctuated and decreased until the end of the deployment to 105 µmol l^{-1} after 5 days.

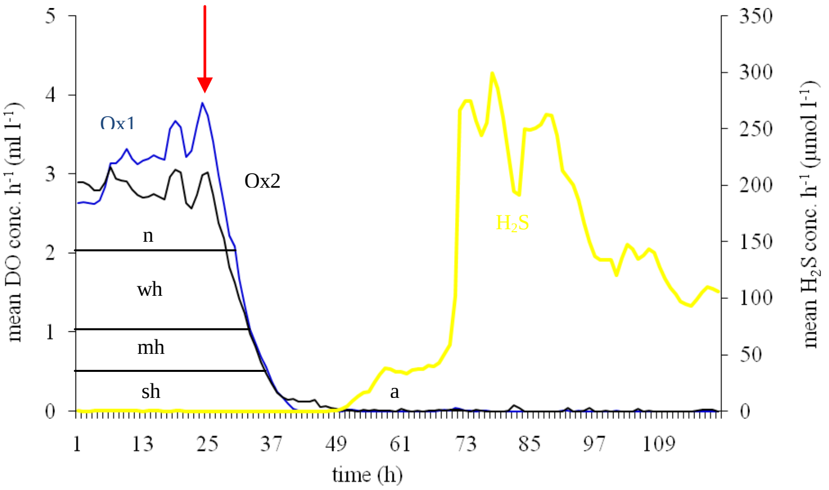


Fig. 5: DO and H_2S values (yellow) during the experiment 11 (5-10 October 2006). Ox1 (blue): lower oxygen sensor, Ox2 (black): higher oxygen sensor. Oxygen categories: n: normoxia, wh: weak hypoxia, mh: moderate hypoxia, sh: severe hypoxia, a: anoxia. Red arrow: switch to closed configuration.

Bottom water temperature during the open and closed configuration averaged 21.3°C and ranged between 20.9 and 21.7°C. The pH was 8.2 during normoxia and dropped to 7.5 at the end of the experiment. The salinity was 38‰.

3.2 Open versus closed configuration

During the open configuration the visibility was poor due to turbid water, suspended particles and marine snow. The color of the sediment inside the frame had the same color as the sediment outside (Fig. 6a). The benthic organisms showed their normal behavior: the brittle star *Ophiothrix quinquemaculata*, for example, was positioned on sponges and ascidians in its typical suspension-feeding position, i.e. arms held upright into the water. The tentacle crown of the sea anemone *Cereus pedunculatus* was normally expanded on the sediment surface and the hermit crabs occupied gastropod shells. Infaunal species, such as the irregular sea urchin *Schizaster canaliferus*, were not visible (i.e. did not emerge from the sediment). No atypical behavioral patterns were observed. Mobile animals (e.g. hermit crabs, gastropods and fish) crossed over the frame without difficulties. Some organisms originally within the frame left the open configuration before the EAGU was closed, others entered the frame, and still others remained and were enclosed in the chamber. No mortalities were recorded in the open configuration.

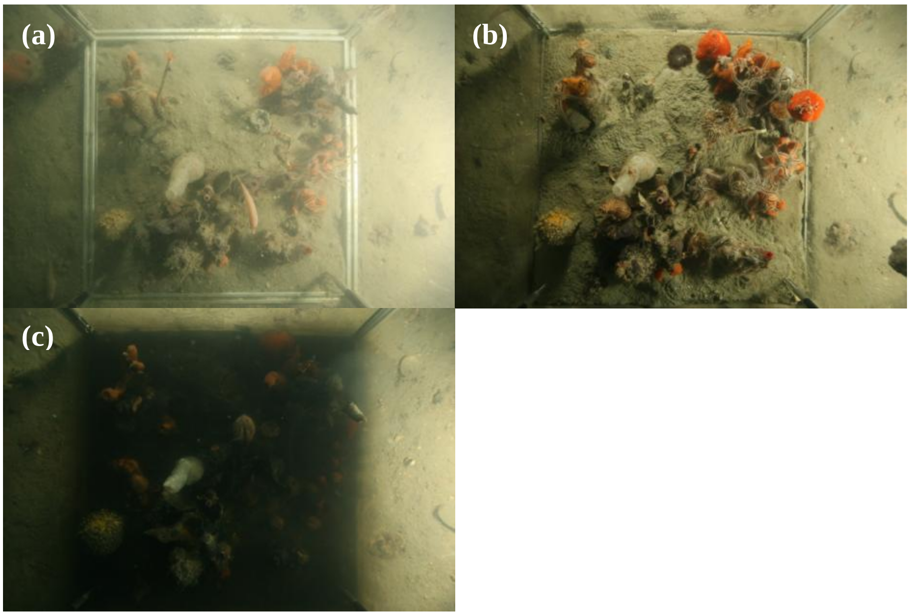


Fig. 6: (a) Open configuration: the aluminum frame is visible (DO 2.6 ml l^{-1}). (b) Closed configuration: 12 hours after switch to chamber. The water rapidly becomes clear (DO 0.6 ml l^{-1}). (c) End of the experiment: 96 hours after chamber deployment (anoxic, H_2S ~107 µmol l^{-1}). The sediment and many decaying organisms have darkened, the bottom water layer has become murkier.

After switching to the closed configuration, the visibility markedly improved, as suspended particles trapped within the chamber rapidly settled (Fig. 6b). The behavioral patterns clearly changed: the organisms either visibly increased or decreased their normal activities or began to show atypical behaviors. Infaunal species (e.g. the bivalve *Abra alba*, the irregular sea urchin *S. canaliferus*) emerged from the sediment, the anemone *C. pedunculatus* extended from the sediment, and cryptic crabs emerged from hiding and aggregated on elevated substrates. Brittle stars were the first to die (early anoxia); the gastropod *Hexaplex trunculus* and the anemone *C. pedunculatus* were among the most tolerant (all specimens survived until the end of the experiment). Two predatory interactions were also observed: one *C. pedunculatus* made contact with, pulled in and consumed two brittle stars during anoxic conditions (for a detailed description see Riedel et al., 2008). The color of the sediment successively became darker and turned black ~50 h after switching to the closed chamber (H_2S ~274 µmol l^{-1}). Twenty-six hours later (20 h before the end of the experiment), the lowermost water layer became murky due to decaying organic matter (Fig. 6c). The wet weight of the total enclosed and collected fauna was 1042.71 g. Almost 80% of this biomass was represented by sponges and ascidians (506.8 g and 291.7 g, respectively).

3.3. Macrofauna responses

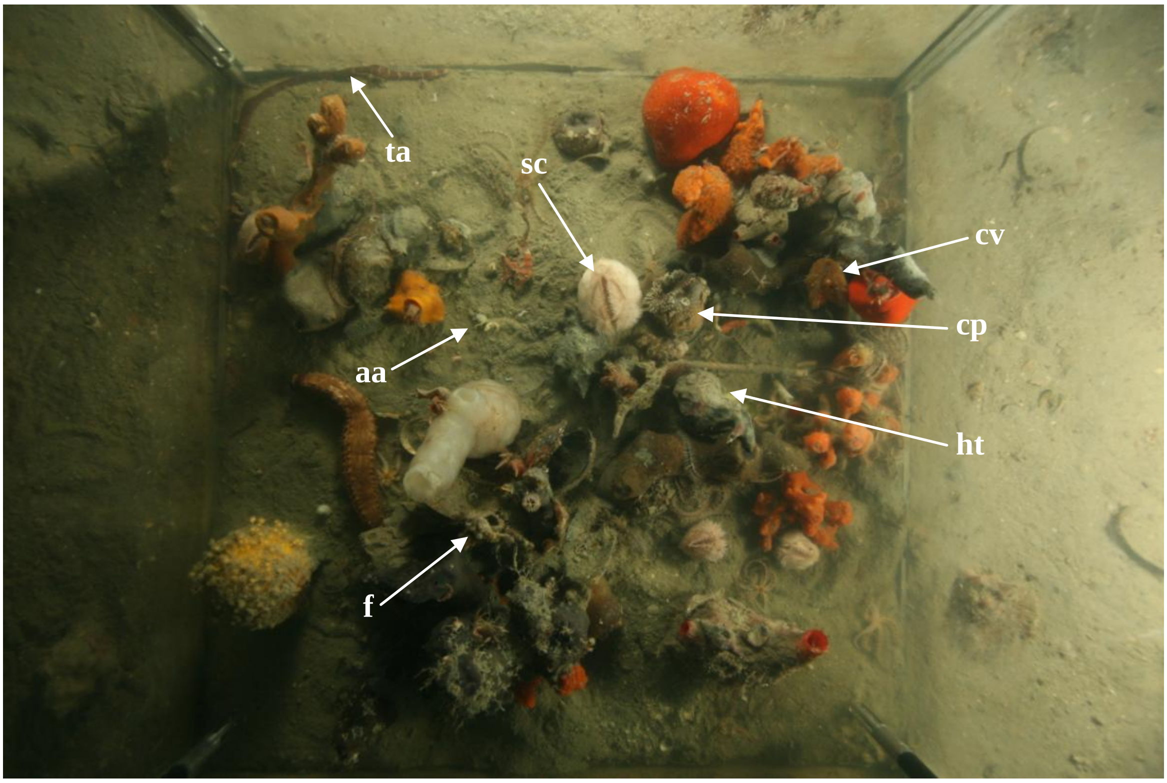


Fig. 7: Image taken on day 4 of the deployment, 39 h after switch to closed configuration (anoxia, H_2S ~39 µmol l^{-1}). Sea anemone *Cereus pedunculatus* extended from the sediment (cp), ribbon worm *Tubulanus annulatus* (ta), gastropods *Fusinus* sp.(f) and *Hexaplex trunculus* (ht), infaunal bivalve *Abra alba* (aa), epifaunal bivalve *Chlamys varia* (cv), and infaunal sea urchin *Schizaster canaliferus* (sc).

3.3.1 *Cereus pedunculatus* (Pennant, 1777; Anthozoa: Sagartiidae)

Two of the sea anemones were positioned in the sediment with the tentacle crown on the sediment surface, two were "integrated" into epifaunal aggregations (Fig. 7 "cp"). Six species-specific behaviors are presented here (see Table 1). Under normoxia the tentacle crown retained its normal color (Fig. 8a). Two of the anemones had a light brown color originally, the other two individuals were dark brown. Hypoxia, however, triggered a successive change from the original color to a transitional tentacle crown color (Fig. 8b): the light brown individuals became brighter and the dark brown anemones had a somewhat reddish color. At anoxia (39 h after chamber deployment, H_2S ~36 µmol l^{-1}) all individuals turned grey (Fig. 8c).

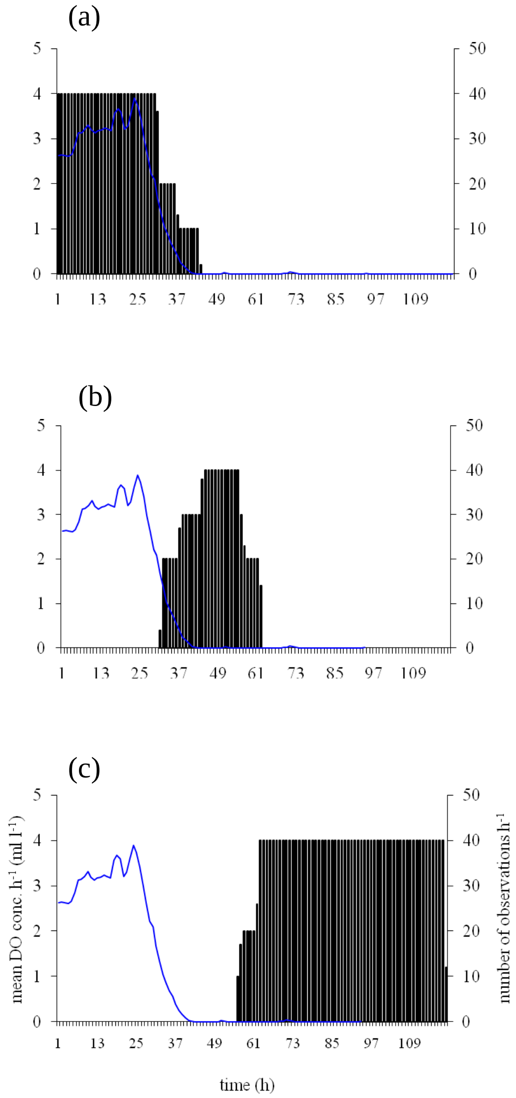


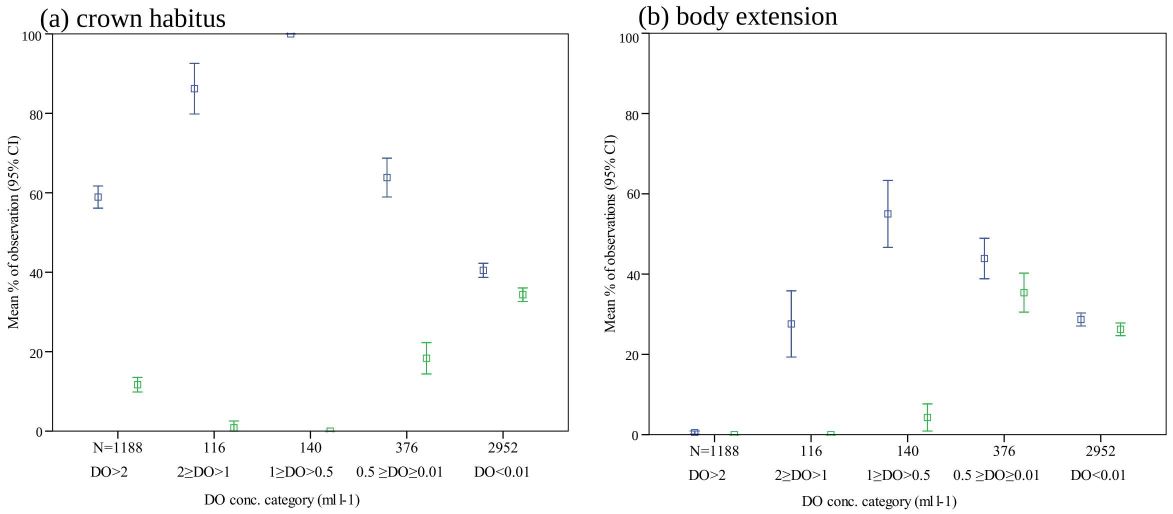
Fig. 8: Color change of *Cereus pedunculatus* (4 individuals): (a) original, (b) transitional and (c) final color. Histograms show the number of observations of the selected behavior per hour, in relation to the dissolved oxygen curve (blue, values averaged per hour).

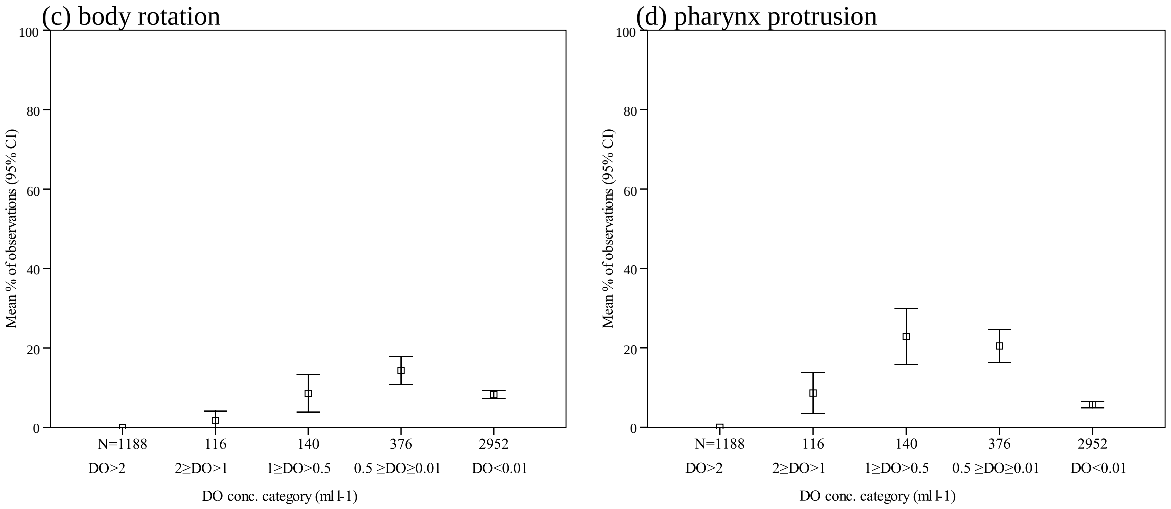
During normoxia, the tentacle crowns were mostly (> 50%) open. At weak hypoxia this behavior increased significantly in all anemones from 59 to 86 % of observations ($p<0.01$, Appendix 1: Table 3), peaking at moderate hypoxia (100% in 140 observations). As oxygen values dropped below 0.5 ml l^{-1}, crown closure gradually increased (Fig. 9a). At h 94 (H_2S ~187 µmol l^{-1}), however, the crowns of the two anemones living in the sediment opened again and stayed in this position until the end of the experiment. From h 111 on (H_2S ~109 µmol l^{-1}), the crowns of the other two individuals also opened somewhat and remained in this half-open state until the end of the recordings (data not shown).

At the onset of hypoxia, the anemones started to slightly extend from the sediment surface. With decreasing oxygen concentrations these minor extensions markedly increased from 28 to 55% ($p<0.01$, Appendix 1: Table 3), followed by major extensions where almost the whole body became visible (35% of observations at severe hypoxia (Fig. 9b). Once extended, the anemones also gradually began to rotate, peaking at severe hypoxia (14 % in 376 observations, Fig. 9c).

Fig. 9d shows an increase in pharynx protrusion at weak hypoxia, peaking at moderate hypoxia (23% in 140 observations), followed by a slight decrease and almost stopping at anoxia.

Body contractions were subdivided into retractions into the sediment and constrictions or inflations of column diameter. Only few retractions were observed during the whole experiment, but the constrictions/inflations began when oxygen values fell below 2 ml l^{-1}. his atypical behavior significantly increased at moderate hypoxia ($p<0.01$, Appendix 1: Table 3), peaking at anoxia (44% in 2952 observations, Fig. 9e). At the end of the experiment (ca. h 105), all *C. pedunculatus* became rather immobile: they no longer extended from the sediment and constriction or inflation activity ceased. All four individuals survived.




(c) body rotation

(d) pharynx protrusion

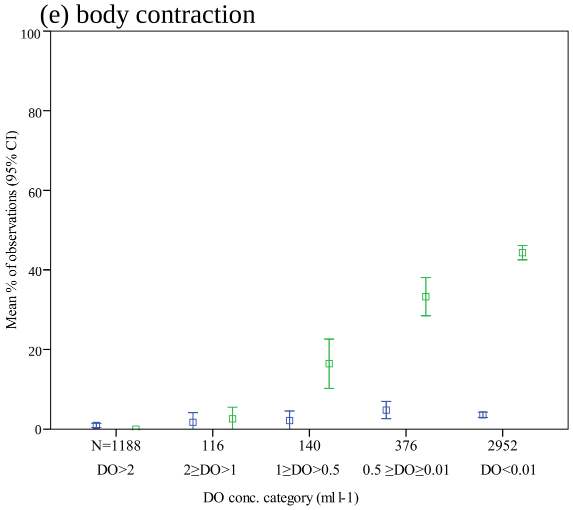

(e) body contraction

Fig. 9: Changes in the behavior of *Cereus pedunculatus* (4 individuals) during the deployment: (a) open (blue error bars) and closed tentacle crown (green error bars); (b) minor (blue error bars) and major (green error bars) extension from the sediment; (c) body rotation; (d) pharynx protrusion and (e) body contractions – retraction (blue error bars), inflation (green error bars). Diagrams show course of selected behaviors during five oxygen categories; N=the number of photographs evaluated per oxygen category. The proportions of behaviors per oxygen category do not necessarily total 100% because not all behavioral states are included. For details see text.

3.3.2 *Tubulanus annulatus* (Montagu, 1804; Anopla (Nemertini): Tubulanidae)

This strikingly colored (brownish red with white longitudinal stripes and rings) nemertean (Fig. 7 “ta”) first became visible at anoxia (h 60, H_2S ~35 µmol l^{-1}), emerging from under a multi-species clump. The worm then glided over the sediment surface for nearly 8 hours (Fig. 10), mostly along the plexiglass wall, before it disappeared from view again (h 68, H_2S ~43 µmol l^{-1}).

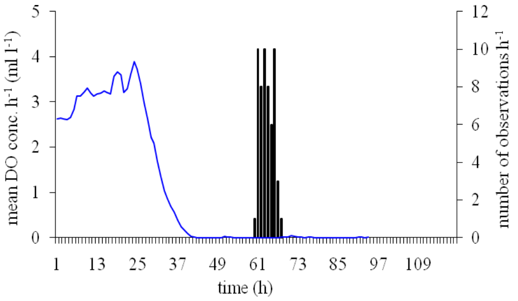
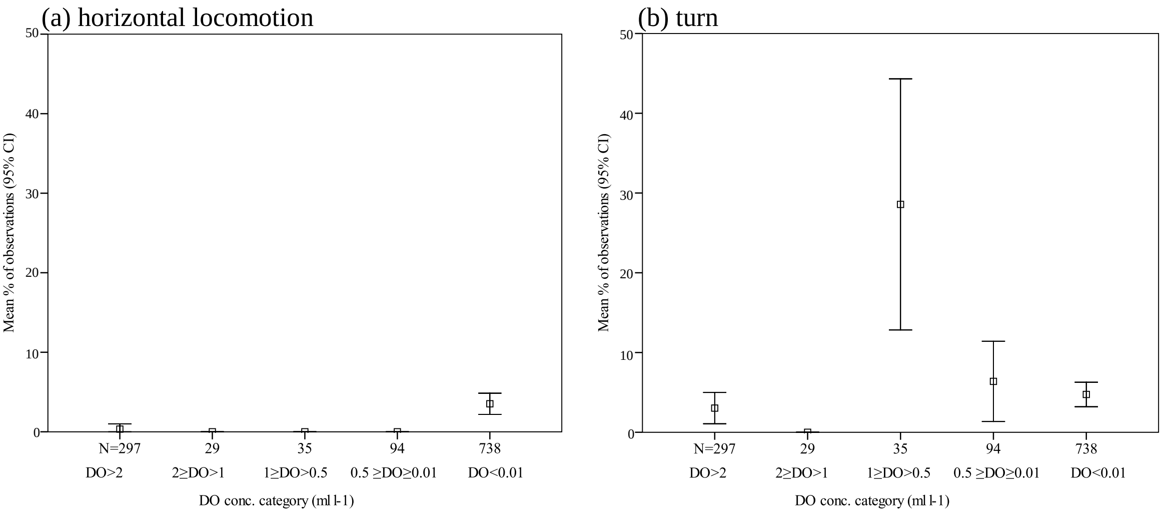


Fig. 10: Horizontal locomotion of *Tubulanus annulatus* (1 individual). For description of diagram see figure legend of Fig. 8.

3.3.3 *Fusinus* sp. (Gastropoda: Fasciolariidae)

This gastropod, originally positioned on a multi-species clump located in the lower right corner of the chamber (Fig. 7 “f”), made only few horizontal and vertical movements throughout the experiment (Fig. 11a). Turns (=changes in direction but no large distances covered) dominated and peaked at moderate hypoxia (29% in 35 observations, Fig. 11b). *Fusinus* extended its foot strongly the first time at h 71 (anoxia, H_2S ~102 µmol l^{-1}). Thereafter, periodically for 11 hours, such foot extensions/retractions were observed (both 10% in 94 observations). This continued even though oxygen concentrations briefly increased to severe hypoxia (Fig. 11c). At h 82, the gastropod disappeared from view, hidden under a bioherm.



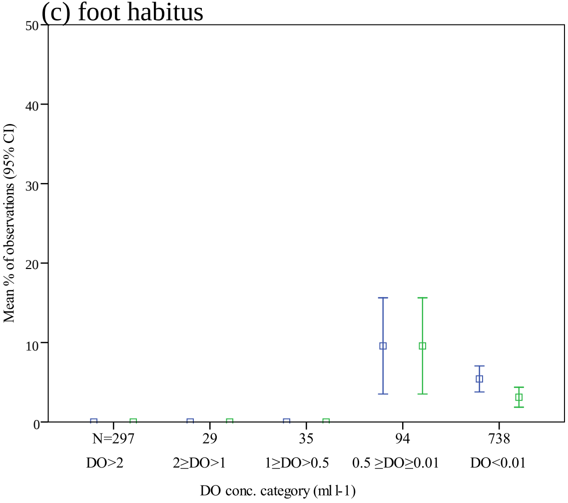


Fig. 11: Behavioral changes of *Fusinus* sp. (1 individual) during the experiment: (a) horizontal locomotion, (b) turn, and (c) foot habitus: retracted (blue error bars) and extended (green error bars). For description of diagram see figure legend of Fig. 9.

3.3.4 *Hexaplex trunculus* (Linnaeus, 1758; Gastropoda: Muricidae)

Three out of 6 of *H. trunculus* evaluated here were initially burrowed shallowly into the soft sediment (Fig. 7 "ht"). During normoxia the two fully exposed *Hexaplex* and the third, juvenile individual moved only sporadically. Weak hypoxia elicited a clear change in locomotion and location: the burrowed individuals fully emerged and moved horizontally in different directions. Vertical locomotion to the chamber lid was observed in 3 individuals, peaking at moderate hypoxia (5% in 210 observations, Fig. 12a). Two *H. trunculus* fell from the lid at anoxia (h 57 and h 83; H_2S ~33 and ~191 µmol l^{-1}, respectively): one remained inactive on the sediment until the end of the experiment, the second retreated again to the top and fell down once again at h 107 (H_2S ~143 µmol l^{-1}). The third individual remained invisible on the lid until the end of the experiment. The other three *Hexaplex* that stayed on the sediment were mostly immobile with the foot extended. The gastropods that fell from the lid also showed foot extension until the end of the experiment (Fig. 12b). All six individuals survived.

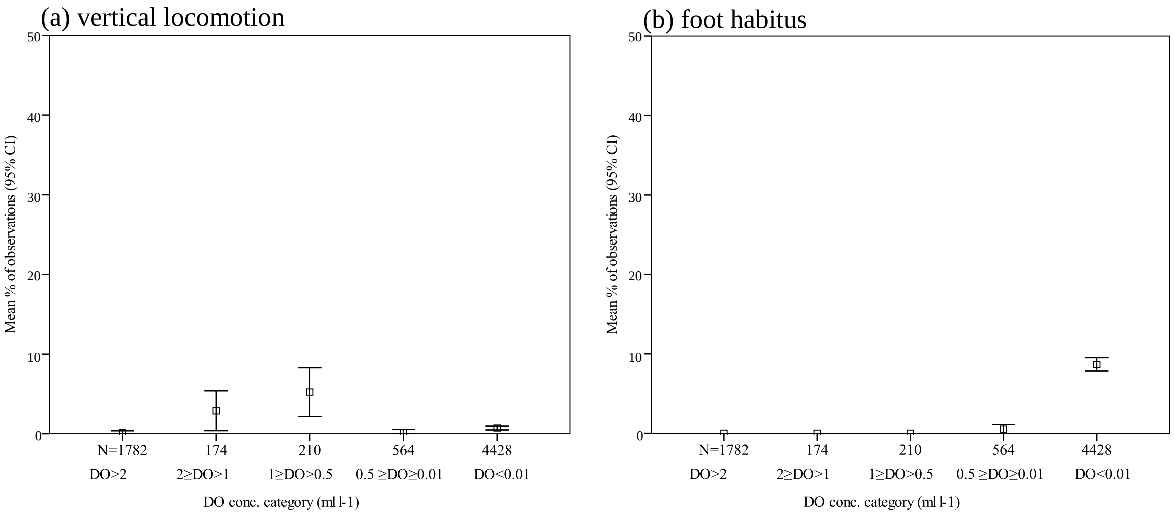
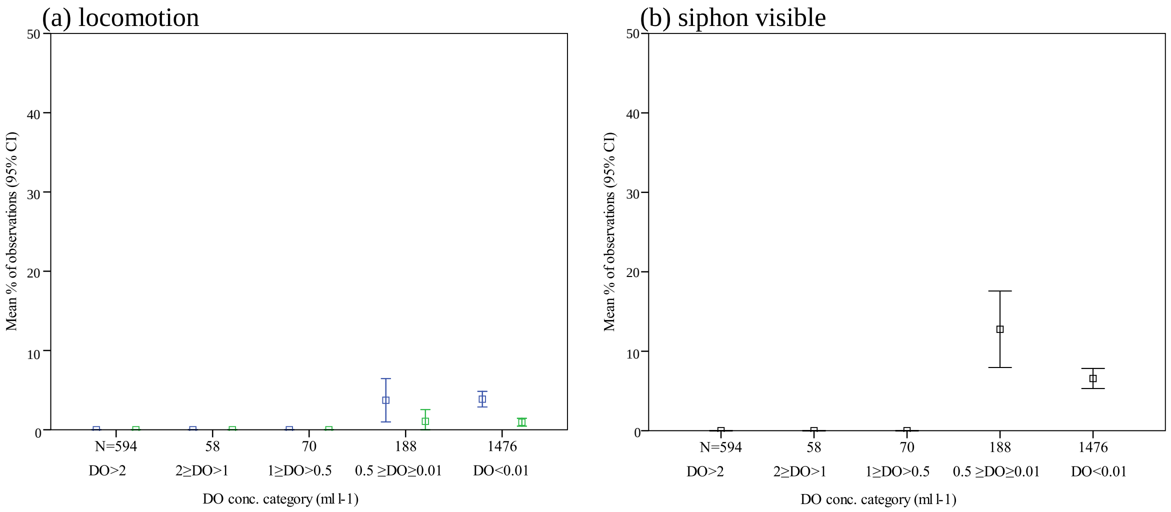


Fig. 12: Behavioral change of *Hexaplex trunculus* (6 individuals) during the deployment: (a) vertical locomotion and (b) foot extension. For description of diagram see figure legend of Fig. 9.

3.3.5 *Abra alba* (Wood W., 1802; Bivalvia: Semelidae)

Both individuals of this infaunal bivalve first emerged from the sediment at severe hypoxia, 32 h after chamber deployment (Fig. 7 “aa”). They then moved horizontally across the sediment (Fig. 13a) and started to stretch out the siphon strongly ($p<0.01$, 13% in 188 observations, Appendix 1: Table 6) at severe hypoxia (Fig. 13b). Siphon extension ceased at h 72 (H_2S ~33 µmol l^{-1}) and the bivalves gradually became motionless. The individuals were not clearly identifiable from h 78 on due to poor overall visibility and, thus, it was not possible to determine the time of death.


(a) locomotion

(b) siphon visible

Fig. 13: Behavioral change of *Abra alba* (2 individuals) during the experiment: (a) minor (blue error bars) and major (green error bars) locomotion and (b) siphon extension. For description of diagram see figure legend of Fig. 9.

3.3.6 *Chlamys varia* (Linnaeus, 1758; Bivalvia: Pectinidae)

This bivalve was positioned on a multi-species clump consisting mainly of the sponge *Reniera* sp. and the ascidian *Microcosmus sulcatus* (Fig. 7 “cv”). Immediately after switching to the closed configuration, *Chlamys* swam a few cm, directly next to the plexiglass wall, by flapping its valves, and then moved back to its original position (still during normoxic conditions). No change in the mantle tissue was observed during the open configuration. In the closed configuration, however, the tissue began to swell already at normoxia (h 29). This behavior dramatically increased from 5% to 100% and remained on the 100% level during moderate hypoxia. At severe hypoxia the swollen tissue significantly decreased ($p<0.01$, Appendix 1: Table 7): this retraction significantly increased and remained until the end of the experiment (Fig. 14a). During normoxia, weak and moderate hypoxia, the bivalve usually showed an “open” (normal) gape, followed by a significant decrease at severe hypoxia and anoxia (68 and 35%, respectively). A closed shell was observed only during anoxia (for a total of 4 h), followed by a sustained wide gape (Fig. 14b), which was interpreted as mortality at h 75 (H_2S ~274 µmol l^{-1}, Table 2).

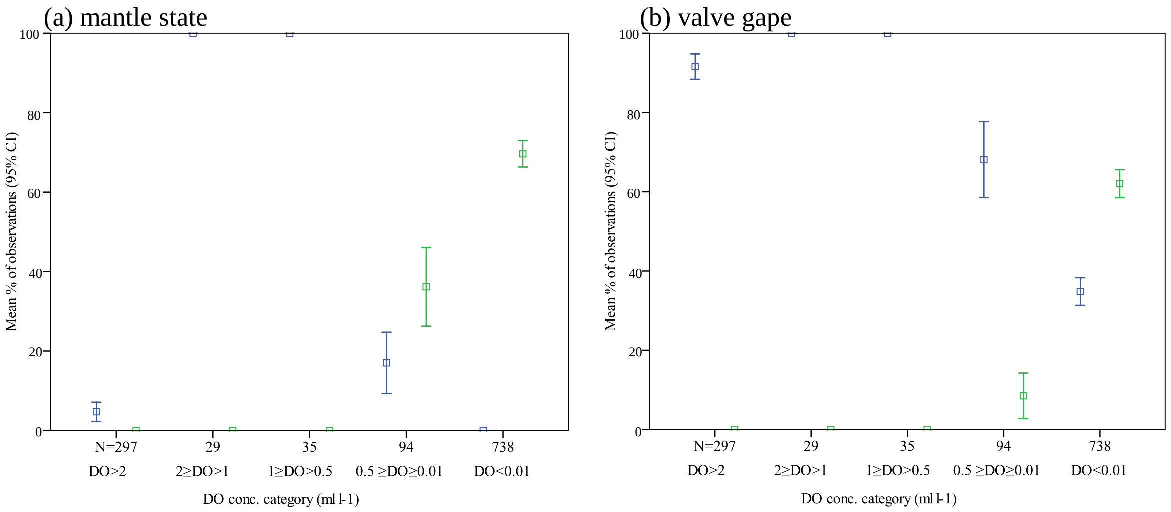


Figure 14: The behavioral change of *Chlamys varia* (1 individual) during the deployment: (a) swollen (blue error bars) and retracted (green error bars) mantle tissue. (b) Open (blue error bars) and widely open (green error bars) gap. For description of diagram see figure legend of Fig. 9.

3.3.7 *Corbula gibba* (Olivi, 1792; Bivalvia: Corbulidae)

Twelve individuals emerged from the sediment (Fig. 16 “cg”). The first “group” of these bivalves (3 individuals) became visible already during the open configuration (between h 11 and 15), the second (5 individuals) throughout weak, moderate and severe hypoxia (between h 32 and h 38) and the two last “groups” (2 individuals in both cases) at anoxia (at h 51, H_2S ~4 µmol l^{-1}, and between h 74 and h 78, H_2S ~275-299 µmol l^{-1}, respectively). The first sign of emerging was the bulging of the sediment above the bivalves. Once fully exposed on the sediment, individuals moved across the sediment surface, mostly less than 10 cm (for periods ranging between 5 and 9 h; Fig. 15). After that period, six individuals remained motionless on the sediment, four re-burrowed into the sediment and two disappeared under the multispecies clumps. All individuals survived based on the observations of the collected animals after the experiment.

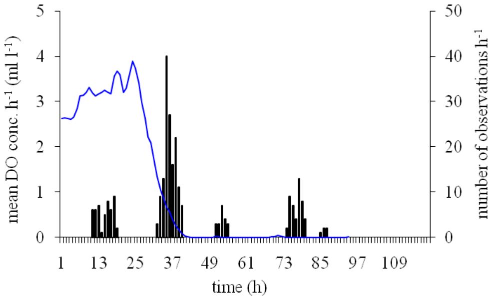


Fig. 15: Horizontal locomotion of *Corbula gibba* (12 individuals) on the sediment during the experiment. For description of diagram see figure legend of Fig. 8.

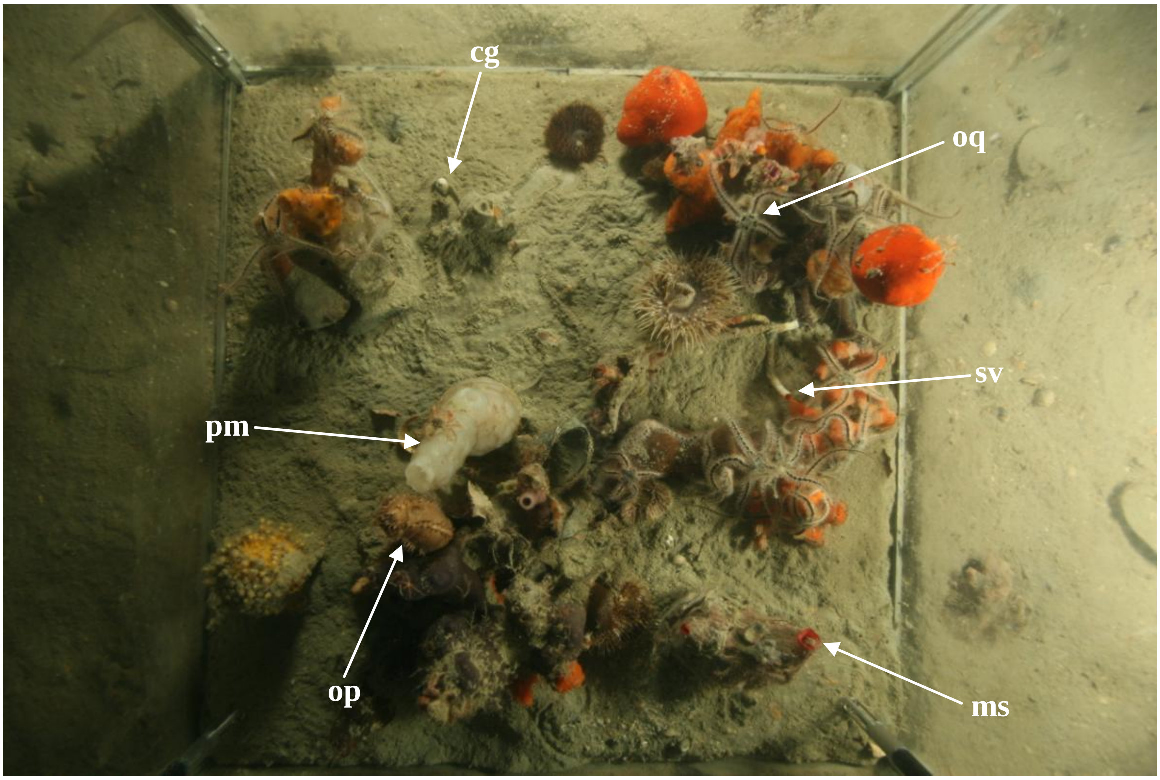


Fig. 16: Image taken on day 2 of the deployment, 12 h after chamber deployment (DO 0.60 ml l^{-1}, no H_2S). Infaunal bivalve *Corbula gibba* (cg), polychaete *Serpula vermicularis* (sv), sea cucumber *Ocnus planci* (op), brittle star *Ophiothrix quinquemaculata* (oq), ascidians *Microcosmus sulcatus* (ms) and *Phallusia mammilata* (pm). Note that brittle stars are no longer in their susupension-feeding position and that a crab (*Pilumnus hirtellus*) has crawled on top of *P. mammilata*.

3.3.8 *Serpula vermicularis* (Linnaeus, 1776; Polychaeta: Serpulidae)

One tube of this serpulid polychaete worm (Fig. 16 “sv”) was attached to a multi-species clump consisting of the sponge *Reniera* sp. and the ascidian *Microcosumus sulcatus*. The second was attached to a gastropod shell inhabited by a hermit crab (and was thus moved about within the chamber). In the early phase of the experiment, both tentacle crowns were fully extended, but they retracted into the tube already during the open configuration (h 18). The crowns remained retracted 100% of the time during weak hypoxia and occasionally extended at moderate and severe hypoxia (11 and 14 %, respectively). At anoxia, this behavior significantly increased (p<0.01, Appendix 1: Table 8) and both crowns remained visible even after the animals became moribund and died (h 84, H_2S ~250 µmol l^{-1}, Fig. 17).

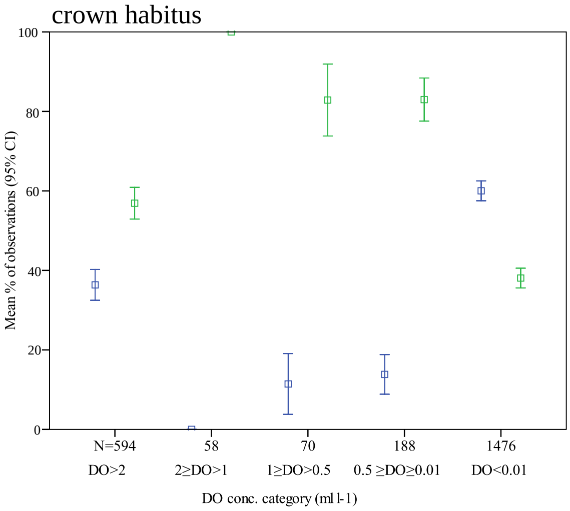
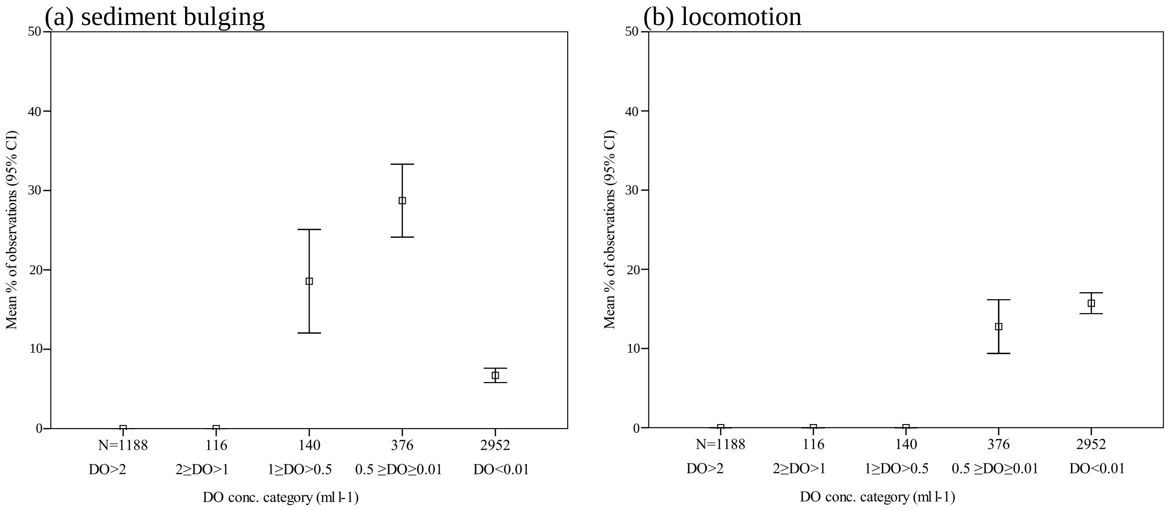


Fig. 17: Behavioral change of *Serpula vermicularis* during deployment: tentacle crown visible (blue error bars) and not visible (green error bars). For description of diagram see figure legend of Fig. 9.

3.3.9 *Schizaster canaliferus* (de Lamarck, 1816; Echinoidea: Schizasteridae)

Four infaunal sea urchins emerged (Fig. 7 “sc”). Sediment bulging was the first sign of emergence and was recorded for all four animals at moderate hypoxia. Every bulging activity ended in emergence. This bulging took between 4 and 18 h (19% of the time during moderate hypoxia, 29% during severe hypoxia and 7% during anoxia**,** Fig. 18a). The last specimen fully emerged at severe hypoxia. Once exposed on the sediment, the sea urchins moved horizontally across the sediment (Fig. 18b). One individual covered a distance of ca. 40 cm, the other three animals, however, moved less than 10 cm. The last locomotion was recorded at h 75 (H_2S ~257 µmol l^{-1}), followed by spine movement (Fig. 18c) until mortality (Table 2).



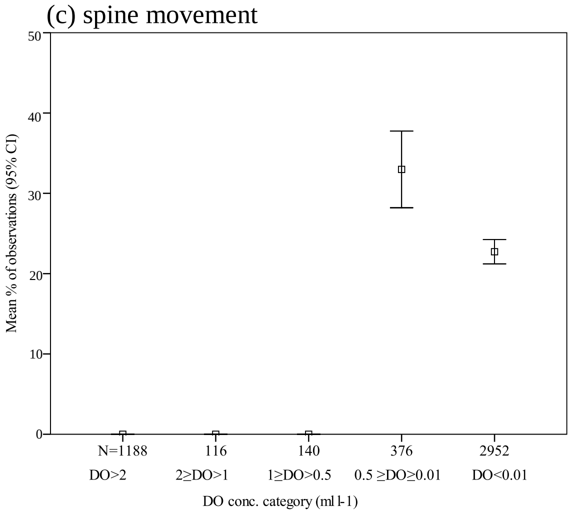


Fig. 18: Behavioral change of *Schizaster canaliferus* during the experiment: (a) sediment bulging, (b) locomotion on the sediment, (c) spine movement. For description of diagram see figure legend of Fig. 9.

3.3.10 *Ocnus planci* (Brandt, 1835; Holothuroidea: Cucumariidae)

From normoxia until moderate hypoxia, the single sea cucumber individual was positioned on a multi-species clump (Fig. 16 "op") in an upward-oriented body position. Then, at severe hypoxia and early anoxia, the posterior end of the body sporadically pointed upwards (13% of 738 observations), before the animal fell onto the sediment (anoxia, H_2S ~16 μmol l^{-1}, Fig. 19a). During normoxia the mouth was also directed upwards, but it increasingly turned sideward, peaking at moderate hypoxia (94% in 35 observations). In 3% of observations during severe hypoxia and 2% during anoxia, the mouth turned toward the sediment (Fig. 19b). *O. planci* also began to actively elongate at weak hypoxia (h 33, DO 1.04 ml l^{-1}): minor elongation significantly increased until moderate hypoxia ($p<0.01$, Appendix 1: Table

10), followed by major elongation (36 and 74% at severe hypoxia and anoxia, respectively, Fig. 19c).

During normoxia, weak and moderate hypoxia, the crown was never completely open; the individual opened its crown at h 64 (anoxia, H_2S ~ 38 µmol l^{-1}) for the first time and remained in this state until the end of the experiment (Fig. 19d). At h 90 the last body movement was recorded, which was interpreted as mortality (H_2S ~ 244 µmol l^{-1}). Three hours later, gradual body inflation was observed. This was interpreted as a decomposition process.

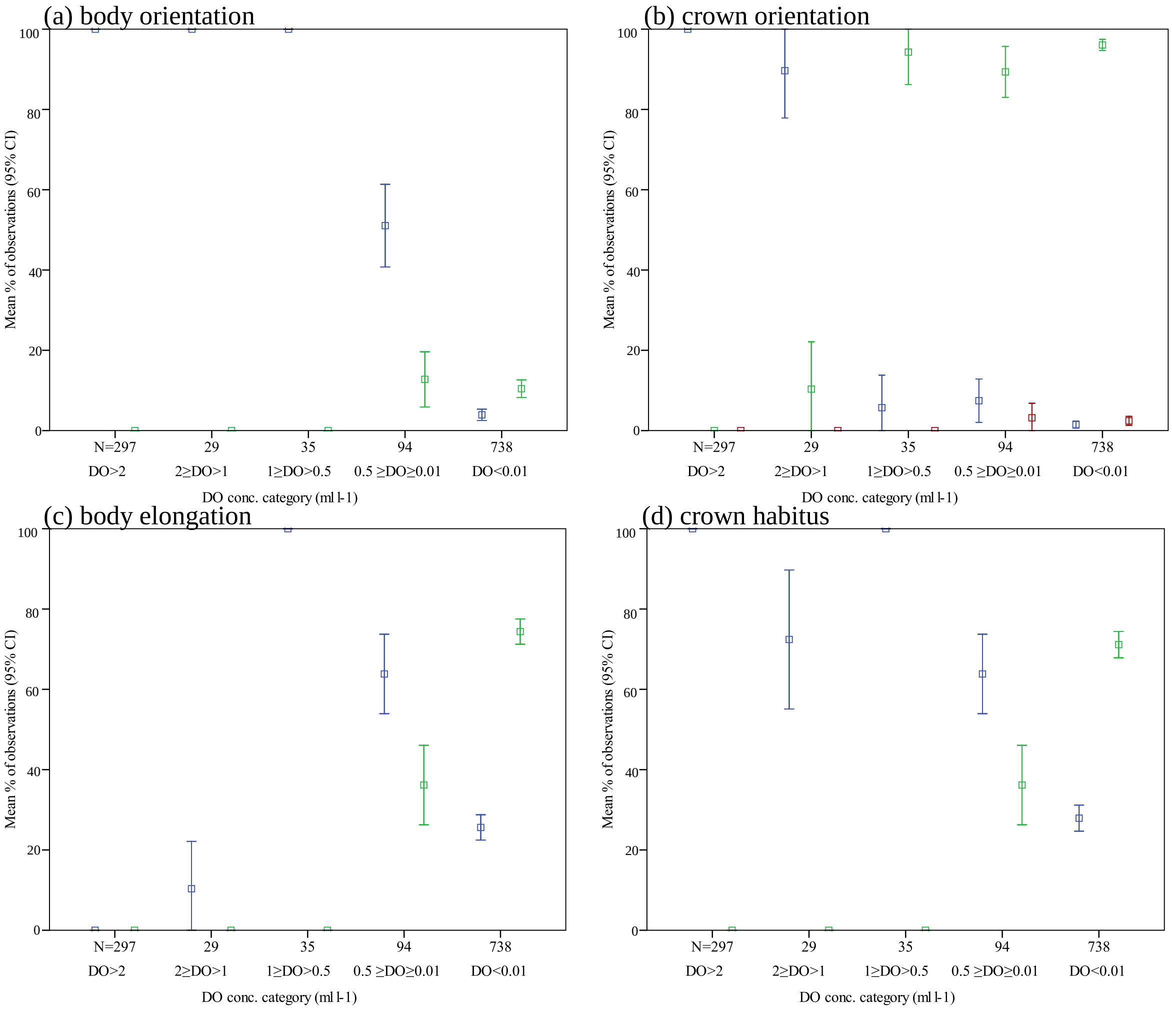


Fig. 19: Behavioral change of *Ocnus planci* during deployment: (a) anterior end (blue error bars) or posterior end upwardly orientated (green error bars), (b) upwards (blue error bars), to the side (green error bars) and downwards (red error bars) orientated mouth, (c) minor (blue error bars) and major (green error bars) body elongation and (d) closed (blue error bars) and open (green error bars) tentacle crown. For description of diagram see figure legend of Fig. 9.

3.3.11 *Ophiothrix quinquemaculata* (Delle Chiaje, 1829; Ophiuroidea: Ophiotrichidae)

From ~40 brittle stars in the chamber (Fig. 16 “oq”), four individuals were evaluated. Initially, all *O. quinquemaculata* were mostly stationary on multi-species clumps in their normal suspension-feeding posture (i.e. arms were directed upwards or lateral, Fig. 20a, b). Weak hypoxia elicited a clear change in both locomotion and arm-posture: the brittle stars became more active, moved horizontally on the multi-species clumps (23% in 116 observations), and upwards/lateral arm-posture gradually began to decrease.

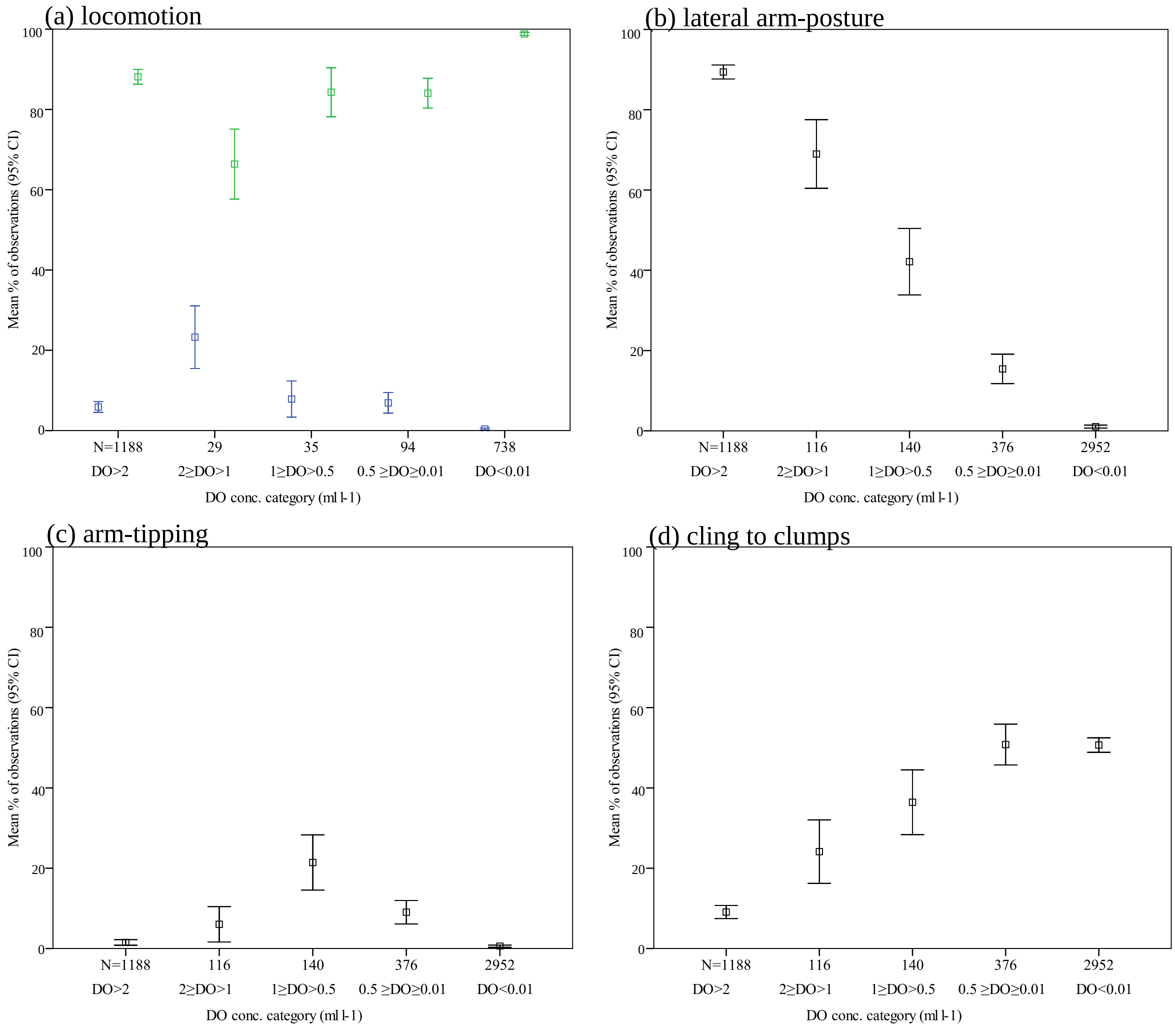


Fig. 20: Behavioral changes of *Ophiothrix quinquemaculata* during the experiment: (a) horizontal (blue error bars) and no locomotion (green error bars); (b) lateral arm posture, (c) arm-tipping and (d) cling to clumps. For description of diagram see figure legend of Fig. 9.

Arm-tipping (i.e. the brittle stars began to stand on their arms and elevated their central disk) peaked at moderate hypoxia (21% of 140 observations, Fig. 20c); *O. quinquemaculata* gradually became motionless and finally clung moribund to their sponge substrate

(Fig. 20a, d). Two individuals fell onto the sediment from the multi-species clump immediately before death. All four brittle stars died at the onset of anoxia, between h 47 and 49 (H_2S 0.01 and 0.18 µmol l^{-1}, respectively). These were the first mortalities in the experiment.

3.3.12 *Microcosmus sulcatus* (Cocquebert, 1797; Ascidiacea: Pyuridea)

Until severe hypoxia, the oral siphon of the three individuals (Fig. 16 “ms”) was mostly open, then siphon closure significantly increased from 14 % at severe hypoxia to 43 % at anoxia (p<0.01, Appendix 1: Table 12; Fig. 21a). A completely open siphon was observed the last time at h 83 (H_2S 191 µmol l^{-1}); after that the opening was either closed or half open. Body contractions were observed throughout the experiment but slightly increased towards the end of the deployment, peaking at anoxia (13% in 2214 observations, Fig. 21b). One individual drooped between h 97 (H_2S ~137 µmol l^{-1}) and h 118 (H_2S ~110 µmol l^{-1}), which is interpreted as the time of death. The two other *M. sulcatus* survived.

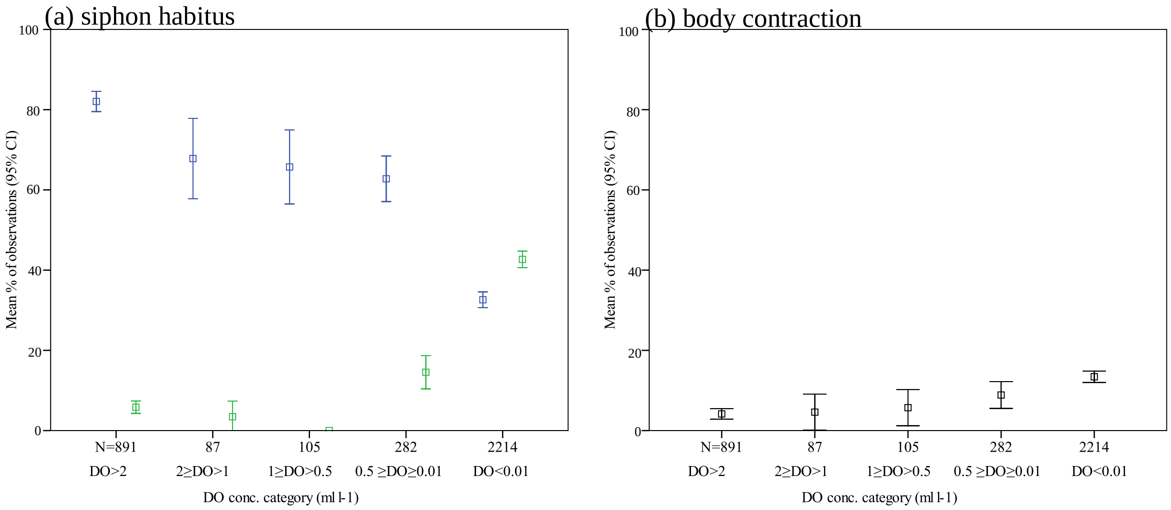


Fig. 21: Behavioral change of *Microcosmus sulcatus* during the experiment: (a) open (blue error bars) and closed (green error bars) siphon, (b) body contractions. For description of diagram see figure legend of Fig. 9.

3.3.13 *Phallusia mammilata* (Cuvier, 1815; Ascidiacea: Ascidiidae)

The change in siphon habitus of the single *P. mammilata* individual (Fig. 16 “pm”) paralleled that of *M. sulcatus*: in more than 72% of observations the aperture was open until severe hypoxia. At anoxia, closed openings dominated (56% in 738 observations, Fig. 22a). This ascidian also contracted its body throughout the experiment (Fig. 22b), but this activity significantly increased at anoxia (p<0.01, Appendix 1: Table 13). At h 77 (H_2S ~255.27 µmol l^{-1}) *P. mammilata* started to droop and died 2 hours later.

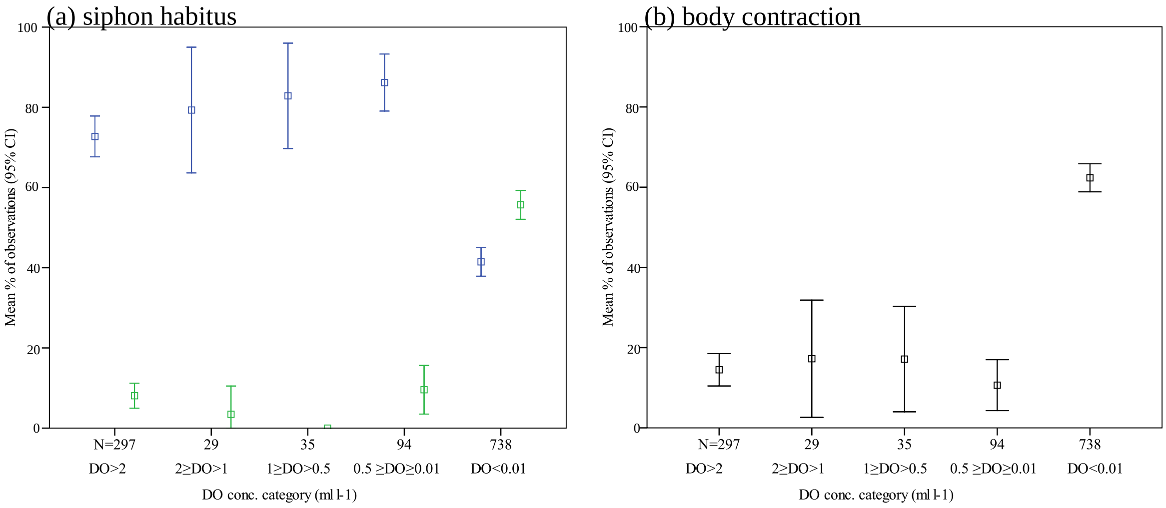


Fig. 22: Behavioral change of *Phallusia mammilata* during the experiment: (a) open (blue error bars) and closed (green error bars) siphon, (b) body contractions. For description of diagram see figure legend of Fig. 9.

3.3.14 Unidentified sipunculan

The one individual visible in the experiment emerged from hiding first at anoxia, at h 76 (H_2S ~244 µmol l^{-1}). The worm moved across the sediment for one hour and disappeared from view (Fig 23, red column).

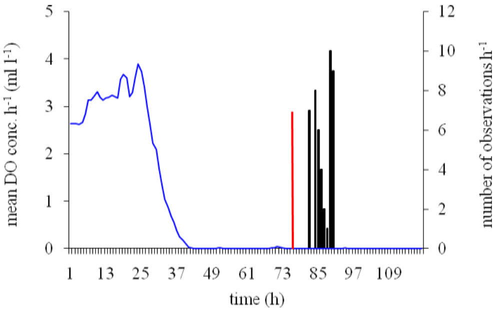


Fig. 23: Visibility of the unidentified sipunculan (red column) and the unidentified worm (black columns). For description of diagram see figure legend of Fig. 8.

3.3.15 Unidentified worm

One individual emerged at h 82 (H_2S ~195 µmol l^{-1}; Fig. 23, black columns). The worm mostly squirmed on the spot and shortly moved horizontally before it disappeared from view 8 h later (h 90, H_2S ~244 µmol l^{-1}).

3.4 Tolerance of evaluated species

Oxygen depletion and increasing H_2S concentrations killed many, but not all animals in this experiment. All mortalities occurred during anoxic conditions (Fig. 24). The brittle star *Ophiothrix quinquemaculata* was the most sensitive species in the experiment: it showed atypical arm-posture already 4 hours after chamber deployment, and was also the first to die (between h 47 and h 49, H_2S <0.2 µmol l^{-1}). *Schizaster canaliferus* individuals were the next in the mortality sequence: sediment bulging was recorded from h 34 on (moderate hypoxia, DO ~0.9 ml l^{-1}) and the infaunal sea urchins died between h 58 and h 77 (H_2S ~38-299 µmol l^{-1}). Mortality of *Chlamys varia* was observed also around this time, at h 75 (H_2S ~257µmol l^{-1}), but the first response to the oxygen depletion, the swollen mantle tissue, was recorded earlier, already at normoxia, at h 29. *Phallusia mammilata* died at h 80 (H_2S ~261 µmol l^{-1}), the two *Serpula vermicularis* individuals at h 84 (H_2S ~250 µmol l^{-1}) and *Ocnus planci* at h 90 (H_2S ~244 µmol l^{-1}). The last observed mortality occurred at h 118, at a H_2S value of ~110 µmol l^{-1}, i.e. one *Microcosmus sulcatus*. The other two *M. sulcatus* survived until the end of the experiment, as did all individuals of *Corbula gibba*, *Cereus pedunculatus* and *Hexaplex trunculus*. These organisms survived 15.8 h of hypoxia, followed by 73.8 h of anoxia and H_2S concentrations of ~304 µl l^{-1} (the highest value during the experiment). However, the resistant species also showed atypical behaviors early on: the first three individuals of *C. gibba* emerged already during normoxia, *C. pedunculatus* changed its color from h 31 on (DO ~1.7 ml l^{-1}) and began to extend its body. As a first response to the dropping oxygen values, *H. trunculus* moved up on the plexiglass to the lid. This behavior was observed the first time already 3 h after chamber deployment. In *Fusinus* sp., *Tubulanus annulatus*, *Abra alba*, the sipunculan and the unidentified worm it was not possible to determine the time of death because the individuals disappeared from the view before the end of the experiment.

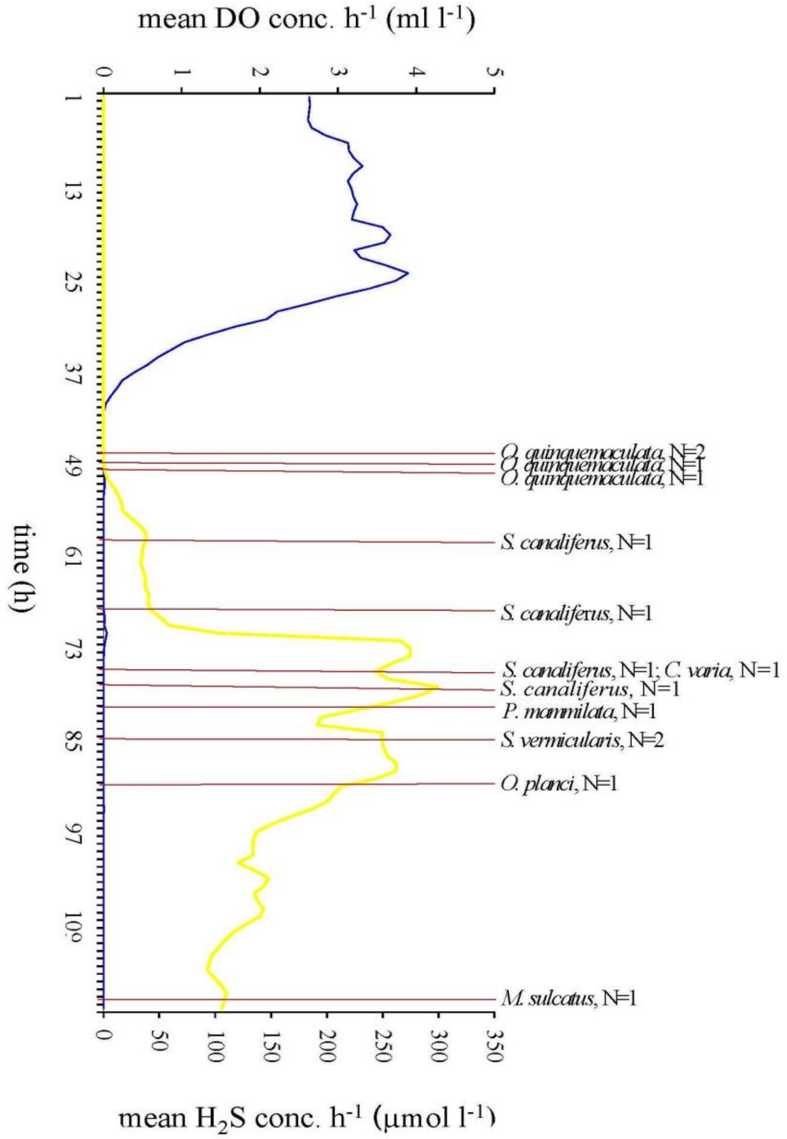


Fig. 24: Sequence of mortality during the experiment. Blue line: DO concentration, yellow line: H_2S concentration.

4. Discussion

Oxygen depletion is probably one of the most widespread deleterious effects induced by humans in marine ecosystems. More than any other impact, it causes extensive mortalities of benthic macrofauna. Over the past decades the number of coastal areas affected by hypoxia has increased rapidly, but our knowledge about the exact behavioral responses and tolerance of macroepi- and infauna to oxygen deficiency is still insufficient. The benthic community investigated in the present study plays a crucial role in the ecosystem; the high loss of biomass and the loss of this ecosystem function (Ott, 1992) during oxygen crises can lead to long-term ecosystem shifts (Gray et al., 2002). This makes it particularly important to understand the phases of its deterioration process in detail. The experimental approach of the EAGU provided results on the sequence of *in situ* reactions to hypoxia and anoxia of the O-R-M community in the Gulf of Trieste. Beyond the definition of behavioral responses and their correlation to distinct oxygen concentrations, mortality times and sequences of the investigated taxa were determined.

4.1 Critical oxygen thresholds and behavioral reactions

The basic responses to declining oxygen values and/or to increasing duration of anoxia and H_2S were: (1) increase or decrease in normal activities, (2) initiation of atypical behaviors, (3) unexpected intraspecific interactions, (4) emergence of infauna, and (5) mortalities. The documented behaviors and mortalities of the selected species were correlated to five oxygen categories: normoxia (>2.0 ml O_2 l^{-1}), weak hypoxia (≤2.0-1.01 ml O_2 l^{-1}), moderate (1.0-0.51 ml O_2 l^{-1}) and severe hypoxia (0.5-0.01 ml O_2 l^{-1}) and anoxia (according to Diaz and Rosenberg, 1995).

The framework of the present study was an experiment in the series of EAGU deployments introduced in Stachowitsch et al. (2007) and initially described in Riedel et al. (2008b). Those earlier results confirmed that reactions are related to specific oxygen levels. The presence of an intermediate oxygen-peak in this experiment (Fig. 1 in Riedel et al., 2008b) showed that the behavior changes of the organisms are strongly linked to the changes in oxygen concentrations: around the re-oxygenation peak, the atypical behaviors were interrupted and normal activities resumed. Subsequently, a return of hypoxia again induced the above atypical reactions during the second oxygen decrease.

4.1.1 Weak hypoxia

In present study an oxygen concentration of 2 ml l^{-1} is considered as the onset of hypoxia, in reference to the categories defined in Diaz and Rosenberg (1995). According to Gray et al. (2002), however, it is likely that different taxa have different susceptibility to hypoxia, and one particular threshold may be, therefore, inadequate to describe a generally valid hypoxia level. Vaquer-Sunyer and Duarte (2008) concluded that reactions to hypoxia occur at a broad range of DO concentrations, including oxygen concentrations well above the generally applied threshold.

My results confirm that some organisms already exhibit responses to oxygen depletion before reaching 2 ml l^{-1} DO. The brittle star *Ophiothrix quinquemaculata* already initiated arm-tipping behavior at ~3.7 ml l^{-1}DO. A similar situation was observed in the infaunal bivalve *Corbula gibba*: 3 individuals emerged from the sediment at ~3.2 ml l^{-1}DO. Interestingly, the epifaunal bivalve *Chlamys varia*, which might have been expected to react earlier than the burrowing species (Hagermann, 1998), showed a swollen mantle tissue at ~2.2 ml l^{-1}. Finally, even sessile species showed early reaction: for example the polychaete tubeworm *Serpula vermicularis* retracted its crown into the tube at ~2.6 ml l^{-1}DO.

The analysis of most other species, however, underlined the validity of the Diaz and Rosenberg threshold: these organisms showed atypical behaviors only below the level of 2 ml l^{-1} DO, which we termed weak hypoxia. The sea anemone *Cereus pedunculatus* started to extend from the sediment, accompanied by pharynx protrusion, body rotation and contractions below ~1.6 ml l^{-1}DO. The column and pharynx extension may increase the surface area to volume ratio and decrease diffusion distances within the tissues to enhance oxygen uptake (Sassaman & Magnum, 1972). Body rotations and constrictions may also improve oxygen delivery and consumption. This behavior pattern parallels that observed by Sagasti et al. (2001) in laboratory experiments for *Diadumene leucolena*, as well as that recorded for *C. pedunculatus* by Stachowitsch (1984) in the 1983 mass mortality in the Gulf of Trieste and by Riedel et al. (2008b) with the same experimental set-up . The sea cucumber *Ocnus planci* started to elongate its body as well, increasing its surface area to volume ratio. The large coelom in holuthurians also “provides a store of oxygen”, which could improve the chance of survival (Astall & Jones, 1991). Weak hypoxia triggered an upward movement of 3 individuals of the gastropod *Hexaplex trunculus* on the plexiglass wall to the chamber lid. This response can be interpreted as an avoidance reaction and as an attempt to reach more oxygenated waters, as in higher water layers higher DO values are likely. According to Hagerman (1998), vertical movement is one of the simplest strategies to avoid hypoxia.

O. quinquemaculata showed an initially high locomotion activity at weak hypoxia and continued the arm-tipping already initiated at normoxia. This behavior was followed by clinging to the multi-species clumps. Standing on the tips of their arms with the disc elevated above the substrate is interpreted as an effort to reach more oxygenated water layers. Such atypical postures of brittle stars during oxygen depletion have been described by Dethlefsen and Westernhagen (1983) in the German Bight. Humped body positions of ophiuroids have been recorded by Rosenberg et al. (1991) and by Visisten & Visman (1997) in laboratory experiments and by Stachowitsch (1984) in the field as well.

During the whole time of weak hypoxia, *Chlamys varia* showed a swollen mantle tissue, paralleling the experiment of Riedel et al. (2008b). This condition was not observed during normoxia and must therefore be interpreted as a stress reaction. It is likely that its surface increase is aimed at longer survival in a stressed environment.

As mentioned above, the first group of 3 individuals of *Corbula gibba* emerged already at normoxic conditions (around 21:00 h). The second group of 5 individuals, however, only became visible one day later around the same time, during weak hypoxia. Emergence and reburial of infaunal bivalves is a frequent response to oxygen deficiency in Scandinavian basins with seasonal hypoxia (Jørgenssen, 1980). Nevertheless, since both intervals between the emergence of group 1 and 2, and group 3 and 4 (both at ~13:00 h, at anoxic conditions) are around 24 h, it is likely that not only the oxygen concentrations but the diurnal rhythm also affects this organism.

4.1.2 Moderate hypoxia

Oxygen levels between 1.0 and 0.5 ml l^{-1} DO, termed moderate hypoxia here, triggered the emergence of the infaunal sea urchin *Schizaster canaliferus*. The animals did not become visible in this oxygen category, but the sediment above them began to bulge, indicating an effect of oxygen depletion at this level. Such a sediment bulging, and its duration, have never been reported previously. Emergence of burrowing species under hypoxic conditions was reported in numerous studies (Diaz & Rosenberg, 1995; Schinner et al., 1997; Wu, 2002; Levin et al., 2009). Laboratory observations (Nilsson and Rosenberg, 1994) revealed similar oxygen thresholds for *Echinocardium cordatum* to my observations of *S. canaliferus*; both species began to leave the sediment before DO values reached 0.7 ml l^{-1}.

The polychaete *Serpula vermicularis* opened its crown at moderate hypoxia after a continuous closed phase during weak hypoxia. The contraction of the tentacles during oxygen depletion has the advantage of reducing oxygen consumption. Further decrease of the DO values, however, triggers the animals to extend the crown again, even at low oxygen concentrations (Theede, 1973).

The gastropod *Fusinus* sp. was mostly stationary during the whole experiment. Enhanced movement was observed only during moderate hypoxia, which ceased as oxygen concentrations dropped below 0.5 ml l^{-1}.

4.1.3 Severe hypoxia and anoxia

The different species of bivalves reacted differently to dropping oxygen concentrations. The infaunal bivalve *Abra alba* first left its natural position in the sediment as DO values dropped below 0.5 ml l^{-1}. Immediately after emerging it moved across the sediment surface and its siphon became visible. After a 20 h-period of locomotion (less than 10 cm), the two specimens of *A. alba* remained immobile, with siphons stretched across the sediment surface. Some bivalves react to hypoxia by stretching their siphons up into the water column where more oxygen may be available. In their experiments, Rosenberg et al. (1991) observed, in *A. alba***,** 3.5 times higher siphon activity in the water column than that of other individuals in normoxic conditions. In Limfjorden (Denmark) during hypoxic conditions, siphons of *Mya arenaria* extended up to 30 cm above the bottom have been recorded by Jørgenssen (1980). In the 1983 mass mortality event in the Gulf of Trieste, *Cardium* sp. showed same behavioral pattern (Stachowitsch, 1986).

Chlamys varia modified its behavior by opening its valve gape widely, followed by a repeated closure. Already before this process the mantle tissue became retracted. In the 1983 mass mortality, *Pecten jacobaeus* and *Cardium echinatum* were found on the sediment with gaping valves (Stachowitsch, 1984). *Hexaplex trunculus* located on the sediment began to periodically extend/retract its foot, as did *Fusinus* sp. Of the three *H. trunculus* individuals located on the plexiglass lid, two fell down during anoxia and showed the same behavior pattern. This agrees with the field observations of Stachowitsch (1984), who found the gastropod *Murex brandaris* with its foot partially retracted, lying on the side.

At severe hypoxia, *Cereus pedunculatus* elongated its body more intensely, accompanied by an enhanced rate of rotations and contractions. This body extension ceased more than three days after reaching anoxic conditions; the rate of the other activities also dropped. The behavior and the tolerance of the two ascidians paralleled each other. *Microcosmus sulcatus* and *Phallusia mammilata* gradually closed their oral siphons during severe hypoxia. Fiala-Medioni (1979) described a decreased pumping and filtration in *P. mammilata* already as oxygen concentrations dropped below 3.85 ml l^{-1}; this may explain the very low, constant rate of body contractions observed in ascidians throughout the experiment. Nevertheless, Riedel et al. (2008b) and Steckbauer (2009) reported enhanced contraction rates in ascidians as DO values began to fall; this, however, might also have been provoked by the aggregation of crustacean at the oral siphon.

Other behaviors observed during severe hypoxia included the locomotion of *Schizaster canaliferus* on the sediment. The sea urchins moved around over a long period on the surface, followed by a moribund phase. One individual covered a distance of ca. 40 cm, the other three animals moved less, similar to the other burrowing species mentioned above.

During severe hypoxia, more intense elongation in *Ocnus planci* was observed. The sea cucumber opened its crown entirely for the first time during severe hypoxia. An open crown with extended tentacles is the feeding position of these animals. In the mass mortality event in the Gulf of Trieste, the same species was reported to lie on the sediment with partially retracted tentacles (Stachowitsch, 1984). The atypical long closed phase of the crown in this case, however, cannot be attributed solely to oxygen depletion because it was already observed during the open configuration of the chamber. This indicates a reaction to some other condition.

All *Ophiothrix quinquemaculata* individuals were found moribund, lying on the multi-species clumps or on the sediment surface, some of them overturned, at the onset of anoxia. The total absence of oxygen, coupled with high H_2S concentrations, triggered the emergence of a sipunculan and an unidentified cryptic worm; however, both organisms were visible only briefly (1 and 8 h, respectively) before hiding again under the multi-species clump.

4.2 Tolerance and mortality

Mortality, particularly mass mortality, is the common response of benthic communities to aperiodic and seasonal hypoxia. The timescale of the events, however, can be very different due to the conditions at the different locations: from months in New York Bight (Swanson & Sindermann, 1979) and weeks in Limfjorden (Jørgenssen, 1980), to days in the Gulf of Trieste (Stachowitsch, 1984). In the latter case, the time interval between the first oxygen-depletion-induced behavioral modifications and the onset of mortalities was very quick; after four days, less than 10 % of the macroepifauna biomass remained and most of the emerged infauna species began to die (Stachowitsch & Fuchs, 1995).

In the present experiment, despite the short, four day-deployment of the closed configuration, almost all organisms died. This may partially reflect the rapid time-course of hypoxia: the closed chamber of the EAGU generated hypoxic conditions within ~7 h and anoxia within ~19 h. The rapidity of oxygen decline can influence mortality thresholds (Haselmair et al., in press). The very high biomass of the enclosed organisms (1042.71 g) might play another key role here: high biomass corresponds with high respiration, and after the mortality the oxygen demand of the microbes is related to the amount of the decomposable dead material. The maximum H_2S value reached during deployment was ~304 $\mu l\ l^{-1}$. All mortalities occurred during anoxia.

The first animals that died, and consequently the most sensitive in the present experiment, were *Ophiothrix quinquemaculata*. The mortality of all four evaluated individuals occurred at the early onset of anoxia, around one day after chamber deployment. *O. quinquemaculata* are known to be very sensitive to natural oxygen depletion events (Stachowitsch, 1984). In laboratory experiments, however, other ophiuroids showed higher tolerance (Visisten & Vismann, 1997): at such low H_2S values where O. *quinquemaculata* died in present study (< 0.2 ml l^{-1}), the individuals of the infaunal *Amphiura filiformis* and the epifaunal *Ophiura albida* had a median lethal time (LT_{50}) of five and two days, respectively. This, beyond the species-specific differences, may also reflect the effect of different experiment setups. The tolerance of a species to specific thresholds may well differ in *in situ* versus laboratory experiments, where the organisms are typically deprived of normal substrates and hiding places etc.

At about the same time the evaluated brittle stars died, two atypical predatory events were observed: two *O. quinquemaculata* were predated by a *Cereus pedunculatus*. This interspecific interaction, induced by oxygen deficiency, was described for the first time by Riedel et al. (2008a). *C. pedunculatus* extended from the sediment, made contact with, pulled in and consumed the brittle stars. The duration of both predatory events was around two and a half hours. *C. pedunculatus* is one of the organisms that survived and remained active until the end of the experiment. Anemones are known to be particularly tolerant to anoxia (Jørgenssen, 1980; Stachowitsch, 1984; Sagasti et al., 2001) due to their ability to use anaerobic pathways and to depress their metabolism (Rutherford & Thuesen, 2005). The combination between the activity of the anemone and the moribund condition of the brittle stars increases the chance of predation. Accordingly, *C. pedunculatus* may also benefit from oxygen deficiency (Riedel et al., 2008a). This supports the statement of Breitburg et al. (1994) that predation interactions could be affected by hypoxia, and that of Sandberg (1994) that oxygen depletion can induce the exploitation of the most intolerant prey.

Another echinoderm, *Schizaster canaliferus*, was the second species in the mortality sequence. The individuals died at the latest two days after chamber deployment. According to Gray et al. (2002), most echinoderms are intolerant of anoxia. In contrast, Vaquer-Sunyer and Duarte (2008) reported a high LT_{50} of echinoderms exposed to acute hypoxia, but this may be a consequence of summarizing different types of experiments, including laboratory setups. The mortality of *Chlamys varia* occurred also around this time. Bivalves are generally tolerant to hypoxia. Here, however, the mortality of other key bioherm components might impact the resistance of theoretically more tolerant species that use them as substrate, in this case *C. varia* (Riedel et al., 2008b). Sponges, for example, were among the most sensitive taxa in the 1983 mass mortality in the Gulf of Trieste (Stachowitsch, 1984), and their death clearly accelerates stress behavior in the associated fauna (Stachowitsch, 1991).

In contrast, the infaunal bivalve *Corbula gibba* and the gastropod *Hexaplex trunculus* survived until the end of the experiment. Vaquer-Sunyer and Duarte (2008) described mollusks and cnidarians as the most resistant groups to hypoxia. Under disadvantageous conditions, most mollusks can close their shells. Bivalves additionally reduce their heart beat and feeding activity. This reduces their oxygen demand, enabling the mollusks survive the oxygen-depleted periods with anaerobic energy production (Theede et al, 1969; Theede, 1973). *Mytilus edulis* and infaunal bivalves have been reported to survive anoxia for one to two weeks (Jørgenssen, 1980). The tolerance of *H. trunculus* to oxygen depletion is high also (field observations; Stachowitsch, 1984). The survival of the individuals in the present experiment, however, can be attributed at least partially to the advantage they gained by reaching the plexiglass lid, where slightly higher DO values are likely.

After the death of *C. varia* the successive mortalities were: *Phallusia mammilata* (h 80), *Serpula vermicularis* (both individuals at h 84) and *Ocnus planci* (h 90). Finally, one *Microcosmus sulcatus* individual died, almost four days after chamber deployment. The other two *M. sulcatus* survived until the end of the experiment.

Ascidians and polychaetes are known to be resistant to oxygen crises. Stachowitsch (1986) found living *Microcosmus*, a designating species in the multi-species clumps, and polychaetes 4 days after the onset of the community deterioration. Polychaetes, due to their ability to reduce their metabolic activity, showed long LT_{50} in several experiments when exposed to anoxia and H_2S (e.g. Theede et al., 1969; Diaz & Rosenberg, 1995; 96 and 99 h respectively).

The long survival of the sea cucumber *Ocnus planci* also indicates that these animals can reduce their metabolic requirements under stress (Astall & Jones, 1991). In the 1983 mass mortality, *O. planci,* as was the case in all other epifaunal sea cucumbers, were reported to be dead on the third day (Stachowitsch, 1986). This parallels the here-observed mortality 66 h after chamber deployment.

Discussing the sublethal/lethal effects of anoxia calls for considering the potential simultaneous presence of sulfide as well. Once anoxia occurs, hydrogen sulfide increases due to the activity of sulfate-reducing bacteria. Several studies deal with the lower resistance of the benthic fauna to oxygen depletion when simultaneously exposed to H_2S (e.g. Theede et al., 1969; Theede, 1973; Visisten & Vismann, 1997; Hagerman, 1998; Vaquer-Sunyer & Duarte, 2010). Sulfide is very toxic to most aerobic organisms due to the inhibition of cytochrome *c* oxidase in the respiratory chain (Visisten & Vismann 1997). Adaptive strategies to H_2S exposure are excluding sulfide from entering the body, oxidizing it to harmless compounds or switching to anaerobic energy production. The anaerobic metabolism protects the animals both against high sulfide concentrations and to anoxic conditions (Hagerman, 1998). These abilities are exhibited by several organisms analyzed in the present study. Nevertheless, according to Vaquer-Sunyer and Duarte (2010) the survival times in benthic communities under hypoxia are reduced by an average of 30% if they are exposed to H_2S. Visisten & Vismann (1997) argue that sulfide is the environmental factor to which benthic animals have the lowest tolerance. This makes it a key factor for distribution and survival. This also means that the threats posed to marine biodiversity by hypoxia are more significant than assumed based solely on the direct effects of low oxygen concentrations (Vaquer-Sunyer & Duarte, 2010).

The increasing number of areas affected by hypoxia and anoxia is significantly changing the structure of the benthic communities. This, in turn, also affects benthic-pelagic coupling. The accompanying mortalities eliminate well-developed communities (Diaz & Rosenberg, 1995), like the O-R-M community investigated in present study. The function of such filter- and suspension-feeding communities as a natural eutrophication control (Officer et al., 1982) is lost. The recolonization process of this epifaunal assemblage in the northern Adriatic Sea was very slow: three years after the 1983 mass mortality event, less than 50% of the original biomass was present and it was dominated by atypical species, including serpulid polychaetes and rapidly growing ascidians (Stachowitsch, 1991). Even after ten years the community still had not recovered from this initial disturbance and had not attained its former status (Stachowitsch & Fuchs, 1995). This was compounded by additional small-scale anoxias and the effects of benthic fisheries in the Gulf of Trieste. The present characteristics of the O-R-M community (low biomass, atypical species composition) most likely hinder its role in maintaining a stabilizing function. This makes the overall ecosystem more vulnerable to further disturbances.

Oxygen depletion events have emerged as major threats to coastal ecosystems globally. The present study, together with ongoing experiments, will help better understand and determine the status of benthic ecosystems exposed to hypoxia and, hopefully, better protect and conserve their biodiversity in the future.

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6. Abstract

Hypoxia and anoxia are key threats to modern shallow coastal ecosystems worldwide. The occurrence of oxygen depletion events has increased dramatically over recent decades both in frequency and intensity. In the Adriatic Sea, hypoxia may affect up to 3000 km² of the seafloor, leading to extensive mass mortalities of the benthos. The onset of these catastrophic events, however, is hard to predict. Deploying an experimental anoxia generating unit (EAGU) we created and fully documented a small-scale anoxia event (0.25 m^2) in 24 m depth, in the northern Adriatic Sea. The instrument combines photo-documentation with detailed chemo-physical recording and allows the analysis of the behaviors and mortalities of benthic organisms before, during and after oxygen depletion. The responses to declining oxygen values and/or to increasing duration of anoxia were: (1) increase or decrease in normal activities, (2) initiation of atypical behaviors, (3) unexpected intraspecific interactions, (4) emergence of infauna, and (5) mortalities. The documented behaviors and mortalities of the selected species were correlated to five oxygen categories: normoxia (>2.0 ml O_2 l^{-1}), weak hypoxia (≤2.0-1.01 ml O_2 l^{-1}), moderate (1.0-0.51 ml O_2 l^{-1}) and severe hypoxia (0.5-0.01 ml O_2 l^{-1}) and anoxia. The results show considerable differences in tolerance to oxygen depletion from species to species. Weak hypoxia triggered increased locomotion in the brittle star *Ophiothrix quinquemaculata* and in the gastropod *Hexaplex trunculus,* which is interpreted as an escape behavior. Different species-specific sublethal responses were observed, such as body extension in the sea anemone *Cereus pedunculatus*, and in the sea cucumber *Ocnus planci*, or swollen mantle tissue in the epifaunal bivalve *Chlamys varia*. Moderate hypoxia elicited the emergence of the infaunal sea urchin *Schizaster canaliferus*. At severe hypoxia ascidians began to close their siphon and the activity of the ophiuroids ceased followed by a moribund phase. The first mortalities occurred at the onset of anoxia. The brittle stars, infaunal sea urchins and *C. varia* were among the first to die; *C. pedunculatus*, *H. trunculus,* the infaunal bivalve *Corbula gibba* survived.

The present study, together with ongoing experiments, is an important step in compiling a generally valid catalogue of reactions and a list of sensitive and tolerant species. This approach will ultimately help to better determine the status of benthic ecosystems exposed to oxygen depletion here and elsewhere.

7. Zusammenfassung

Hypoxie und Anoxie sind Schlüsselfaktoren, welche die heutigen flachen Küstenökosysteme weltweit bedrohen. Das Auftreten von Sauerstoffarmut hat in den letzten Jahrzehnten sowohl in der Häufigkeit als auch in der Intensität stark zugenommen. In der Adria können bis zu 3000 km² des Meeresgrundes von Hypoxie betroffen sein, was zu ausgedehnten Massensterben des Benthos führt. Der Beginn solcher Katastrophen ist kaum vorherzusagen. Mit Hilfe eines Gerätes, das experimentell Anoxie erzeugt, dokumentierten wir in der nördlichen Adria in 24 m Tiefe eine Sauerstoffkrise in kleinem Maßstab (0.25 m²). Das Gerät kombiniert fotografische Dokumentation mit detaillierter chemo-physikalischer Messung und ermöglicht Verhaltens- und Mortalitätsanalysen der benthischen Organismen vor, während und nach der Sauerstoffarmut. Die Reaktionen auf den abnehmenden Sauerstoffgehalt und/oder die ansteigende Dauer der Anoxie waren: (1) vermehrte oder verminderte Aktivität, (2) Auftreten von atypischem Verhalten, (3) unerwartete intraspezifische Interaktionen, (4) Hervorkommen von Infauna und (5) Mortalität. Die Verhaltensweisen und Sterberaten der ausgewählten Arten wurden mit fünf Sauerstoffkategorien in Korrelation gesetzt: normale Sauerstoffkonzentration (>2.0 ml O_2 l^{-1}), leichte (≤ 2.0-1.01 ml O_2 l^{-1}), moderate (1.0-0.51 ml O_2 l^{-1}) und ernste Hypoxie (0.5-0.01 ml O_2 l^{-1}) und Anoxie. Die Ergebnisse zeigten große Unterschiede zwischen den ausgewählten Arten im Bezug auf die Hypoxie- bzw. Anoxietoleranz. Leichte Hypoxie, zum Beispiel, löste eine erhöhte Lokomotionsaktivität des Schlangensterns *Ophiothrix quinquemaculata* und des Gastropoden *Hexaplex trunculus* aus, was als Fluchtverhalten gedeutet wird. Zudem wurden verschiedene artspezifische subletale Reaktionen dokumentiert, wie etwa die Körperausdehnung bei der Seeanemone *Cereus pedunculatus* und der Seegurke *Ocnus planci* oder die Mantelschwellung bei der epifaunalen Bivalve *Chlamys varia*. Moderate Hypoxie forcierte das Hervorkommen des infaunalen Seeigels *Schizaster canaliferus*. Bei ernster Hypoxie begannen die Seescheiden ihre Siphonen zu schließen und die Aktivität der Schlangensterne sank deutlich, bis sie letztendlich starben. Bei Anoxie wurden die ersten Mortalitäten dokumentiert, unter anderem die Schlangensterne, infaunale Seeigel und *C. varia*; *C. pedunculatus*, *H. trunculus* und die infaunale Bivalve *Corbula gibba* überlebten.

Die vorliegende Studie, in Kombination mit laufenden Experimenten, ist ein wichtiger Schritt, um einen allgemein gültigen Katalog von Verhaltensreaktionen und eine Liste von empfindlichen und toleranten Arten erstellen zu können. Dieses Herangehen wird letztendlich dazu beitragen, den Status benthischer Ökosysteme, welche einer Sauerstoffarmut ausgesetzt sind, besser einzuschätzen.

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Curriculum Vitae

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Education

2007-2010	Master studies of Ecology/Marine biology at the University of Vienna, Vienna, Austria. Thesis: A time-lapse camera experiment on benthic reactions to anoxia in the Northern Adriatic Sea (Supervisor: Doz. Dr. M. Stachowitsch)
2002-2007	Diploma studies of Biology/Ecology at the Eötvös Lorand University, Budapest, Hungary (not finished, accepted at the University of Vienna as Bachelor of Science)

Research activities and training courses attended

2010	FISH course – Fluorescent *in situ* hybridization
2010	Stable isotope tracing course
2009	Specific techniques in electron microscopy and ultra-structure research
2009	Submicroscopic anatomy and preparation techniques – Electron microscopy course
2008	Reef ecology course, Dahab, Egypt
2008	Nature conservation course – Sea turtles in Turkey, Yaniklar, Turkey
2007	Data collection in the project "The Interrelation between Social Learning and Physical Cognition in Kea", Konrad Lorenz Institute for Ethology, Vienna, Austria
2007	Marine ecology course, Station de Recherches Sous-Marines et Océanographiques, Calvi, Corsica – "The Cleaning Behavior of the Mediterranean Cleaner Wrasse *Symphodus melanocercus*"

2007	Marine biological field course on the Mediterranean fauna and flora, Centre for Marine Research, Rovinj, Croatia
2006	Laboratory class- Introduction into marine laboratory work
2006	Data collection and data analysis in the project “The Effects of the Various Food Quality and Quantity on the Vocalization in *Callithrix jacchus*”, University of Vienna, Vienna, Austria (within the Erasmus Scholarship)
2004-2006	Data collection and data analysis in the project “Vocalization in Various Situations in Belgian Shepherd Dog”, Eötvös Lorand University, Budapest, Hungary
2004	Summer Internship Program in the Cetacean Research and Rescue Unit, Moray Firth, Scotland

Personal skills

- Languages:
  Hungarian (native), German (fluent), English (fluent), Turkish (good) and Spanish (good)
- Computer skills:
  Microsoft Office applications, EndNote, statistics (SPSS, Statistica), image processing (Adobe Photoshop, Adobe Illustrator), Adobe Audition, ArcGis
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  Electron microscopy techniques: TEM, SEM, ESEM, EELS, ESI, EDX, Dot mapping
  FISH
  Stable isotope tracing
  Analogue, digital and underwater photography
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Conferences

2010	3rd International Paleontological Congress (IPC), London, UK, 28 June-3 July (poster) European Geosciences Union (EGU), Vienna, Austria, 2-7 May (poster) Ecology Science Day, University of Vienna, Austria, 12 March (talk)

Appendix 1

Table 3: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Cereus pedunculatus* (results for not shown data are omitted). Numbers 1-5 under „comparison" refer to oxygen categories: 1: normoxia; 2: weak hypoxia; 3: moderate hypoxia; 4: severe hypoxia; 5: anoxia. Bold numbers indicate highly significant (P<0.01); underlined numbers indicate significant (P<0.05) differences.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Closed crown	Mann-Whitney *U*	61436.0	73430.0	208490.0	1356336.0	8050.0	17994.0	113880.0	21490.0	135660.0	466188.0
	Wilcoxon *W*	68222.0	83300.0	914756.0	2062602.0	17920.0	24780.0	120666.0	31360.0	145530.0	537064.0
	Z	-3.598	-4.276	-3.309	-14.704	-1.099	-4.709	-7.518	-5.441	-8.458	-6.235
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.272	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Open crown	Mann-Whitney *U*	50104.0	49000.0	212384.0	1430118.0	7000.0	16928.0	92926.0	16800.0	83650.0	425396.0
	Wilcoxon *W*	756370.0	755266.0	918650.0	5788746.0	13786.0	87804.0	4451554.0	87676.0	4442278.0	4784024.0
	Z	-5.758	-9.532	-1.693	-10.772	-4.530	-4.555	-9.780	-8.284	-13.890	-8.609
	P - value	**<0.01**	**<0.01**	0.090	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Retraction	Mann-Whitney *U*	68354.000	82148.000	214720.000	1706760.000	8086.000	21140.000	168020.000	25624.000	203648.000	548336.000
	Wilcoxon *W*	774620.0	788414.0	920986.0	2413026.0	14872.0	27926.0	174806.0	35494.0	213518.0	4906964.0
	Z	-0.826	-1.333	-4.836	-4.680	-0.241	-1.459	-1.070	-1.350	-0.908	-1.153
	P - value	0.409	0.183	**<0.01**	**<0.01**	0.810	0.145	0.285	0.177	0.364	0.249
Constriction	Mann-Whitney *U*	67122.0	69498.0	149094.0	976536.0	6996.0	15122.0	99780.0	21894.0	149028.0	493572.0
	Wilcoxon *W*	773388.0	775764.0	855360.0	1682802.0	13782.0	21908.0	106566.0	31764.0	158898.0	564448.0
	Z	-5.547	-14.088	-20.712	-27.737	-3.643	-6.573	-8.909	-3.752	-6.509	-4.080
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Minor extension	Mann-Whitney *U*	50244.0	37842.0	126462.0	1259226.0	5894.0	18254.0	169322.0	23394.0	152278.0	470672.0
	Wilcoxon *W*	756510.0	744108.0	832728.0	1965492.0	12680.0	25040.0	176108.0	94270.0	4510906.0	4829300.0
	Z	-16.545	-25.185	-23.484	-20.281	-4.407	-3.128	-0.258	-2.248	-6.643	-6.030
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.796	0.025	**<0.01**	**<0.01**
Major extension	Mann-Whitney *U*	68904.0	79596.0	144342.0	1293138.0	7772.0	14094.0	126266.0	18138.0	161246.0	504368.0
	Wilcoxon *W*	75690.0	785862.0	850608.0	1999404.0	14558.0	20880.0	133052.0	28008.0	171116.0	4862996.0
	Z	0.000	-7.149	-21.424	-19.586	-2.252	-7.491	-6.382	-7.071	-5.844	-3.738
	P - value	1.000	**<0.01**	**<0.01**	**<0.01**	0.024	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Pharynx protrusion	Mann-Whitney *U*	62964.0	64152.0	177606.0	1653102.0	6964.0	19222.0	166258.0	25694.0	171238.0	473096.0
	Wilcoxon *W*	769230.0	770418.0	883872.0	2359368.0	13750.0	26008.0	4524886.0	96570.0	4529866.0	4831724.0
	Z	-10.155	-16.674	-15.991	-8.420	-3.056	-2.923	-1.305	-0.588	-8.033	-10.297
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.192	0.557	**<0.01**	**<0.01**
Rotation	Mann-Whitney *U*	67716.0	76032.0	191268.0	1608552.0	7564.0	19052.0	160016.0	24796.0	206008.0	521144.0
	Wilcoxon *W*	773982.0	782298.0	897534.0	2314818.0	14350.0	25838.0	166802.0	34666.0	4564636.0	4879772.0
	Z	-4.528	-10.133	-13.289	-10.214	-2.394	-3.743	-2.544	-1.749	-0.128	-3.899
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.017	**<0.01**	0.011	0.080	0.898	**<0.01**

Table 4: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Fusinus* sp. (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Horizontal locomotion	Mann-Whitney *U*	4292.0	5180.0	13912.0	106101.0	507.5	1363.0	10324.0	1645.0	12460.0	33464.0
	Wilcoxon *W*	4727.0	5810.0	18377.0	150354.0	1137.5	5828.0	10759.0	6110.0	13090.0	37929.0
	Z	-0.312	-0.343	-0.563	-2.908	0.000	0.000	-1.028	0.000	-1.129	-1.848
	P - value	0.755	0.731	0.574	**<0.01**	1.000	1.000	0.304	1.000	0.259	0.065
Turn	Mann-Whitney *U*	4176.0	3870.0	13491.0	107716.5	362.5	1276.0	10193.5	1280.0	1280.0	9837.5
	Wilcoxon *W*	4611.0	48123.0	57744.0	151969.5	797.5	1711.0	10628.5	5745.0	5745.0	282528.5
	Z	-0.949	-6.144	-1.473	-1.234	-3.109	-1.389	-1.200	-3.386	-3.386	-5.879
	P - value	0.343	**<0.01**	0.141	0.217	**<0.01**	0.165	0.230	**<0.01**	**<0.01**	**<0.01**
Retracted foot	Mann-Whitney *U*	4306.5	5197.5	12622.5	107716.5	507.5	1232.5	10121.0	1487.5	1487.5	12215.0
	Wilcoxon *W*	4741.5	5827.5	56875.5	151969.5	1137.5	1667.5	10556.0	2117.5	2117.5	12845.0
	Z	0.000	0.000	-5.388	-1.234	0.000	-1.724	-1.287	-1.891	-1.891	-1.413
	P - value	1.000	1.000	**<0.01**	0.217	1.000	0.085	0.198	0.059	0.059	0.158
Extended foot	Mann-Whitney *U*	4306.5	5197.5	12622.5	106177.5	507.5	1232.5	10367.5	1487.5	1487.5	12512.5
	Wilcoxon *W*	4741.5	5827.5	56875.5	150430.5	1137.5	1667.5	10802.5	2117.5	2117.5	13142.5
	Z	0.000	0.000	-5.388	-3.075	0.000	-1.724	-0.965	-1.891	-1.891	-1.060
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.085	0.335	0.059	0.059	0.289

Table 5: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Hexaplex trunculus* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Vertical locomotion	Mann-Whitney *U*	150840.0	177624.0	502479.0	3923478.0	17838.0	47745.0	376950.0	56223.0	443946.0	1241886.0
	Wilcoxon *W*	1739493.0	1766277.0	2091132.0	5512131.0	33063.0	207075.0	10182756.0	215553.0	10249752.0	1401216.0
	Z	-5.335	-8.316	-0.045	-2.639	-1.153	-3.460	-3.116	-5.064	-6.670	-1.505
	P - value	**<0.01**	**<0.01**	0.964	**<0.01**	0.249	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.132
Extended foot	Mann-Whitney *U*	155034.0	187110.0	499851.0	3603204.0	18270.0	48807.0	351828.0	58905.0	424620.0	1147050.0
	Wilcoxon *W*	170259.0	209265.0	2088504.0	5191857.0	40425.0	64032.0	367053.0	81060.0	446775.0	1306380.0
	Z	0.000	0.000	-3.080	-12.833	0.000	-0.963	-4.057	-1.058	-4.455	-6.808
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.335	**<0.01**	0.290	**<0.01**	**<0.01**

Table 6: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Abra alba* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Minor locomotion	Mann-Whitney *U*	17226.0	20790.0	53757.0	421443.0	2030.0	5249.0	41151.0	6335.0	49665.0	138552.0
	Wilcoxon *W*	18937.0	23275.0	230472.0	598158.0	4515.0	6960.0	42862.0	8820.0	52150.0	156318.0
	Z	0.000	0.000	-4.721	-4.856	0.000	-1.488	-1.525	-1.634	-1.675	-0.093
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.137	0.127	0.102	0.094	0.926
Major locomotion	Mann-Whitney *U*	17226.0	20790.0	55242.0	434214.0	2030.0	5394.0	42398.0	6510.0	51170.0	138584.0
	Wilcoxon *W*	18937.0	23275.0	231957.0	610929.0	4515.0	7105.0	44109.0	8995.0	53655.0	1228610.0
	Z	0.000	0.000	-2.515	-2.381	0.000	-0.787	-0.745	-0.865	-0.818	-0.153
	P - value	1.000	1.000	0.012	0.017	1.000	0.431	0.456	0.387	0.413	0.879
Siphon visible	Mann-Whitney *U*	17226.0	20790.0	48707.0	409563.0	2030.0	4756.0	39991.0	5740.0	48265.0	130150.0
	Wilcoxon *W*	18937.0	23275.0	225423.0	586278.0	4515.0	6467.0	41702.0	8225.0	50750.0	1220176.0
	Z	0.000	0.000	-8.839	-6.398	0.000	-2.859	-2.017	-3.133	-2.215	-3.079
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	0.044	**<0.01**	0.027	**<0.01**

Table 7: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Chlamys varia* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Open gape	Mann-Whitney *U*	3944.0	4760.0	10679.0	47389.5	507.5	928.0	3726.5	1120.0	4497.5	23149.0
	Wilcoxon *W*	48197.0	49013.0	15144.0	320080.5	1137.5	5393.0	276417.5	5585.0	277188.5	295840.0
	Z	-1.623	-1.782	-5.704	-16.516	0.000	-3.484	-7.115	-3.800	-7.766	-6.235
	P - value	0.104	0.075	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Widely open gape	Mann-Whitney *U*	4306.5	5197.5	12771.0	41580.0	507.5	1247.0	4060.0	1505.0	4900.0	16112.0
	Wilcoxon *W*	4741.5	5827.5	57024.0	85833.0	1137.5	1682.0	4495.0	2135.0	5530.0	20577.0
	Z	0.000	0.000	-5.073	-18.174	0.000	-1.618	-6.679	-1.775	-7.296	-9.845
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.106	**<0.01**	0.076	**<0.01**	**<0.01**
Swollen mantle	Mann-Whitney *U*	203.0	245.0	12241.0	104427.0	507.5	232.0	0.0	280.0	0.0	28782.0
	Wilcoxon *W*	44456.0	44498.0	56494.0	377118.0	1137.5	4697.0	272691.0	4745.0	272691.0	301473.0
	Z	-14.452	-15.009	-3.902	-5.936	0.000	-8.077	-27.677	-8.538	-27.785	-11.310
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Retracted mantle	Mann-Whitney *U*	4306.5	5197.5	8910.0	33264.0	507.5	870.0	3248.0	1050.0	3920.0	23074.0
	Wilcoxon *W*	4741.5	5827.5	53163.0	77517.0	1137.5	1305.0	3683.0	1680.0	4550.0	27539.0
	Z	0.000	0.000	-10.833	-20.262	0.000	-3.792	-7.820	-4.130	-8.524	-6.443
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Table 8: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Serpula vermicularis* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Tentacle crown open	Mann-Whitney *U*	10962.0	15606.0	43254.0	334638.0	1798.0	4698.0	17110.0	6422.0	26554.0	74648.0
	Wilcoxon *W*	12673.0	18091.0	61020.0	511353.0	3509.0	6409.0	18821.0	8907.0	29039.0	92414.0
	Z	-5.612	-4.170	-5.821	-9.758	-2.649	-2.989	-9.076	-0.506	-8.042	-11.983
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.613	**<0.01**	**<0.01**
Tentacle crown closed	Mann-Whitney *U*	9802.0	15394.0	41276.0	355842.0	1682.0	4524.0	16298.0	6572.0	28526.0	76444.0
	Wilcoxon *W*	186517.0	192109.0	217991.0	1445868.0	4167.0	22290.0	1106324.0	9057.0	1118552.0	1166470.0
	Z	-6.410	-4.183	-6.456	-7.814	-3.299	-3.362	-9.424	-0.023	-7.467	-11.704
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.982	**<0.01**	**<0.01**

Table 9: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Schizaster canaliferus* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Sediment bulging	Mann-Whitney *U*	68904.0	67716.0	159192.0	1635876.0	6612.0	15544.0	159732.0	23648.0	182124.0	432792.0
	Wilcoxon *W*	75690.0	773982.0	865458.0	2342142.0	13398.0	22330.0	166518.0	33518.0	4540752.0	4791420.0
	Z	0.000	-14.995	-19.139	-9.147	-4.887	-6.527	-2.883	-2.336	-5.290	-13.913
	P - value	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.019	**<0.01**	**<0.01**
Horizontal locomotion	Mann-Whitney *U*	68904.0	83160.0	194832.0	1477872.0	8120.0	19024.0	144304.0	22960.0	174160.0	538592.0
	Wilcoxon *W*	75690.0	93030.0	901098.0	2184138.0	17990.0	25810.0	151090.0	32830.0	184030.0	609468.0
	Z	0.000	0.000	-12.504	-14.500	0.000	-4.047	-4.634	-4.435	-5.087	-1.494
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.135
Spine movement	Mann-Whitney *U*	68904.0	83160.0	149688.0	1354914.0	8120.0	14616.0	132298.0	17640.0	159670.0	498100.0
	Wilcoxon *W*	75690.0	93030.0	855954.0	2061180.0	17990.0	21402.0	139084.0	27510.0	169540.0	4856728.0
	Z	0.000	0.000	-20.622	-17.950	0.000	-7.144	-5.808	-7.788	-6.374	-4.389
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Table 10: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Ocnus planci* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Closed crown	Mann-Whitney *U*	3118.5	5197.5	8910.0	30591.0	367.5	1246.0	5939.0	1050.0	3605.0	22228.0
	Wilcoxon *W*	3553.5	5827.5	13375.0	303282.0	802.5	5711.0	278630.0	5515.0	276296.0	294919.0
	Z	-9.151	0.000	-10.833	-20.979	-3.296	-0.849	-5.146	-4.130	-8.990	-7.028
	P - value	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	0.396	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Open crown	Mann-Whitney *U*	4306.5	5197.5	8910.0	31630.5	507.5	870.0	3088.5	1050.0	3727.5	22557.0
	Wilcoxon *W*	4741.5	5827.5	53163.0	75883.5	1137.5	1305.0	3523.5	1680.0	4357.5	27022.0
	Z	0.000	0.000	-10.833	-20.697	0.000	-3.792	-8.081	-4.130	-8.804	-6.796
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Crown upwards	Mann-Whitney *U*	3861.0	297.0	1039.5	1633.5	81.5	242.5	1266.5	1616.5	12369.5	32620.0
	Wilcoxon *W*	48114.0	927.0	5504.5	274324.5	711.5	4707.5	273957.5	2246.5	285060.5	305311.0
	Z	-5.560	-17.607	-18.779	-31.340	-6.686	-8.699	-21.721	-0.342	-1.898	-3.736
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.732	0.058	**<0.01**
Crown to the side	Mann-Whitney *U*	3861.0	297.0	1485.0	4306.5	81.5	286.0	1527.5	1564.0	12684.5	32359.0
	Wilcoxon *W*	48114.0	44550.0	45738.0	48559.5	516.5	721.0	1962.5	6029.0	13314.5	36824.0
	Z	-5.560	-17.607	-18.362	-30.083	-6.686	-8.142	-17.540	-0.853	-0.525	-2.896
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.394	0.599	**<0.01**
Crown downwards	Mann-Whitney *U*	4306.5	5197.5	13513.5	106920.0	507.5	1319.5	10440.0	1592.5	12600.0	34425.0
	Wilcoxon *W*	4741.5	5827.5	57766.5	151173.0	1137.5	1754.5	10875.0	2222.5	13230.0	307116.0
	Z	0.000	0.000	-3.087	-2.714	0.000	-0.970	-0.851	-1.065	-0.934	-0.438
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.332	0.395	0.287	0.350	0.662
Minor extension	Mann-Whitney *U*	3861.0	0.0	5049.0	81526.5	52.5	634.0	9067.5	1050.0	3307.5	21429.0
	Wilcoxon *W*	48114.0	44253.0	49302.0	125779.5	487.5	1069.0	9502.5	5515.0	275998.5	294120.0
	Z	-5.560	-18.193	-14.945	-9.642	-7.213	-5.017	-1.860	-4.130	-9.473	-7.616
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.063	**<0.01**	**<0.01**	**<0.01**
Major extension	Mann-Whitney *U*	4306.5	5197.5	8910.0	28066.5	507.5	870.0	2740.5	1050.0	3307.5	21429.0
	Wilcoxon *W*	4741.5	5827.5	53163.0	72319.5	1137.5	1305.0	3175.5	1680.0	3937.5	25894.0
	Z	0.000	0.000	-10.833	-21.681	0.000	-3.792	-8.706	-4.130	-9.473	-7.616
	P - value	1.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
Anterior end upwards	Mann-Whitney *U*	4306.5	5197.5	7128.0	4306.5	507.5	696.0	420.5	840.0	507.5	18337.0
	Wilcoxon *W*	4741.5	5827.5	11593.0	276997.5	1137.5	5161.0	273111.5	5305.0	273198.5	291028.0
	Z	0.000	0.000	-12.818	-30.083	0.000	-4.742	-19.182	-5.139	-20.139	-14.843
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Posterior end upwards	Mann-Whitney *U*	4306.5	5197.5	12177.0	98158.5	507.5	1189.0	9584.5	1435.0	11567.5	33877.0
	Wilcoxon *W*	4741.5	5827.5	56430.0	142411.5	1137.5	1624.0	10019.5	2065.0	12197.5	306568.0
	Z	0.000	0.000	-6.246	-5.783	0.000	-2.017	-1.833	-2.211	-2.013	-0.689
	P - value	1.000	1.000	**<0.01**	**<0.01**	1.000	0.044	0.067	0.027	0.044	0.491

Table 11: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Ophiothrix quinquemaculata* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Horizontal locomotion	Mann-Whitney *U*	56926.0	81526.0	221060.0	1657296.0	6868.0	18240.0	132060.0	26072.0	191244.0	518856.0
	Wilcoxon *W*	763192.0	787792.0	927326.0	6015924.0	16738.0	89116.0	4490688.0	96948.0	4549872.0	4877484.0
	Z	-6.808	-0.918	-0.720	-11.457	-3.447	-4.964	-21.563	-0.369	-10.023	-11.186
	P - value	**<0.01**	0.358	0.472	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.712	**<0.01**	**<0.01**
No locomotion	Mann-Whitney *U*	53916.0	79962.0	214212.0	1566162.0	6666.0	17956.0	115682.0	26256.0	176618.0	472996.0
	Wilcoxon *W*	60702.0	89832.0	285088.0	2272428.0	13452.0	24742.0	122468.0	97132.0	186488.0	543872.0
	Z	-6.480	-1.311	-2.064	-15.409	-3.341	-4.144	-22.331	-0.067	-12.485	-16.197
	P - value	**<0.01**	0.190	0.039	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.946	**<0.01**	**<0.01**
Lateral arm-posture	Mann-Whitney *U*	54828.0	43866.0	58140.0	204390.0	5942.0	10132.0	54934.0	19288.0	121726.0	475196.0
	Wilcoxon *W*	61614.0	53736.0	129016.0	4563018.0	15812.0	81008.0	4413562.0	90164.0	4480354.0	4833824.0
	Z	-6.364	-14.572	-27.716	-58.324	-4.280	-11.210	-38.417	-6.438	-28.256	-16.270
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Clinging to clumps	Mann-Whitney *U*	58536.0	60426.0	130194.0	1024272.0	7122.0	15994.0	125776.0	22538.0	177196.0	554308.0
	Wilcoxon *W*	764802.0	766692.0	836460.0	1730538.0	13908.0	22780.0	132562.0	32408.0	187066.0	4912936.0
	Z	-5.059	-9.420	-17.919	-24.842	-2.115	-5.046	-5.607	-2.905	-3.294	-0.044
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.034	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.965
Arm-tipping	Mann-Whitney *U*	65790.0	66600.0	206532.0	1737018.0	6870.0	21152.0	161870.0	23060.0	163550.0	507988.0
	Wilcoxon *W*	772056.0	772866.0	912798.0	6095646.0	13656.0	27938.0	4520498.0	93936.0	4522178.0	4866616.0
	Z	-3.387	-11.935	-7.093	-2.985	-3.480	-1.024	-6.545	-3.792	-19.701	-12.585
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.306	**<0.01**	**<0.01**	**<0.01**	**<0.01**

Table 12: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Microcosmus sulcatus* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Closed crown	Mann-Whitney *U*	37833.0	44047.5	114697.5	622903.5	4410.0	10906.5	58522.5	12652.5	66622.5	224316.0
	Wilcoxon *W*	41661.0	49612.5	512083.5	1020289.5	9975.0	14734.5	62350.5	18217.5	72187.5	264219.0
	Z	-0.922	-2.541	-4.712	-19.889	-1.913	-2.787	-7.292	-4.127	-8.695	-9.103
	P - value	0.356	0.011	**<0.01**	**<0.01**	0.056	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Open crown	Mann-Whitney *U*	33244.5	39139.5	101413.5	498771.0	4471.5	11647.5	62403.0	14368.5	77757.0	218037.0
	Wilcoxon *W*	37072.5	44704.5	141316.5	2950776.0	10036.5	51550.5	2514408.0	54271.5	2529762.0	2670042.0
	Z	-3.213	-3.978	-6.744	-24.967	-0.307	-0.857	-6.801	-0.535	-6.990	-9.933
	P - value	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.759	0.392	**<0.01**	0.593	**<0.01**	**<0.01**
Body contraction	Mann-Whitney *U*	38586.0	46047.0	119710.5	894982.5	4516.5	11743.5	87817.5	14338.5	107284.5	297972.0
	Wilcoxon *W*	435972.0	443433.0	517096.5	1292368.5	8344.5	15571.5	91645.5	19903.5	112849.5	337875.0
	Z	-0.198	-0.744	-3.081	-7.534	-0.346	-1.291	-2.392	-1.014	-2.287	-2.146
	P - value	0.843	0.457	**<0.01**	**<0.01**	0.730	0.197	0.017	0.311	0.022	0.032

Table 13: Results of Mann Whitney *U* Test for differences in the number of observed behaviors in the five oxygen categories in *Phallusia mammilata* (results for not shown data are omitted). For description of table see table legend of Table 4.

	Comparison	**1 vs. 2**	**1 vs. 3**	**1 vs. 4**	**1 vs. 5**	**2 vs. 3**	**2 vs. 4**	**2 vs. 5**	**3 vs. 4**	**3 vs. 5**	**4 vs. 5**
Closed crown	Mann-Whitney *U*	4107.0	4777.5	13750.5	57415.5	490.0	1279.5	5110.5	1487.5	5722.5	18690.0
	Wilcoxon *W*	4542.0	5407.5	58003.5	101668.5	1120.0	1714.5	5545.5	2117.5	6352.5	23155.0
	Z	-0.893	-1.743	-0.453	-14.030	-1.099	-1.051	-5.531	-1.891	-6.447	-8.417
	P - value	0.372	0.081	0.650	**<0.01**	0.272	0.293	**<0.01**	0.059	**<0.01**	**<0.01**
Open crown	Mann-Whitney *U*	4023.0	4671.0	12082.5	75330.0	489.5	1269.5	6651.0	1590.5	7569.0	19179.0
	Wilcoxon *W*	48276.0	48924.0	56335.5	348021.0	924.5	1704.5	279342.0	2220.5	280260.0	291870.0
	Z	-0.764	-1.287	-2.655	-9.095	-0.359	-0.890	-4.037	-0.470	-4.826	-8.180
	P - value	0.445	0.198	**<0.01**	**<0.01**	0.720	0.373	**<0.01**	0.638	**<0.01**	**<0.01**
Body contraction	Mann-Whitney *U*	4187.5	5059.0	13423.0	57150.0	507.0	1273.0	5876.0	1538.0	7079.0	16756.0
	Wilcoxon *W*	48440.5	49312.0	17888.0	101403.0	1137.0	5738.0	6311.0	6003.0	7709.0	21221.0
	Z	-0.400	-0.420	-0.947	-13.926	-0.010	-0.946	-4.872	-0.993	-5.335	-9.515
	P - value	0.689	0.675	0.344	**<0.01**	0.992	0.344	**<0.01**	0.321	**<0.01**	**<0.01**