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# DIPLOMARBEIT

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Reactions of a macrobenthic community to anoxia:  
behaviour and mortality sequences in a time-lapse camera experiment  
in the Gulf of Trieste

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Ich widme diese Arbeit meiner Mutter,  
die mich gelehrt hat,  
dass man nie die Hoffnung aufgeben darf,  
was auch geschieht.



„Der höchste Lohn für unsere Bemühungen  
ist nicht das, was wir dafür bekommen,  
sondern das, was wir dadurch werden.“

*John Ruskin (1819-1900)*



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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



## Table of contents

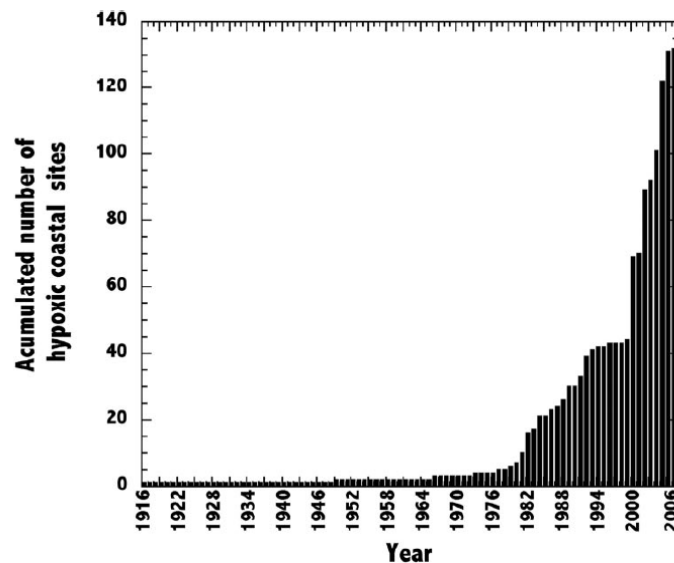
<b>1. Introduction</b> .....	1
<b>2. Material and Methods</b> .....	6
<b>2.1 Study site and deployment</b> .....	6
<b>2.2 Experimental design and sampling</b> .....	7
<b>2.3 Data analyses</b> .....	10
2.3.1 Investigated behaviours and reactions.....	10
2.3.2 Statistical analyses.....	11
<b>3. Results</b> .....	12
<b>3.1 Sensor data - Physical parameters</b> .....	12
<b>3.2 Photograph analyses and investigated taxa</b> .....	13
<b>3.3 Behavioural analyses</b> .....	16
3.3.1 Porifera.....	17
3.3.2 Bivalvia.....	18
3.3.2 (i) <i>Chlamys varia</i> .....	18
3.3.2 (ii) <i>Corbula gibba</i> .....	19
3.3.3 Polychaeta.....	21
3.3.3 (i) Infaunal polychaetes.....	21
3.3.3 (ii) <i>Serpula vermicularis</i> .....	22
3.3.4 Crustacea - <i>Paguristes eremita</i> .....	23
3.3.5 Echinoidea.....	25
3.3.5 (i) <i>Psammechinus microtuberculatus</i> .....	25
3.3.5 (ii) <i>Schizaster canaliferus</i> .....	27
3.3.6 Ophiuroidea.....	29
3.3.6 (i) <i>Ophiothrix quinque maculata</i> .....	29
3.3.6 (ii) <i>Ophiura</i> spp.....	32
3.3.7 Ascidiacea.....	33
3.3.7 (i) <i>Microcosmus sulcatus</i> .....	33
3.3.7 (ii) <i>Phallusia mammilata</i> .....	34
<b>3.4 Mortality</b> .....	35

<b>4. Discussion</b> .....	38
<b>4.1 Critical oxygen levels and behavioural reactions</b> .....	38
4.1.1 Beginning hypoxia ( $\leq 2 \text{ ml l}^{-1} \text{ DO}$ ).....	39
4.1.2 Moderate hypoxia ( $\leq 1 \text{ ml l}^{-1} \text{ DO}$ ).....	40
4.1.3 Severe hypoxia ( $\leq 0.5 \text{ ml l}^{-1} \text{ DO}$ ) and anoxia.....	41
<b>4.2 Mortality</b> .....	43
<b>5. References</b> .....	46
<b>6. Abstract</b> .....	51
<b>7. Zusammenfassung</b> .....	52
<b>Danksagung</b> .....	55
<b>Curriculum Vitae</b> .....	57



# 1. Introduction

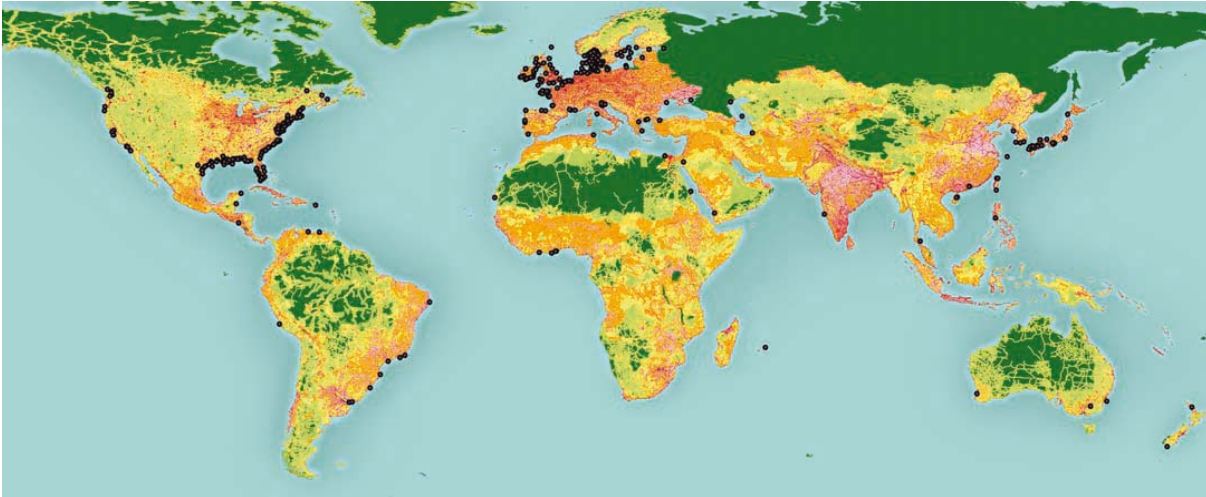
Dissolved oxygen (DO) is a key environmental variable in estuarine and coastal marine ecosystems around the world, and its concentration has changed drastically in the past decades (Diaz 2001; Diaz and Rosenberg 2008). Hypoxia around the world is defined as dissolved oxygen values below  $2 \text{ ml l}^{-1}$  DO (equivalent to  $2.8 \text{ mg O}_2 \text{ l}^{-1}$  or  $91.4 \text{ mM}$ ; Diaz and Rosenberg 1995; Wu 2002) and is often associated with semi-enclosed water bodies that, combined with water-column stratification, hinder full water exchange. At that value, benthic fauna start to show aberrant behaviour, culminating in mass mortality when DO declines below  $0.5 \text{ ml l}^{-1}$  (Diaz and Rosenberg 1995). If anoxia, defined as  $0 \text{ ml l}^{-1}$  DO, is established on the sea floor, microbially generated  $\text{H}_2\text{S}$  often occurs. Due to its toxic effects, sulphide shortens the survival times of organisms (Hagerman 1998).



**Fig. 1:** Accumulated number of coastal sites where hypoxia has been reported (Vaquer-Sunyer and Duarte 2008).

The number of hypoxic and anoxic environments in shallow coastal areas is increasing due to anthropogenic eutrophication (Diaz 2001), with an exponential growth rate of  $5.54\% (\pm 0.23\%)$  per year (Fig. 1; Vaquer-Sunyer and Duarte 2008). More than 400 so-called dead zones have now been reported from all over the world (Fig. 2). When these areas were first studied they were not hypoxic (Diaz and

Rosenberg 2008), but, after 1955, eutrophication increased due to the use of phosphorus compounds by the detergent industry and of nitrogen and phosphorus by an expanding intensive agriculture. As a consequence, primary production has increased from that time on in the Adriatic (Justic et al. 1987).



**Fig. 2:** Global distribution of more than 400 systems that have scientific reports of being eutrophication-associated dead zones (Diaz and Rosenberg 2008).

Oxygen from the atmosphere or produced by phytoplankton is dissolved into the water column and mixed down into bottom waters, where it sustains the life of benthic fishes and invertebrates. This process can be disturbed, for example through water column stratification or decomposition of organic matter in the bottom water. In such cases, the oxygen can be depleted entirely. Values below  $2 \text{ ml l}^{-1}$  DO (about 18% of air saturation; Diaz 2001) are designated as being hypoxic. Physical and hydrodynamic processes control stratification and the renewal of oxygen in bottom waters. If there is water exchange and/or no stratification, hypoxia is less likely to occur (Conley et al. 2009a,b).

One signal that a system has reached a critical point of eutrophication is episodic oxygen depletion. The next potential step is the onset of annual hypoxia. Diaz and Rosenberg (2008) reported that about 50% of the 400 so-called dead zones (Fig. 2) occur once a year in the summer after spring bloom, when the water reaches higher temperatures and stratification is strongest. This leads to mortality of benthic organisms, followed by some level of recolonization after the return of normal oxygen

conditions. Approximately 17 % of the dead zones are characterized by less than one such event per year, sometimes with years elapsing between events, and another 8% exhibit persistent hypoxic conditions (e.g. deeper parts of the Baltic Sea) due to extremely limited water exchange and excessive anthropogenic inputs of nutrients.

The Adriatic Sea – an elongated basin in the Central Mediterranean – stretches NW to SE from the Gulf of Venice to the Strait of Otranto (800 km) and has an extremely long, geometrically complex coastline which creates a high diversity of hydrodynamic and sedimentary environments (Giordani et al. 2002). Those authors and Zavatarelli et al. (1988) subdivided the Adriatic into three parts: (1) the shallow northern part with an average depth of 35 m, which reaches from the Gulf of Venice to the Ancona-Zadar transect, (2) the central Adriatic with an average depth of 140 m and (3) the southern part (max. depth 1200 m), extending down to the Otranto Strait.

Especially the northern Adriatic Sea has been described several times as a sensitive ecosystem (Kollmann and Stachowitsch 2001; Schinner et al. 1997; Stachowitsch 1986). Hypoxic events have been noted here periodically for centuries (Crema et al. 1991), but the frequency and extent have increased during recent decades. Stachowitsch (1984, 1986), was among the first to describe the course of such oxygen crises in detail. The position at fairly high latitude makes the northern Adriatic subject to considerable seasonal fluctuations in temperature and radiation (Ott and Fedra 1977). A semi-enclosed, shallow water body (< 35 m), soft substrate, a high riverine input (e.g. from the Po River), high productivity and stratification favour the development of seasonal low DO events (Stachowitsch and Avcin 1987; Ott 1992). Thus, the shallowness and the nutrient-rich freshwater inflow make it one of the most productive areas in the Mediterranean (Stirn et al. 1974), while at the same time exhibiting the greatest amplitude of climatic factors during the year (Ott and Fedra 1977).

Degobbis et al. (2000) described the Po waters as a significant influence on the northern Adriatic Sea, because the freshwater inflow leads to higher water column stratification and changes both the general circulation and water exchange rate between the northern and the central Adriatic. The physical and chemical influences of this river, combined with meteorological conditions, such as calm weather along with an increased nutrient input in highly stratified water column, have a significant

biological effect on marine ecosystems. This promotes phytoplankton blooms followed by organic matter sedimentation, microbial decomposition and decreasing oxygen concentration.

The soft-bottom of the northern Adriatic consists of muddy sand and is inhabited by well-known macrobenthic communities. Infaunal species are very common, for example the infaunal sea urchin *Schizaster canaliferus* and the brittle star *Amphiura chiajei*, which led to the description of the *Schizaster chiajei* zoocenosis in a large part of the northern Adriatic Sea, including the Gulf of Trieste, or the *Schizaster-Turritella* community in the Limski Canal (Vatova 1931, 1949). The macroepibenthos suspension-feeders in those parts of the Gulf of Trieste and further south along the Istrian coast are formed largely by the sponge *Reniera* spp., the ascidian *Microcosmus sulcatus* and the brittle star *Ophiothrix quinquemaculata*, an assemblage termed the *Ophiothrix-Reniera-Microcosmus* community (ORM-community) by Fedra et al. (1976). The epifauna is aggregated into so-called multi-species clumps or bioherms (Zuschin and Pervesler, 1996), with an average biomass of  $370 \pm 73 \text{ g m}^{-2}$  (wet weight). They are capable of removing approximately 5% of the pelagic biomass from the water column each day (Ott and Fedra 1977). Thus, the benthos can be described as a buffer that plays an important role as a natural eutrophication control (Officer et al. 1982).

As reviewed by Ott (1992) and Ott and Stachowitsch (1992), the large amount of organic matter (high primary production) can be removed by the suspension feeders or utilized by deposit feeders. As a result, many species grow rapidly and the biomass of the benthos increases. After spring, water column stratification isolates the surface layer from the sub-pycnocline layer, ultimately leading to decreased oxygen concentrations in the bottom layer. This prolonged stratification can promote hypoxic conditions during the summer and autumn months. Afterwards, in winter, the water column becomes homogeneous by vertical mixing, and macrobenthos recover again. Eutrophication compounds the effects of stratification. It leads to higher nutrient levels in the sea, therefore increasing the production of particulate organic matter (POM) and dissolved organic matter (DOM; Gray et al. 2002). This is often associated with a large amount of marine snow (stringers, macroflocs clouds; specified by Stachowitsch et al. 1990) in the upper layer. This sinking material can be temporarily retained at the pycnocline to form the so-called "false benthos". After stratification break-up due to

storms, these aggregates settle down and cover the sea floor, promoting widespread anoxia and thereby mass mortalities. As a consequence, the reduced macrobenthos is incapable of exerting its normal control over pelagic production (Ott 1992 and Ott and Stachowitsch 1992).

The behaviour and physiology of benthic organisms are influenced by both hypoxia and hydrogen sulphide (Mangum 1973; Vismann 1991). "Natural" oxygen crises are unpredictable and full documentation from the onset nearly impossible. To learn more about responses under decreasing oxygen conditions, a new underwater device, the Experimental Anoxia Generating Unit (EAGU), was built and deployed (Stachowitsch et al. 2007). It is equipped with a time-lapse camera and sensor equipment and successfully induced anoxia in a sublittoral macrobenthic community *in situ*. The "open" configuration (aluminum frame open on all sides) makes it possible to observe behaviours under normoxia. The "closed" configuration (plexiglass walls on all four sides) reveals the behavioural succession from the onset of hypoxia and anoxia. The novel feature is the combination of both time-lapse documentation and physico-chemical data (dissolved oxygen, hydrogen sulphide, temperature and pH). Such parallel recording makes it possible to correlate atypical behaviours of various species to specific oxygen concentrations.

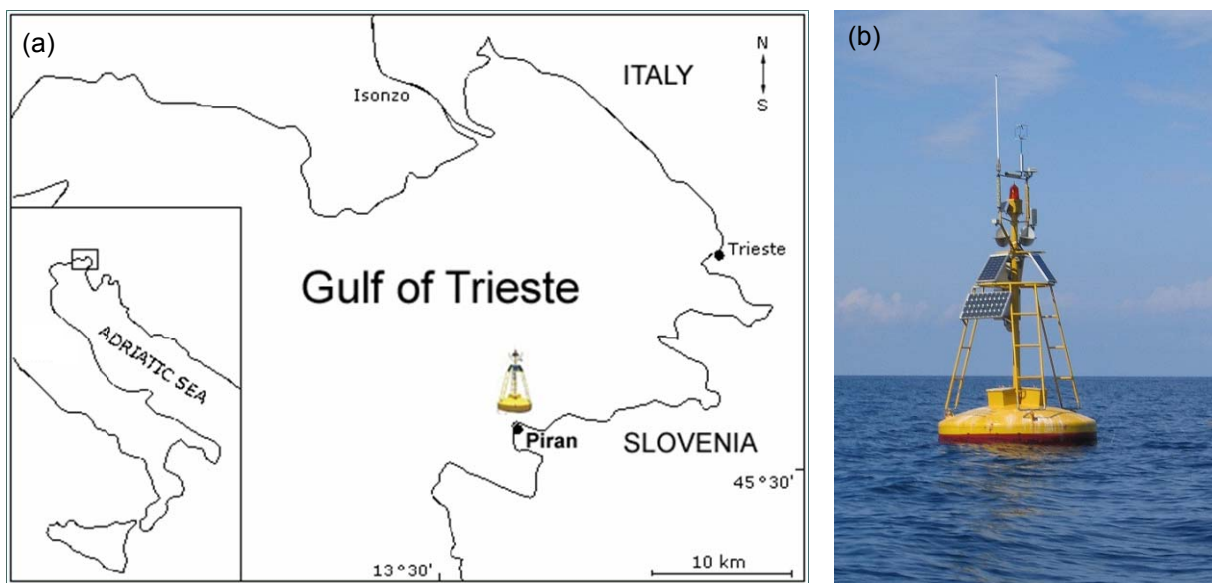
The present study focuses on a range of organisms making up and surrounding the multi-species clumps. It is based on one of 13 experiments and was conducted from 10.10.2006 - 14.10.2006. We used the oxygen thresholds 2, 1, and 0.5 ml l<sup>-1</sup> DO proposed by Diaz and Rosenberg (1995). These oxygen values were successively associated with escape patterns in the epifauna, emerging infauna and initial mortalities. Whereas some behaviours are reversible, mortalities can lead to long-term ecosystem shifts (Gray et al. 2002, Wu 2002).

This work is a step forward in compiling a list of sensitive and tolerant species and a generally valid catalogue of behaviours to determine *in situ* ecosystem status and stability here and elsewhere.

## 2. Material and Methods

### 2.1 Study site and deployment

The study site is located in the Gulf of Trieste, northern Adriatic Sea ( $45^{\circ} 32' 55.68''$  N,  $13^{\circ} 33' 1.89''$  E) off Cape Madona in Piran (Slovenia; Fig. 3a). The diving site is about 2 km off shore at a depth of 24 m and positioned under the oceanographic buoy of the Marine Biology Station (Fig. 3b) to minimize potential damage by commercial fisheries. The salinity was 38‰ and the bottom water temperature at the time in which the evaluated photo series averaged  $21.5^{\circ}\text{C}$ .



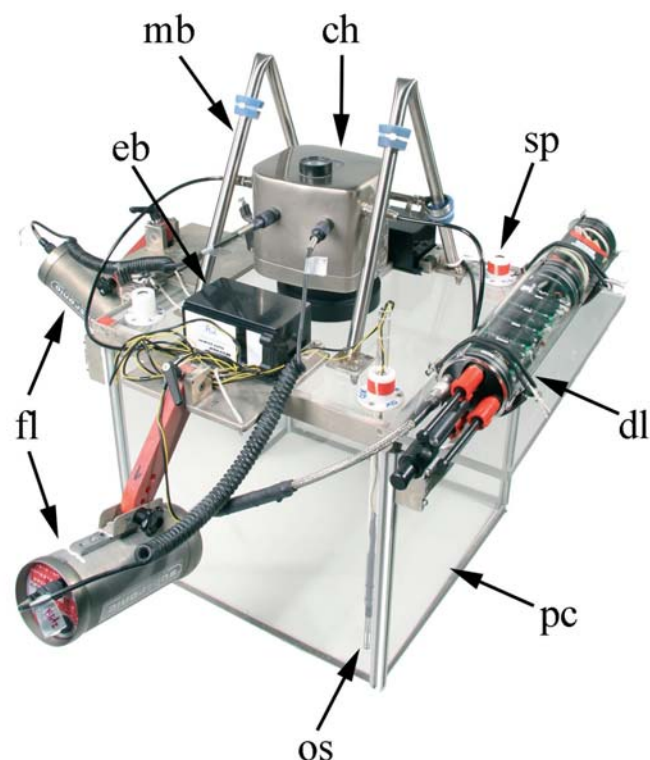
**Fig. 3:** Study site in the Gulf of Trieste (a) and the oceanographic buoy (b).

The diving site is composed of a soft bottom (poorly sorted silty sand) and is dominated by stable, high-biomass macroepibenthic communities (Stachowitsch et al. 2007). They consist largely of sponges, ascidians and brittle stars, and one of the most widespread is known as the *Ophiothrix-Reniera-Microcosmus* community (ORM-community; Fedra et al. 1976). In most cases the organisms are aggregated in so-called multi-species clumps or bioherms that consist of a shelly base overgrown by sessile species (Zuschin et al. 1999).

## 2.2 Experimental design and sampling

The Experimental Anoxia Generating Unit, in short EAGU (Fig. 4), is a new underwater device that induces anoxia *in situ* in marine benthic communities (Stachowitsch et al. 2007). In the initial deployments, i.e. also in the presently evaluated experiment, the emphasis was on including a wide range of representative organisms within the 50 x 50 cm view of the camera.

The unit consists of two interchangeable bases and a separate lid that houses a time-lapse camera, two flashes and a datalogger with a sensor array (Stachowitsch et al. 2007). The frame consists of a 2 cm thick aluminum frame (L x W x H = 50 x 50 x 50 cm) that was used in the first step, the "open" configuration (see detail of deployment below). For the second step, the "closed" configuration, the aluminum frame was switched with the plexiglass chamber and pushed approximately 2 cm into the sediment to hinder water exchange through the sediment. This plexiglass chamber consists of an aluminum frame and 6-mm-thick plexiglass walls on four sides, so it is open at the top and at the bottom.



**Fig. 4:** The Experimental Anoxia Generating Unit (EAGU) with the instrumental lid positioned on top of the plexiglass chamber and one sensor connected to the datalogger and inserted through a sensor port. ch: camera housing, dl: datalogger, eb: external battery, fl: flashes, mb: metal brackets, os: oxygen sensor, pc: plexiglass chamber, sp: sensor port (Stachowitsch et al. 2007).

In both configurations a lid is placed on the top of the aluminum frame. This 12 cm thick plexiglass lid measures 51 x 70 cm and contains the whole instrumentation for the documentation: a digital camera (Canon EOS 30D, 8.2 MP) with a zoom lens (Canon EFS 10-22 mm, f/3.5-4.5 USM), a Canon Timer Remote Controller (TC-80N3) and a 1 GB flashcard (Figure 5b). Two underwater flashes ("midi analog" series 11897; Subtronic, Germany) are attached to the lid by PVC-swivel arms on two adjoining sides. Moreover, four sensors, two battery packs (9Ah Panasonic. Werner light power, Unterwassertechnik, Germany) and the datalogger unit (PA3000UD, Unisense, Denmark) are placed on the lid. The camera was timed to take one image every 6 minutes and thus allowed us to document the full anoxia event over 3-5 days. All four sensors – two oxygen Clark-type microsensors (sensor type: OX-100, outside tip diameter 90-110  $\mu\text{m}$ ), one hydrogen sulphide Clark-type microsensor (sensor type: H2S-50, outside tip diameter 40-60  $\mu\text{m}$ ) and one temperature microsensor (sensor type: TP-200, outside tip diameter 180-220  $\mu\text{m}$ ; consists of a thermocouple within a tapered glass capillary) (Unisense) – were placed in plexiglass tubes (15 mm diameter; 40 cm length) and were pushed through O-ring-equipped sensor ports in the four corners of the lid. To calibrate the sensors at the beginning and end of the experiment the oxygen microelectrodes were inserted into fully oxygenated seawater from the surface and then in seawater that had been deoxygenated with sodium hydroxide. The microelectrode for hydrogen sulphide was calibrated in seawater and in a standard pH buffer reduced with a stock solution of total sulphide (for more information see Unisense instruction manual).

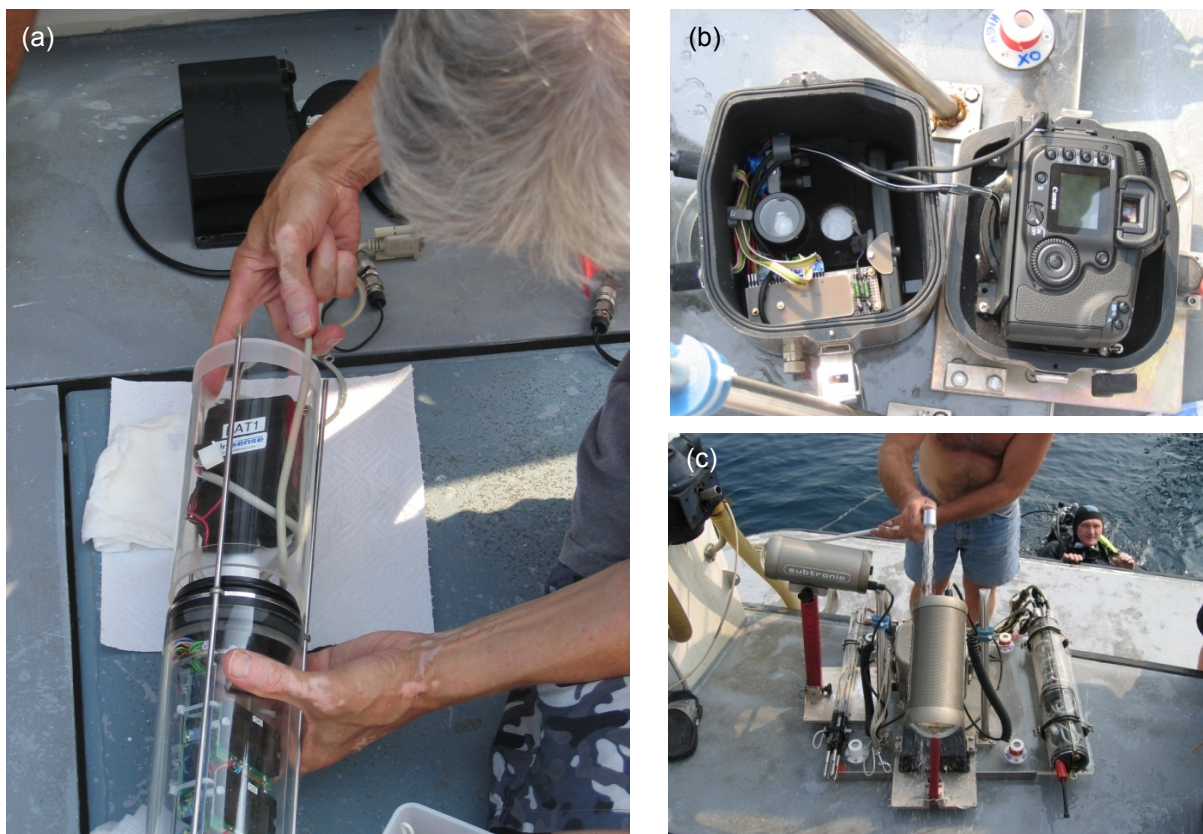
The tips of the two oxygen sensors were positioned 2 cm (Ox1) and 20 cm (Ox2) above the sediment to detect potential oxygen stratification in the water. The temperature sensor was placed 20 cm above the sediment and the hydrogen sulphide sensor at 2 cm height.

The two-channel datalogger unit (PA3000UD, Unisense, Germany) consists of one compartment containing four amplifier circuits with displays and datalogger, and one compartment containing the battery and communication cable (Fig. 5a). The datalogger has a memory capacity of 4000 samples per channel and allowed us to log sensor data every minute.

The pH-value was measured separately with a pH-sensor (TA 197-pH, WTW, Germany) once a day during daily control dives. The sensor was inserted into the



chamber through a 2 cm diameter opening, which was closed with a rubber stopper during regular operation. The sensor is connected to a datalogger (Multi 197i, WTW) on the dive boat by a 60 m cable.



**Fig. 5:** The datalogger and battery packs (a), the time-lapse camera in its housing (b) and the instrument-equipped lid (c) of the EAGU-system on board the research vessel *Sagitta* of the Marine Biology Station, Piran.

Every experiment was subdivided into two configurations. The first is the "open" configuration, designed to observe the activities of the benthic fauna under normoxia for about 24 h. The second is the "closed" configuration; this involved exchanging the aluminum frame against the plexiglass chamber. Over a period of approximately 72 h we observed the onset of hypoxia and anoxia along with the onset of unusual behaviours, the emergence of infaunal organisms, colour changes of organisms and the sediment and the sequence of mortality.

After approximately 72 h the plexiglass chamber and the lid (Figure 5c) were removed and transported to the boat and serviced. The photos and datalogger values were downloaded on a computer, the batteries exchanged and the sensors calibrated again (for more information see Unisense instruction manual).

As many organisms as possible were collected by hand from the 50 x 50 cm surface after the experiment with a wide-mouthed syringe or with small hand sieves. They were transported to the laboratory and placed in plastic trays or small aquaria with seawater to separate living from dead organisms. Afterwards, they were all preserved in 4% formaldehyde:seawater solution (for further information see Stachowitsch et al. 2007).

## **2.3 Data analyses**

### **2.3.1 Investigated behaviours and reactions**

The present thesis evaluates one of 13 experiments, specifically experiment number 12. The fieldwork was done by SCUBA diving and included deployment on 10.10.2006 at 13:37 and termination on 14.10.2006 at 19:37. The experiment yielded a total of 1010 images and had an overall documentation time of 100.6 h. These images were also combined into a time-lapse movie using the Adobe Premier 6.5 program. The whole movie is available at <http://www.marine-hypoxia.com>.

The evaluated organisms were analysed image per image, and behaviours were registered in excel-sheets. If there were less than 5 individuals per species, all of them were evaluated; otherwise, up to 9 individuals were selected. To obtain an overview of different behaviours, sponges, bivalves, polychaetes, serpulids, crustaceans, echinoids, ophiuroids, ascidians were evaluated (Table 1). Bioherm-associated crustaceans have been evaluated by Haselmair (2008). General behavioural categories included visibility (i.e. visible on the surface versus hiding under/in a bioherm or in sediment), locomotion, body movements, interactions and mortality. Some behaviours were further subdivided into different stages: horizontal and vertical locomotion or minor and major moves. Species-specific categories included visibility of the tentacle crown (serpulids), arm-tipping (brittle stars) or presence of camouflage (regular echinoids). As long as living organisms were visible, behaviours were recorded, i.e. until decomposition created poor visibility or until mortality or predation. Table 2 (see results) shows all individuals from the different class and genus, the number of observed organisms and the species-specific behaviours which were evaluated. The last observed locomotion or body movement (in some species plus two hours to account for a potential moribund phase; Table 3)

were equated with mortality. All behaviours were evaluated with respect to the values of the lower oxygen sensor.

**Table 1:** List of evaluated behaviours associated with the different taxa.

Taxonomic group	N	Species-specific behaviours evaluated
Porifera	5	colour change <sup>1</sup>
Bivalvia		
<i>Chlamys varia</i>	1	visibility, valve gape <sup>2</sup> , mantle shape <sup>3</sup>
<i>Corbula gibba</i>	7	sediment movement, visibility, locomotion <sup>4</sup>
Polychaeta		
Infaunal polychaetes	9	visibility, locomotion <sup>4</sup> , squirms in place
<i>Serpula vermicularis</i>	2	tentacle crown <sup>5</sup>
Crustacea		
<i>Paguristes eremita</i>	5	visibility, locomotion <sup>4</sup> , extension/shell <sup>6</sup> , body movement <sup>7</sup>
Echinoidea		
<i>Psammechinus microtuberculatus</i>	1	visibility, locomotion <sup>4</sup> , camouflage
<i>Schizaster canaliferus</i>	3	sediment movement, visibility, locomotion <sup>4</sup> , spine movement
Ophiuroidea		
<i>Ophiothrix quinquemaculata</i>	4	visibility, locomotion <sup>4</sup> , arm position <sup>8</sup>
<i>Ophiura</i> spp.	2	visibility, locomotion <sup>4</sup>
Ascidiacea		
<i>Microcosmus sulcatus</i>	1	visibility, siphon habitus <sup>9</sup> , body contraction
<i>Phallusia mammilata</i>	2	visibility, siphon habitus <sup>9</sup> , body contraction

Behaviour subdivisions: <sup>1</sup> original, transforming, final colour; <sup>2</sup> closed, half, open, normal, widely gaping valves; <sup>3</sup> normal, swollen, retracted mantle tissue; <sup>4</sup> horizontal, vertical locomotion; <sup>5</sup> visible, retracted tentacle crown; <sup>6</sup> normal, extended, out from shell; <sup>7</sup> while the crabs themselves were immobile; <sup>8</sup> upward arm-posture, arm-tipping, cling to clumps, on sediment; <sup>9</sup> open, half open, closed tentacle crown.

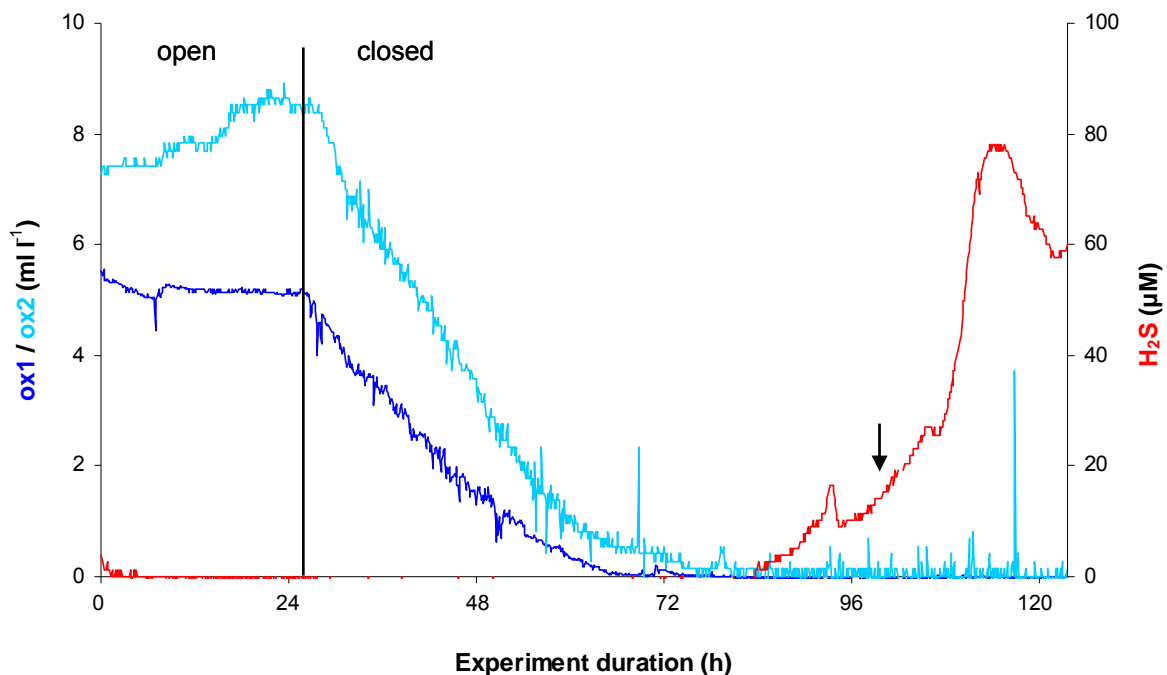
### 2.3.2 Statistical analyses

The observed behaviours were associated to five DO categories: normoxia (> 2.0 ml l<sup>-1</sup> DO), beginning hypoxia (2.0- 1.01 ml l<sup>-1</sup> DO), moderate (1.0-0.51 ml l<sup>-1</sup> DO), severe hypoxia (0.5- 0.01 ml l<sup>-1</sup>) DO and anoxia (no oxygen). Behaviours were recorded on Microsoft Excel sheets and further processed using an SPSS software package (version 11.5). The non-parametric Kruskal-Wallis test was used to determine if oxygen concentration significantly changes behaviour. To determine significant differences in behavioural reactions between oxygen categories, the Mann-Whitney *U* test was chosen.

### 3. Results

#### 3.1 Sensor data - Chemophysical parameters

Dissolved oxygen and hydrogen sulphide values for experiment 12 are shown in Figure 6. During the open configuration (h0 - h25), the DO values of the lower sensor varied from 4.5-5.5 ml l<sup>-1</sup> (Ox1) and those of the higher sensor from 7.3-8.8 ml l<sup>-1</sup> (20 cm above the sediment; Ox2). Stormy weather probably explains the difference between the higher and lower sensors. After switching to the closed configuration, oxygen values of both sensors decreased continuously, reaching hypoxia after ~ 18 h and anoxia after ~ 48 h. Hydrogen sulphide value then increased rapidly, peaking at 79.4 µM. The arrow in Fig. 6 marks the time of the final photographic image (both oxygen values: 0 ml l<sup>-1</sup> DO; H<sub>2</sub>S: 17.9 µM).

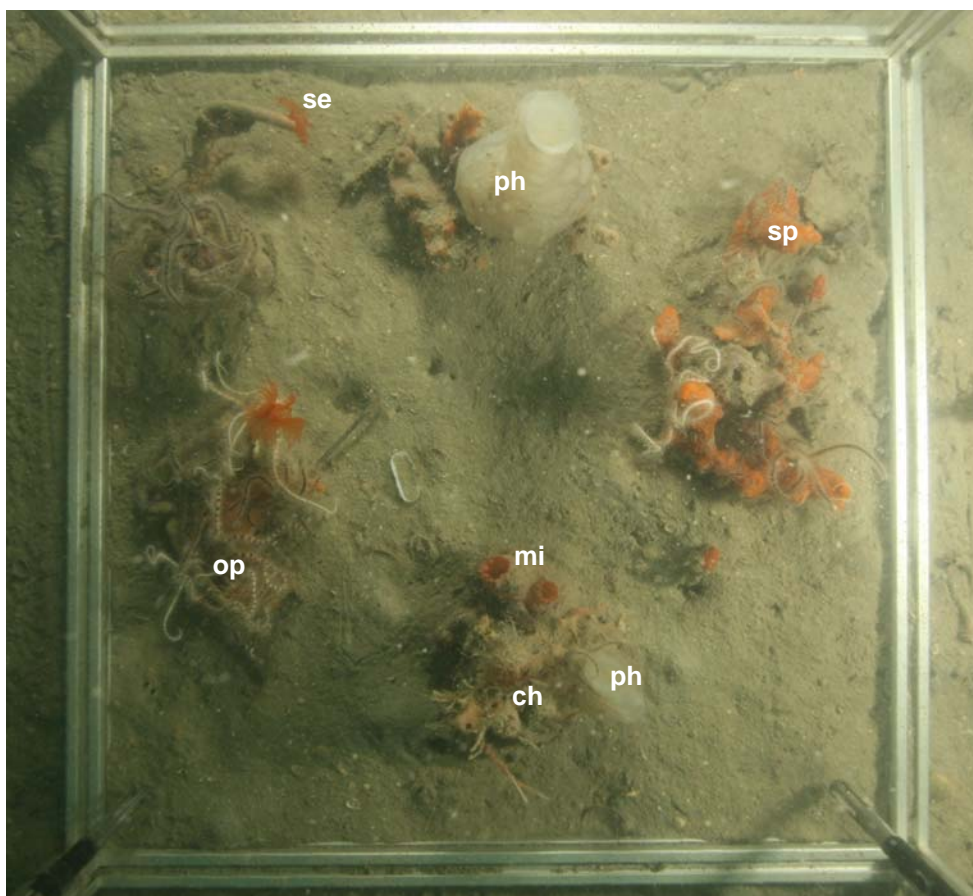


**Fig. 6:** Sensor data from experiment 12 conducted from 10-14 Oct 2006. Corresponding to the image intervals, every sixth value was entered. The vertical line marks the time when the frame was switched to the chamber. The arrow marks the final photographic image. Ox2 (light blue, 20 cm above the sediment) is consistently higher than Ox1 (dark blue line, 2 cm above the sediment). H<sub>2</sub>S begins to increase half a day after attainment of anoxia.

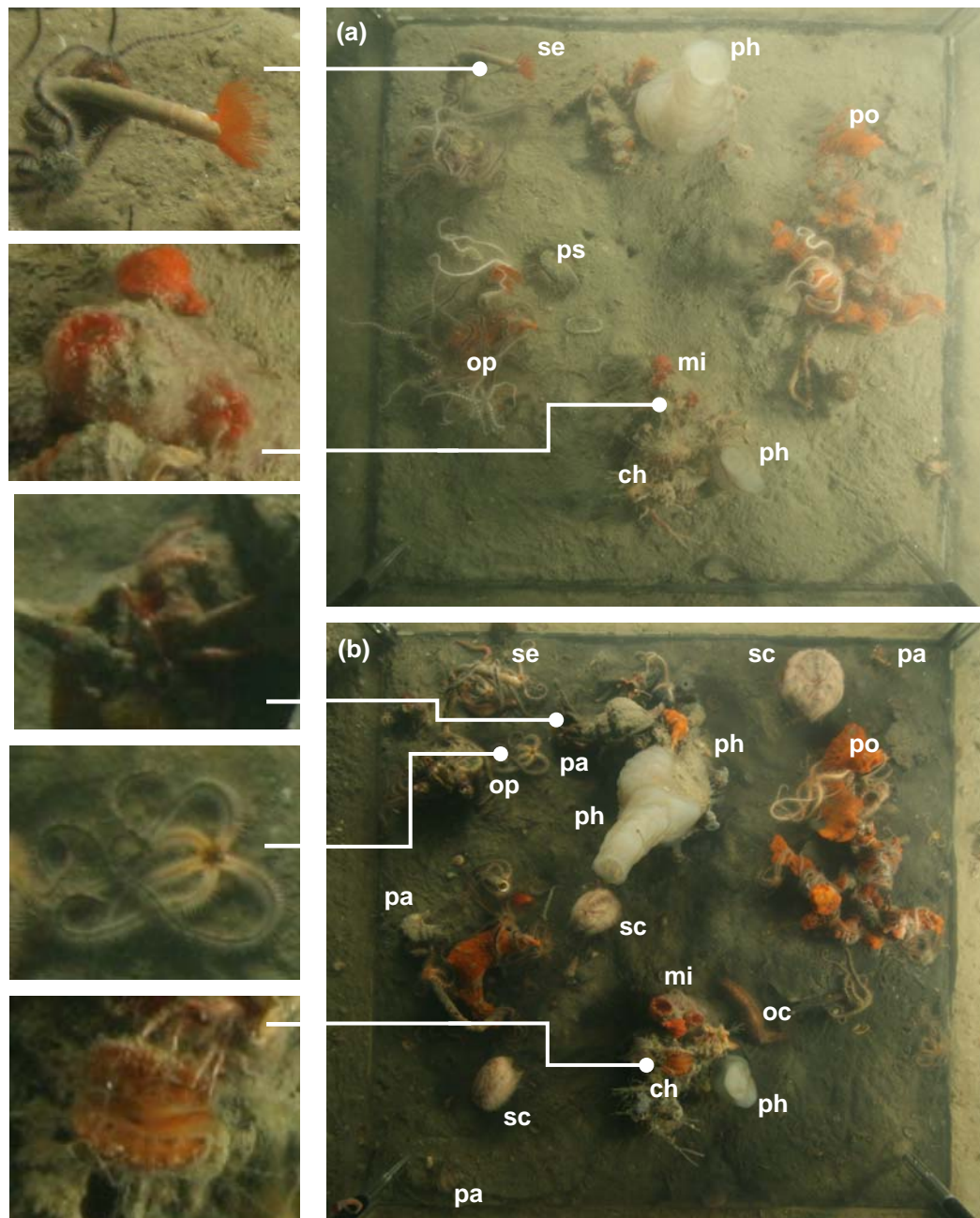
The whole experiment lasted over 5 days, but the photographic documentation stopped at h101 (4.2 days). The pH value was 8.26 at the beginning of the experiment and decreased to 7.68 on the final day. The temperature averaged 21.5°C.

### 3.2 Photograph analyses and investigated taxa

The macrobenthic assemblage during the initial "open" configuration is shown in Figure 7. The aluminum frame was open-sided and contained no plexiglass walls. The organisms showed their normal behaviour (e.g. brittle stars with their arms in upward suspension-feeding position, the tubeworms extended in filter-feeding position). Infaunal and cryptic species were not visible. The sediment had the same colour both in- and outside the frame. In the lower left- and right-hand corner the lower oxygen- and the H<sub>2</sub>S sensors are visible.



**Fig. 7:** Initial, open configuration of experiment 12 showing the ascidians *Phallusia mammilata* (ph) and *Microcosmus sulcatus* (mi), brittle stars *Ophiothrix quinquemaculata* (op), *Serpula vermicularis* (se), sponges (sp) and the bivalve *Chlamys varia* (ch).



**Fig. 8:** First (a) and final (b) image after switch to closed configuration (chamber) in experiment 12. The frame contains the ascidians *Phallusia mammilata* (ph) and *Microcosmus* sp. (mi), the brittle star *Ophiothrix quinquemaculata* (op), the tubeworm *Serpula vermicularis* (se), sponges (sp), the bivalve *Chlamys varia* (ch), emerged infaunal sea urchins *Schizaster canaliferus* (sc). Note broadly gaping *C. varia*, retracted tube worms, and the sea cucumber *Ocnus planci* (oc) exposed on sediment surface. Some hermit crabs (*Paguristes eremita*: pa), have left their hiding places and extended from their shells (upper right-hand corner). Lower oxygen- and H<sub>2</sub>S sensor are visible in the lower left- and right-hand corner. Note dark colour of the inside versus outside sediment due to H<sub>2</sub>S.

At the beginning of the "closed" configuration (Figure 8 a) the behaviour was still normal. With decreasing oxygen, organisms showed numerous atypical behaviours. Figure 8 b shows the changes recorded in the behaviour and appearance of every

organism/species. Also, the sediment colour had changed at the end of the experiment. The hermit crabs emerged from their initial hiding places and the cryptic crabs aggregated on the highest point (e.g. on the sea squirt). The tentacle crowns of the tubeworms were retracted and the infaunal worms came out of the sediment. After bulging of the sediment, the infaunal sea urchin *Schizaster canaliferus* were visible. At the end, most organisms were lying motionless on the sediment, which was clearly darker than outside.

The experiment included a wide range of epi- or infaunal organisms. Those species collected at the end of the experiment (Nx) and evaluated (N), the life habit (epi = epifaunal, in = infaunal) and the mobility are shown in Table 2.

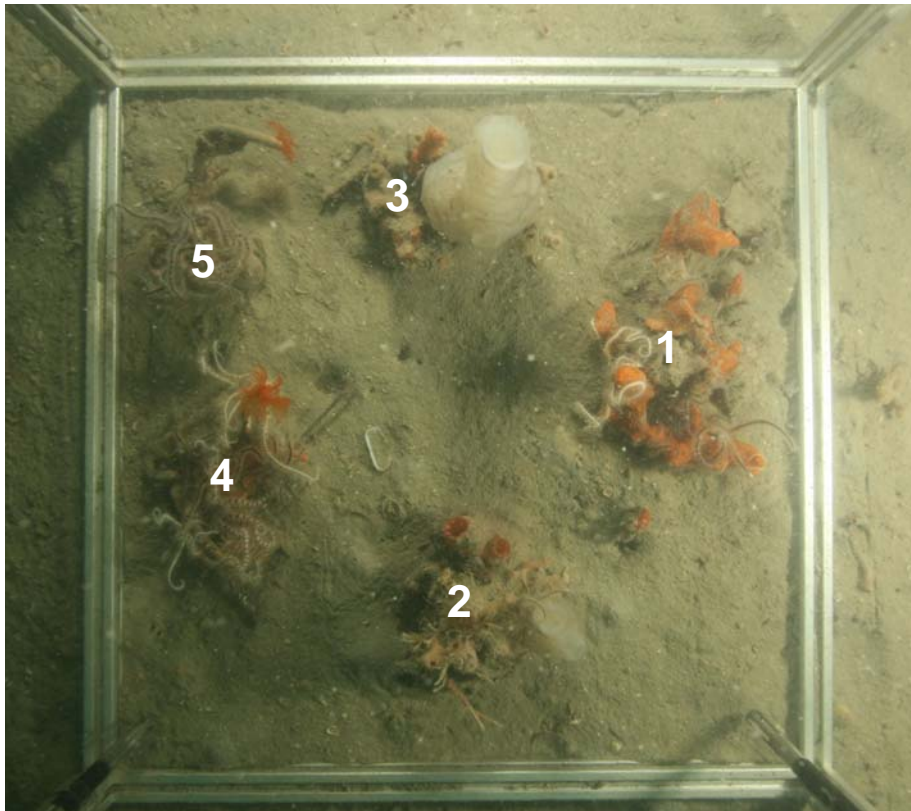
**Table 2:** List of species evaluated in experiment 12. Nx = number of collected specimens; N = number of evaluated organisms; life habit (epi = epifaunal, in = infaunal).

Taxonomic group	Species or organism	Nx	N	Life habit	Mobility
Porifera		5	5	epi	sessile
Bivalvia					
	<i>Chlamys varia</i>	1	1	epi	hemisessile
	<i>Corbula gibba</i>	15	7	in	mobile
Polychaeta					
	Infaunal polychaetes (not identified)	29	9	in	mobile
	<i>Serpula vermicularis</i>	2	2	epi	sessile
Crustacea					
	<i>Paguristes eremita</i>	9	5	epi	mobile
Echinoidea					
	<i>Psammechinus microtuberculatus</i>	1	1	epi	mobile
	<i>Schizaster canaliferus</i>	3	3	in	mobile
Ophiuroidea					
	<i>Ophiothrix quinque maculata</i>	20	4	epi	mobile
	<i>Ophiura</i> spp.	2	2	epi	mobile
Ascidiacea					
	<i>Microcosmus sulcatus</i>	1	1	epi	sessile
	<i>Phallusia mammilata</i>	2	2	epi	sessile

### 3.3 Behavioural analyses

Under normoxia, no atypical behaviours were observed. With decreasing oxygen values, however, the organisms showed unusual behaviours correlated to the different oxygen thresholds 2, 1, and 0.5 ml l<sup>-1</sup> DO. Observed behaviours differed not only between species but also within species, for example between juvenile and adult individuals in the infaunal sea urchin *Schizaster canaliferus*. With the exception of the bivalves *Corbula gibba*, all organisms died during the experiment.

To simplify the evaluation, I numbered all multi-species clumps in the 50 x 50 cm area from 1 to 5. This made it easier to associate them with different organisms and to find the individuals again (Fig. 9).



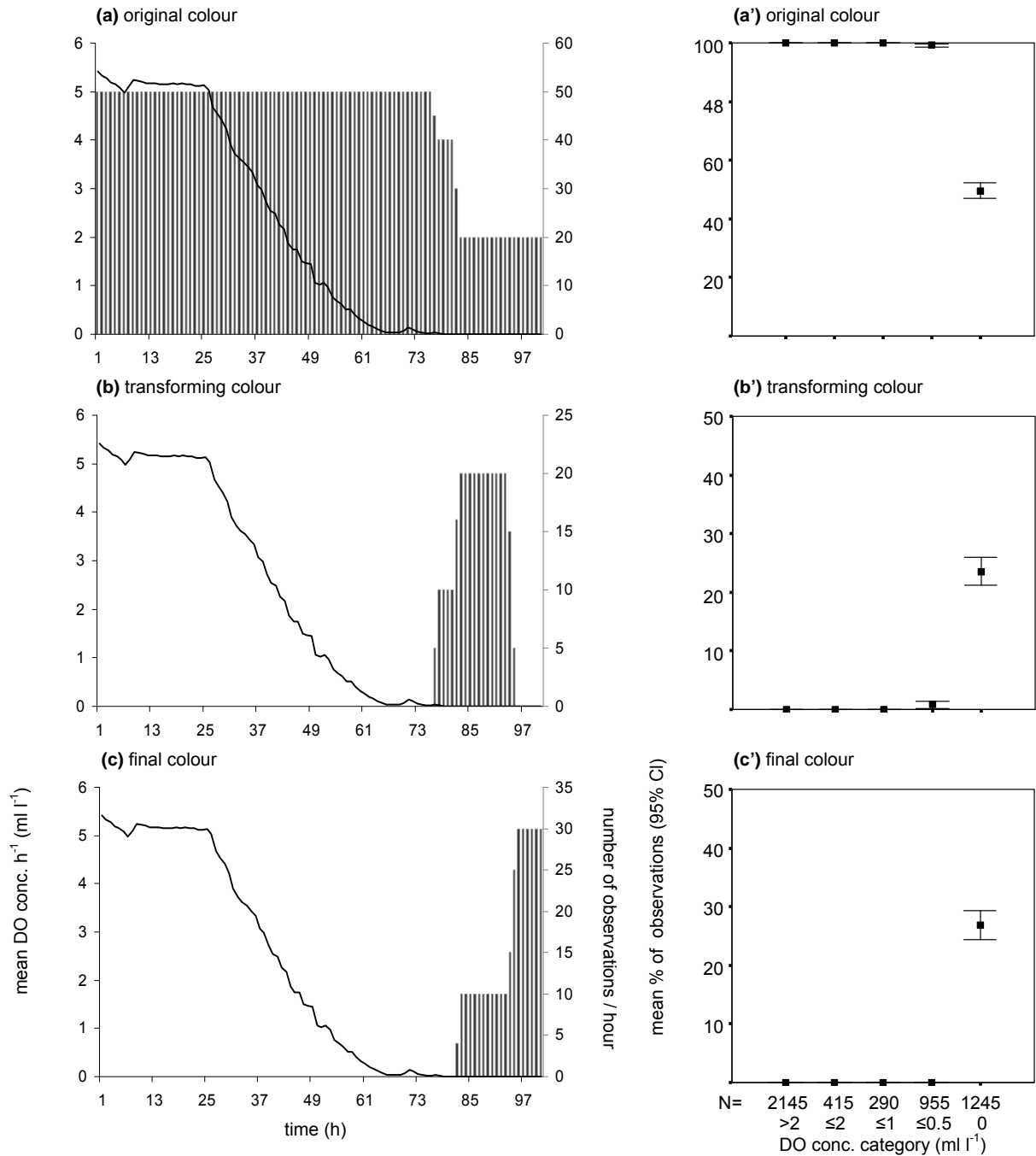
**Fig. 9:** Experiment 12, open configuration, numeration of the multi-species clumps.

#### 3.3.1 Porifera (not identified)

There were five sponge colonies in experiment 12. Three orange colonies belong to clumps 1, 3 and 4, and two light brown colonies belong to clump 3. Only colour changes were documented (subdivided into three stages): original, transforming and



final colour. At h75 the colour started to change from the original light brown into grey (Fig. 10b-b'), and at h80 the first colony showed the final dark grey colour. All three of the grey colonies showed this final colour at h95 (Fig. 10c-c'). The colour of the two orange colonies at clumps 1 and 4 did not change at all.

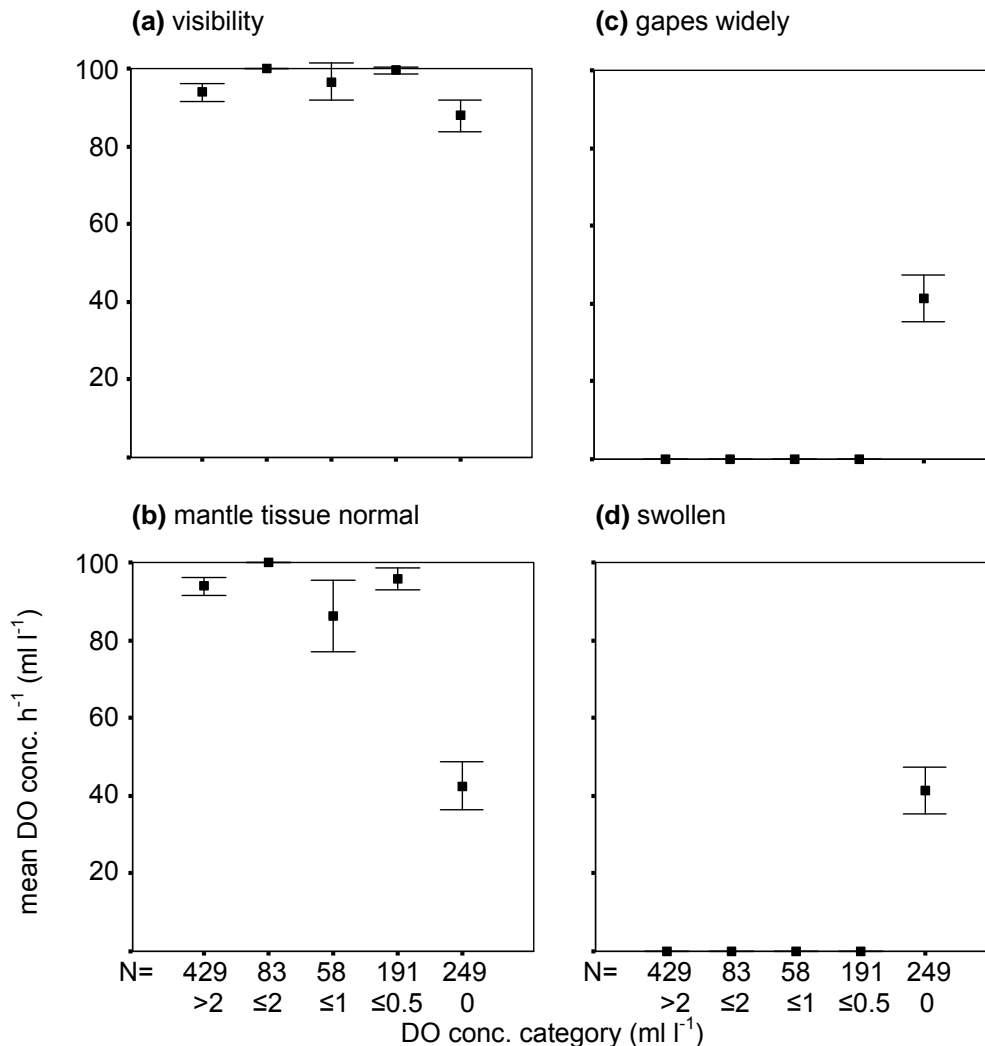


**Fig. 10:** Colour changes of sponges during induced hypoxia and anoxia. The histograms (a-c) show the number of behaviours observed per hour in relation to the lower oxygen sensor (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-c') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.3.2 Bivalvia

#### 3.3.2 (i) *Chlamys varia*

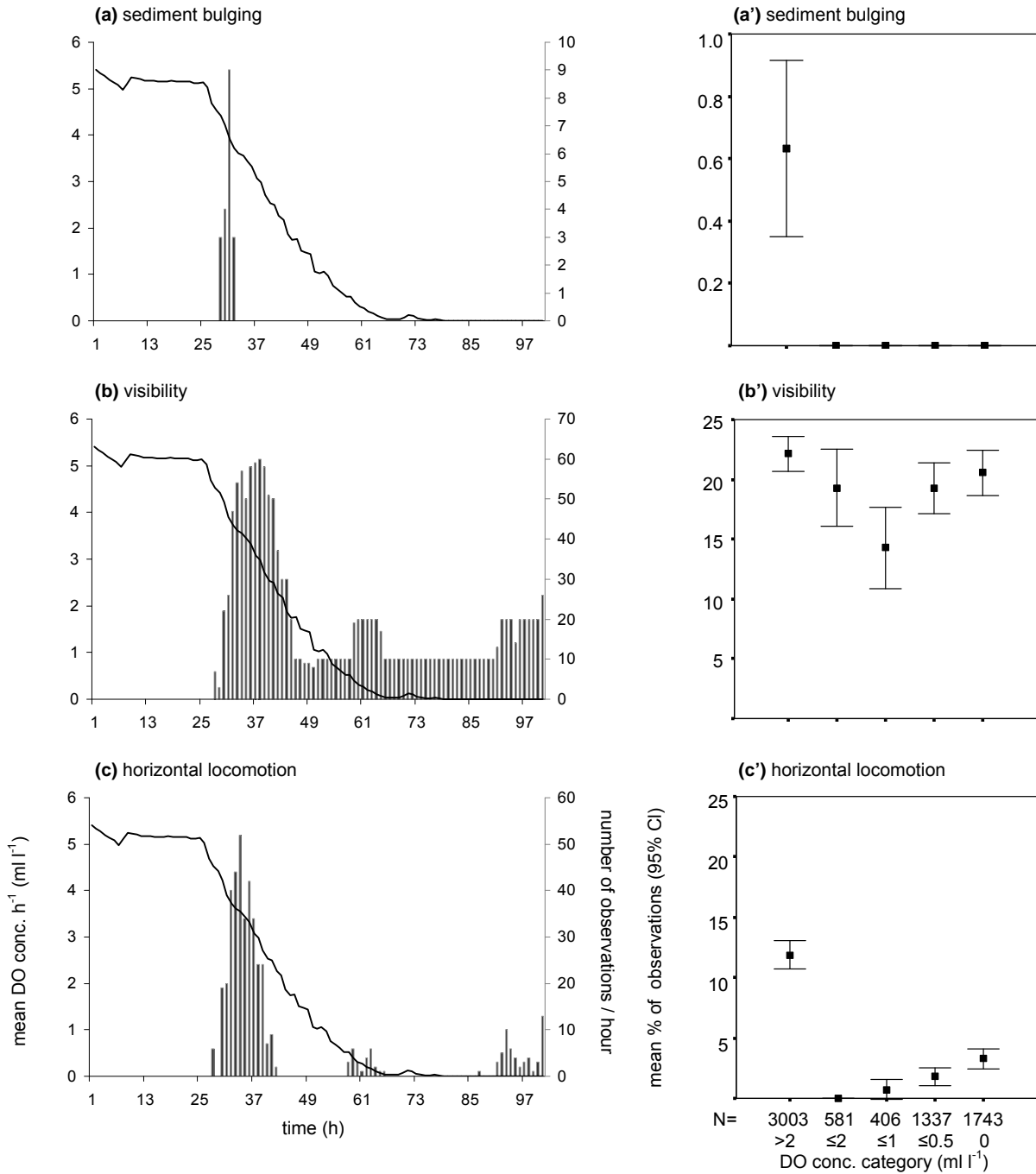
One *Chlamys varia* individual was positioned on the top of clump 2 and remained in the same position throughout the experiment. A hermit crab occasionally sat on the bivalve and so there were fluctuations in visibility (Fig. 11a). Its normal behaviour (shell gape open) was shown up until severe hypoxia was reached (Fig. 11b). At anoxia, the bivalve showed two atypical behaviours at the same time: the number of observations in which the mantle tissue of the bivalve was in its normal position dropped sharply (h91), simultaneously the shells opened widely and the mantle tissue began to swell (highly significant;  $p < 0.01$ ; Fig. 11c+d).



**Fig. 11:** Changes in the species-specific behaviour of *Chlamys varia* during induced hypoxia and anoxia. The behaviours visibility, valve gaps and mantle shape were evaluated. The course of the selected behaviours during the five oxygen categories is shown by the error bar diagrams. Numbers below the x-axes (N) show the number of photographs per oxygen category.

### 3.3.2 (ii) *Corbula gibba*

The infaunal bivalve *Corbula gibba* reacted very sensitively to decreasing oxygen. They emerged from the sediment when the dissolved oxygen dropped below 4 ml l<sup>-1</sup> DO at h29 (Fig. 12a). At this point the sediment over the bivalves started to move and bulge. Below 2 ml l<sup>-1</sup> DO, four individuals reburrowed into the sediment but emerged again at h59 and h92 (small peaks in Fig. 12b). Once exposed on the sediment, 12% moved around as soon as they became visible (Fig 12c-c'), but after peaking during late normoxia the number of observed locomotions decreased significantly ( $p < 0.05$ ). Some remained visible on surface but did not move. In this experiment, all individuals survived.



**Fig. 12:** Changes in the species-specific behaviour of *Corbula gibba* during induced hypoxia and anoxia. The histograms (a-c) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-c') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

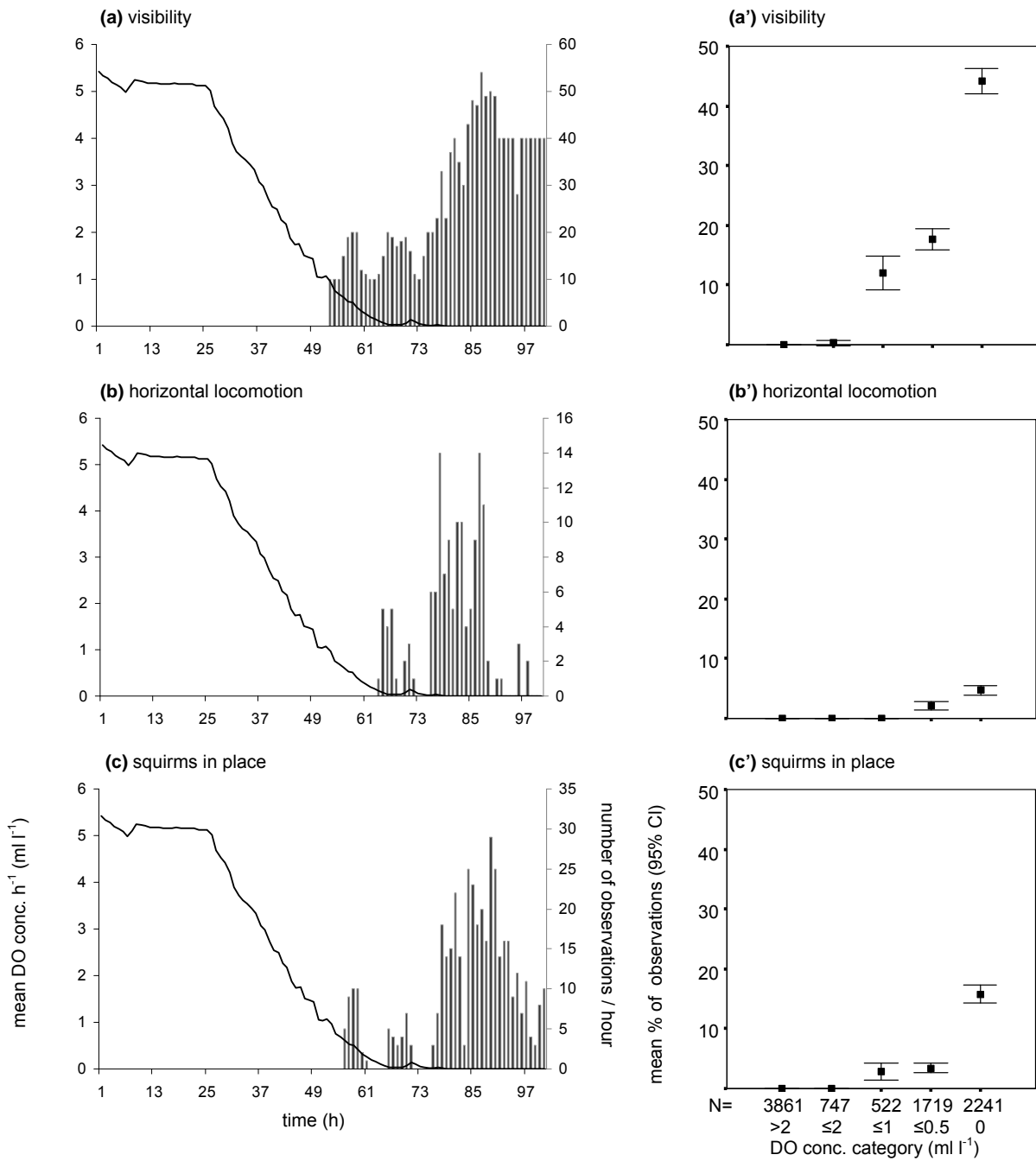
### 3.3.3 Polychaeta

#### 3.3.3 (i) Infaunal polychaetes (not identified)

Nine individuals out of 29 infaunal polychaetes that emerged on the sediment surface were evaluated. After exposure, the polychaetes remained visible just for a few hours. They either reburrowed or hid behind the sensors. The first individuals emerged at moderate hypoxia (h53). Figure 13a clearly shows that the number of visible individuals fluctuated between h52-72. After h73 the number of visible individuals increased constantly and 45 % were visible (after h85).

Horizontal locomotion was rare, but most polychaetes showed the behaviour "squirms in place" at anoxia: they squirmed and writhed at one particular spot.

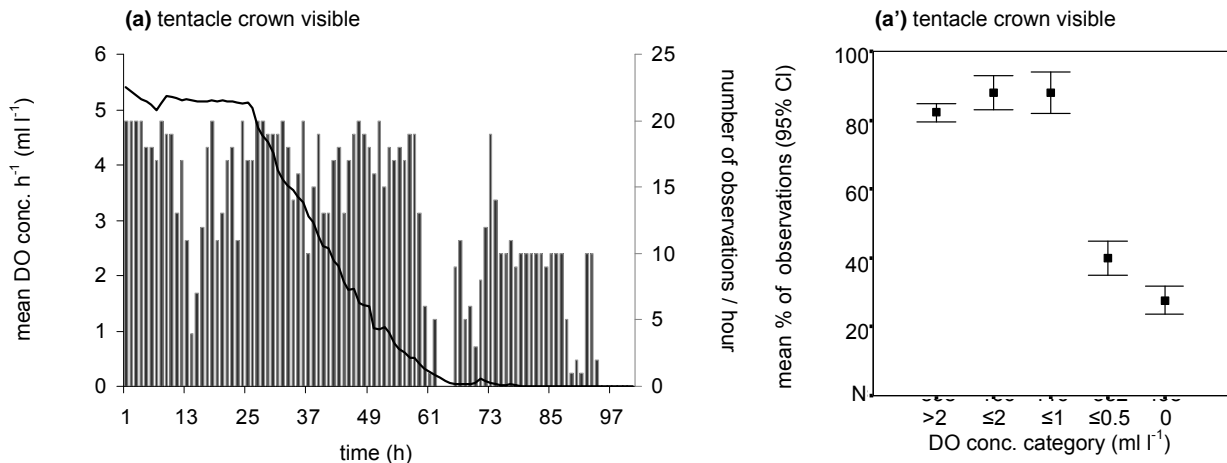
One individual moved in the sediment in the left half of the 50x50 cm frame: it was successively visible (1) at h77 midway along the upper plexiglass wall, (2) midway along the left plexiglass wall, (3) between clumps 4 and 5 and (4) it crawled down to the lower wall, where it was visible until h90.



**Fig. 13:** Changes in the species-specific behaviour of infaunal polychaetes during induced hypoxia and anoxia. The histograms (a-c) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-c') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.3.3 (ii) *Serpula vermicularis*

Two of these tubeworm individuals were located in the left half of the chamber; one was part of clump 4 and the other a part of clump 5. The tubes remained visible throughout the experiment. Whereas under normoxia and until moderate hypoxia the tentacle crowns were visible most of the time, below  $0.5 \text{ ml l}^{-1}$  DO (h59) the worms started markedly to retract their crown into the tube (highly significant;  $p < 0.01$ ; Fig. 14a').



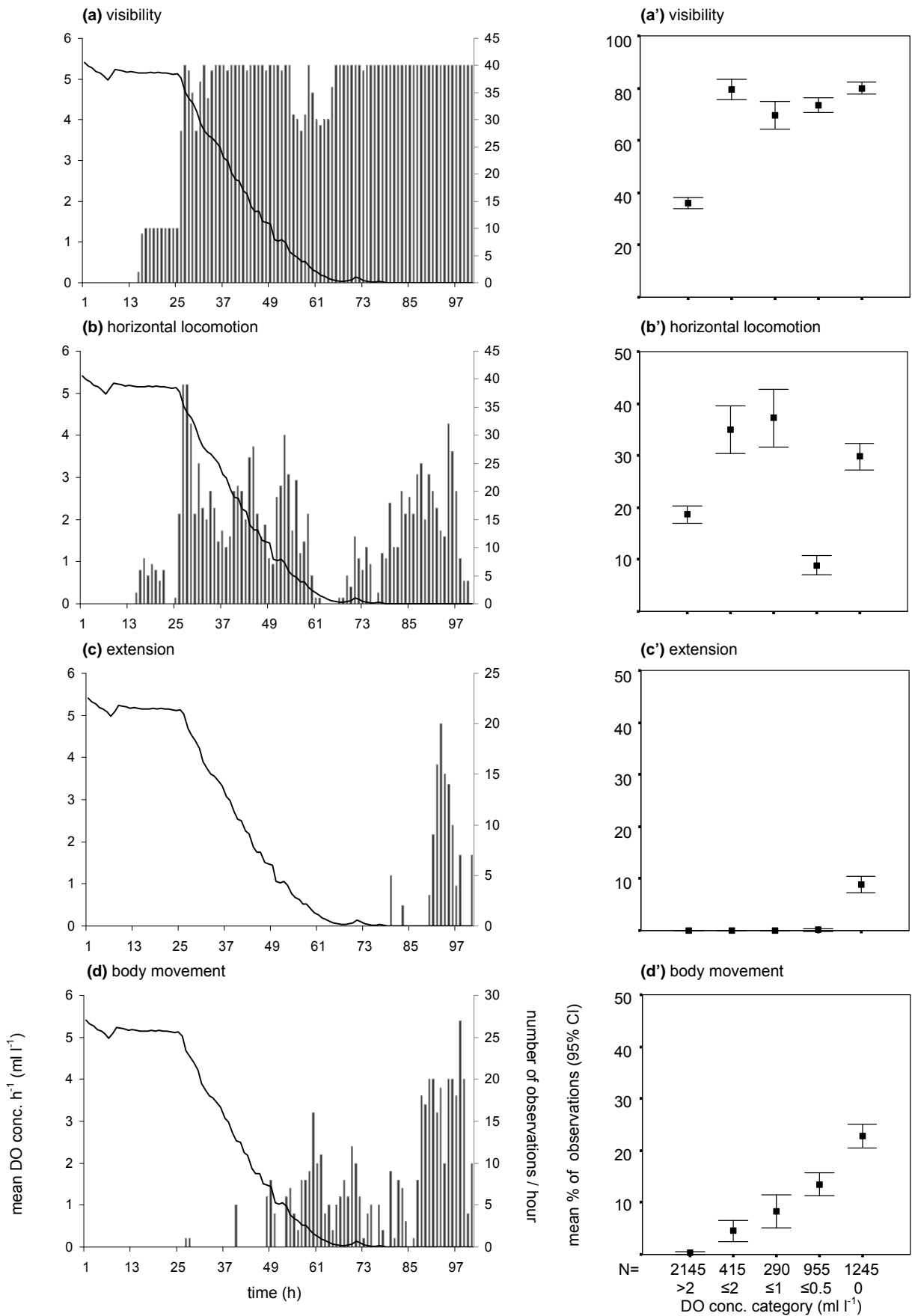
**Fig. 14:** Changes in the species-specific behaviour of *Serpula vermicularis* during induced hypoxia and anoxia. The histograms (a) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). The error bar diagrams (a') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.3.4 Crustacea - *Paguristes eremita*

Under normoxia, the five evaluated hermit crabs were mostly hidden under multi-species clumps. With beginning hypoxia they started to leave their hiding places (Fig. 15a+a') and moved on the sediment or on the clumps (Fig. 15b+b'). There was a highly significant ( $p < 0.01$ ) increase in the number of visible crabs from normoxia to beginning hypoxia. Throughout the experiment, individual crabs hid under a multi-species clump.

Body movements (Fig. 15d-d'), i.e. movements while the crabs themselves were immobile, were recorded and slightly increased after h40.

The hermit crabs extended from their shells only under anoxia (after h79; Fig. 15c+c'). At the end of the experiment (h100), two individuals were completely outside their shells and lying on the sediment in the upper right-hand corner.



**Fig. 15:** Changes in species-specific behavioural of *Paguristes eremita* responses during induced hypoxia and anoxia. The histograms (a-d) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-d') show course of selected behaviours during the five oxygen categories.

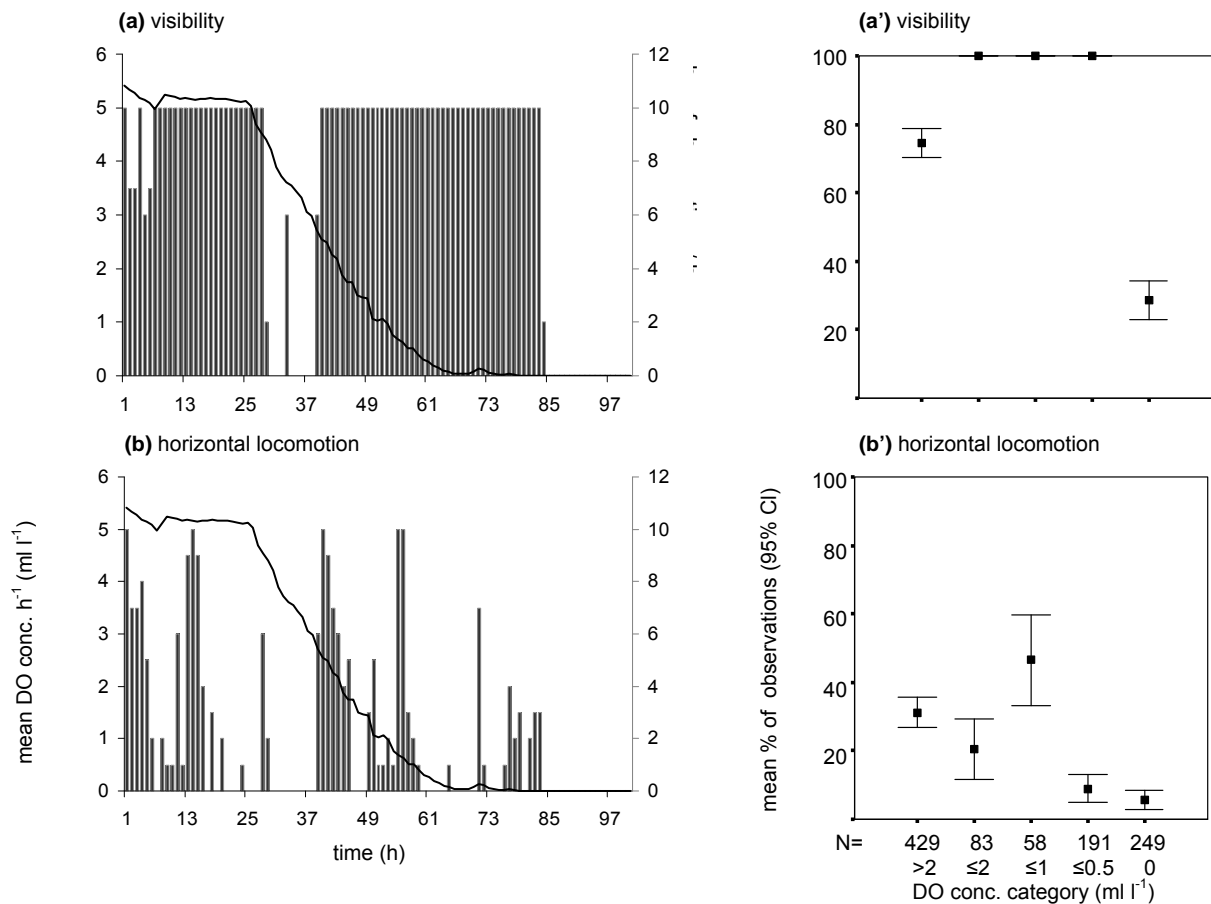


### 3.3.5 Echinoidea

#### 3.3.5 (i) *Psammechinus microtuberculatus*

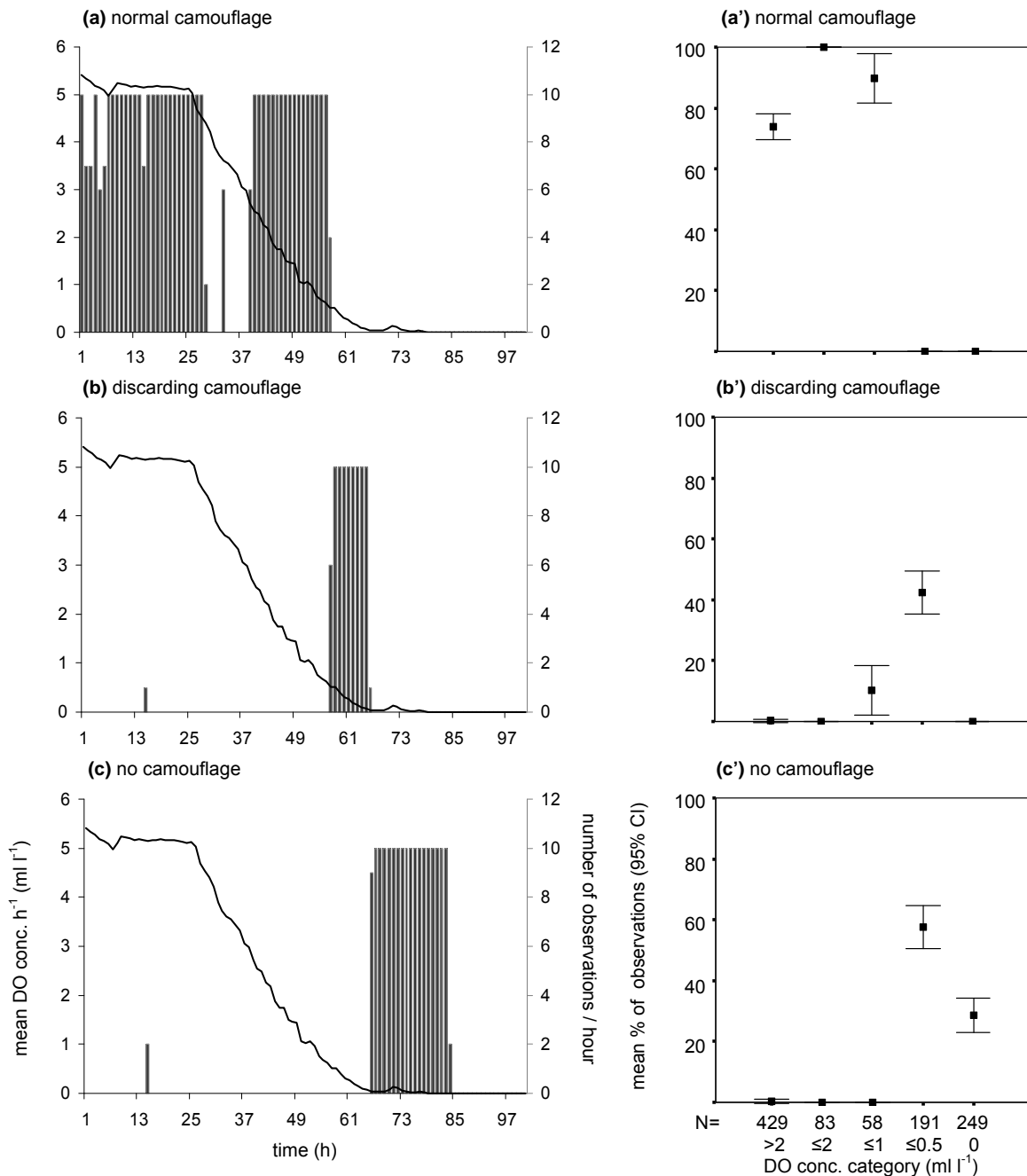
Only one individual of the epifaunal sea urchin *Psammechinus microtuberculatus* was present in experiment 12. At the beginning it was positioned outside the frame, but during the "open" configuration entered it and was visible 75% of the time (Fig. 16a); when not visible it was hiding under the multi-species clumps number 4 and 5 (e.g. h29 to h38).

Horizontal locomotion was observed in phases (Fig. 16b) but decreased markedly with severe hypoxia (Fig. 16b'). From h85 on, the sea cucumber *Ocnus planci* was lying on the sea urchin and no further evaluation was possible.



**Fig. 16:** Changes in the species-specific behaviour of *Psammechinus microtuberculatus* during induced hypoxia and anoxia. The histograms (a-b) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). The error bar diagrams (a'-b') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

Under normoxia the sea urchin was camouflaged with 2 pieces of shell debris (Fig. 17a). With moderate hypoxia it started to discard the camouflage, which slowly moved down the test from the aboral side (peak at severe hypoxia; Fig. 17b-b'). At the end of severe hypoxia (h66) it had lost all its camouflage (Fig 17c).

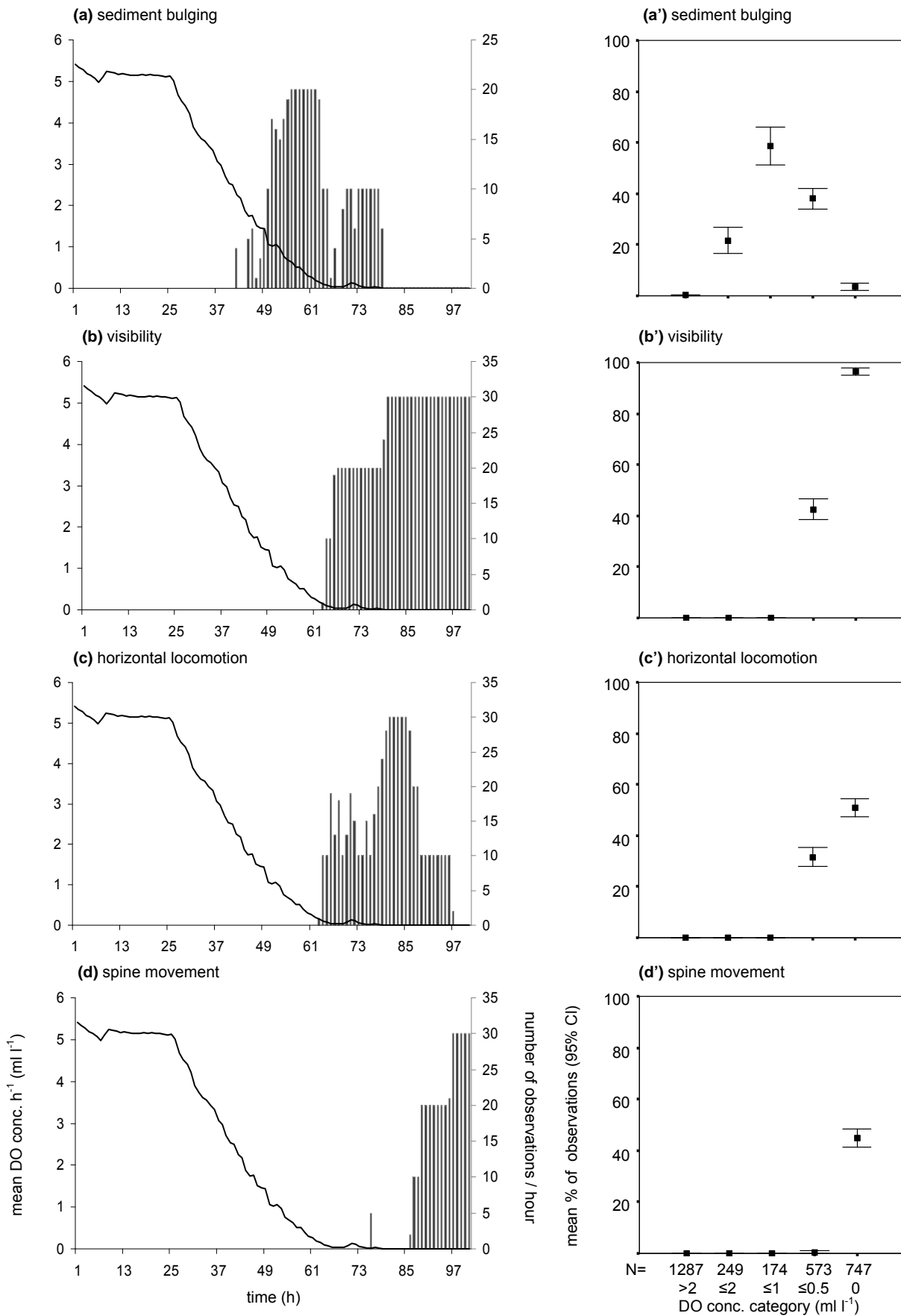


**Fig. 17:** Changes in the species-specific behaviour of *Psammechinus microtuberculatus* during induced hypoxia and anoxia. The histograms (a-c) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). The error bar diagrams (a'-c') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.3.5 (ii) *Schizaster canaliferus*

Three individuals of this infaunal, irregular sea urchin emerged from the sediment: two small juveniles and one large adult. Under normoxia they were not visible because of their infaunal life habit. With beginning hypoxia (h44) the sediment above them started to bulge and clump 2 began to wobble (one sea urchin was apparently under this clump), peaking at moderate hypoxia (Fig. 18a). During emergence the adult individual pushed over clump 3. At h64 and h66 the adult and one juvenile were visible (Fig. 18b) and moved on the sediment (Fig. 18c). The second juvenile emerged at h90. The adult moved in the upper right-hand corner between the upper plexiglass wall and clumps 1 and 3. The first juvenile moved around in the middle of the deployment between the clumps 1 to 4, and the second juvenile moved between the lower plexiglass wall and clumps 2 and 4. Mortality occurred at h88, 91 and 99 when the last body movement (plus 2h) was recorded. While lying motionless on the sediment, spine moves (Fig. 18d) were still observed.

In this species, differences were evident between the two juveniles and the adult. The adult was the first to emerge from the sediment but showed longer body movement (h99) and therefore apparently lived longer than the two younger individuals.



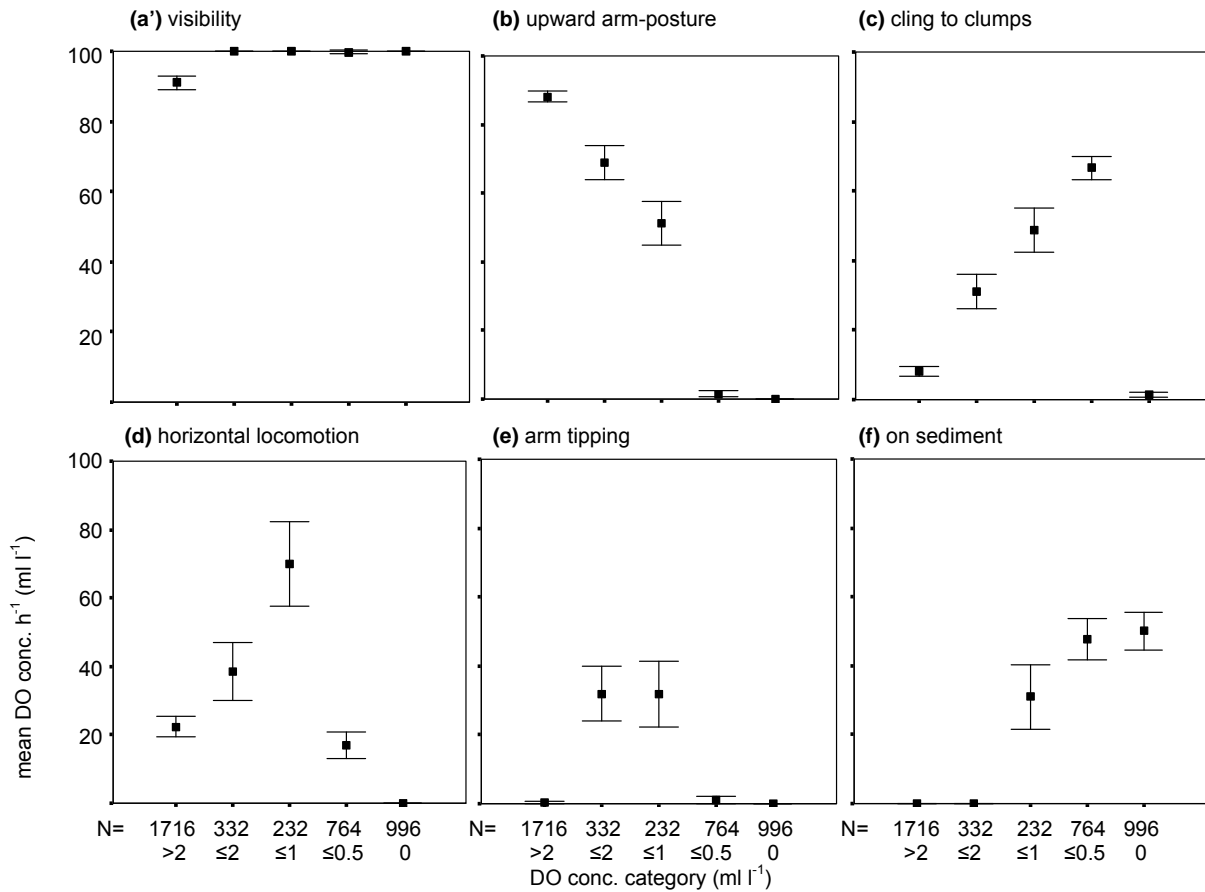
**Fig. 18:** Changes in the species-specific behaviour of *Schizaster canaliferus* during induced hypoxia and anoxia. The histograms (a-d) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-d') show course of selected behaviours during the five oxygen categories.

### 3.3.6 Ophiuroidea

#### 3.3.6 (i) *Ophiothrix quinquemaculata*

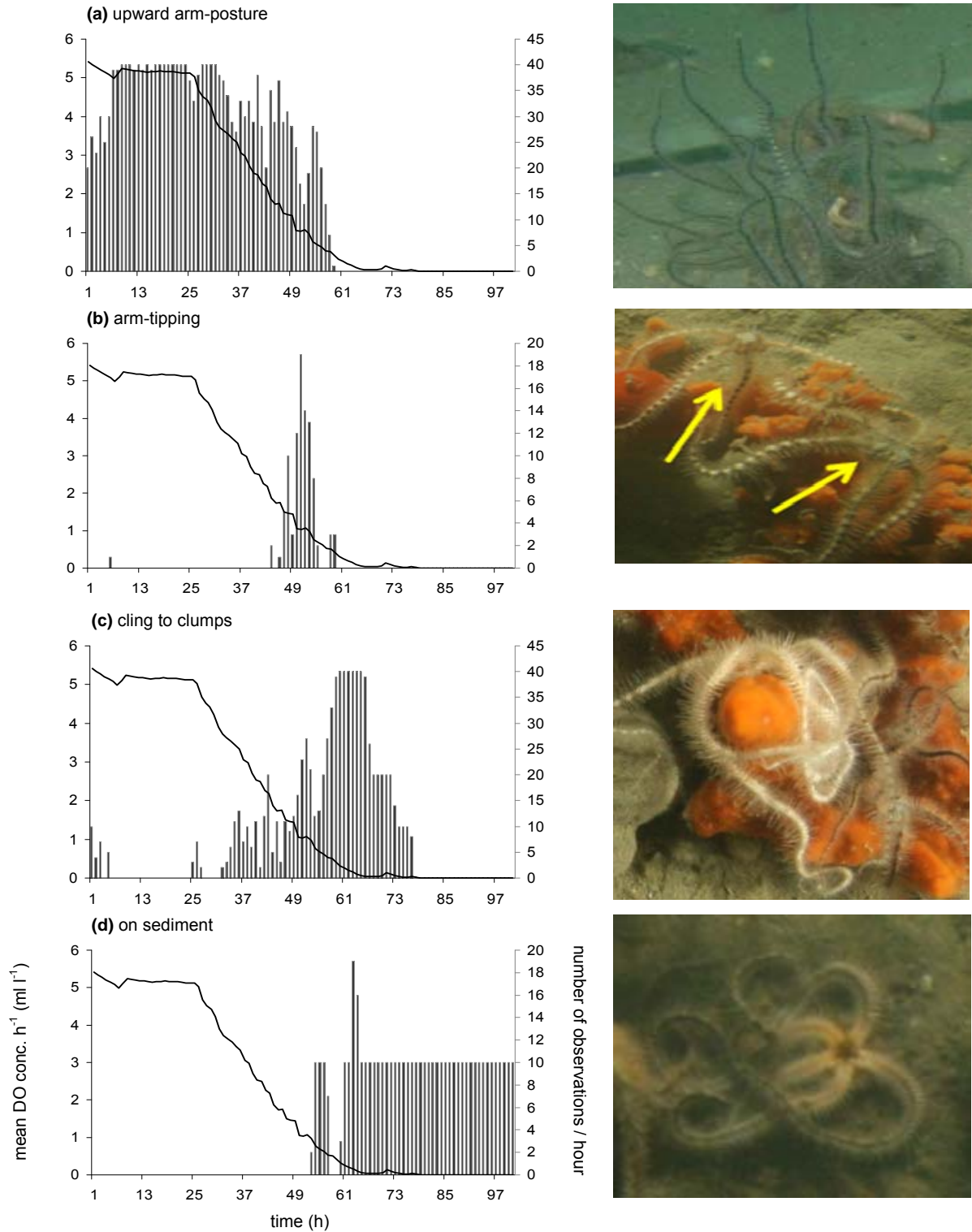
All evaluated brittle stars were visible throughout the experiment (Fig. 19a). Under normoxia they were mostly stationary and positioned on multi-species clumps. Up to seven individuals sat on one bioherm, so the discs were sometimes observed. With beginning hypoxia, they started to move horizontally on the bioherms, peaking at moderate hypoxia (Fig. 19d). At moderate hypoxia, 30% of the evaluated individuals left the substrate and moved onto the sediment (Fig. 19f).

Also a marked change in arm-posture was documented. Under normoxia they showed typical suspension-feeding behaviour with the arms stretched upwards to filter the water (Fig. 19b and Fig. 20a). With beginning hypoxia, arm-posture changed: the brittle stars either frequently stood on their arms (so-called arm-tipping) between h44 to h59 to elevate their central disc (Fig. 19e and Fig 20b) or clung to the multi-species clumps. The latter behaviour increased constantly and peaked at severe hypoxia between h56 to h67 (Fig. 19c and Fig 20c). At anoxia, 50 % of the evaluated individuals were lying motionless on the sediment (Fig. 19f and Fig. 20d). All evaluated *O. quinquemaculata* individuals died at the onset of anoxia (h66, 67, 73 and 77), making them the most sensitive species in the experiment.



**Fig. 19:** Changes in the species-specific behaviour of *Ophiothrix quinquemaculata* during induced hypoxia and anoxia. The error bar diagrams show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

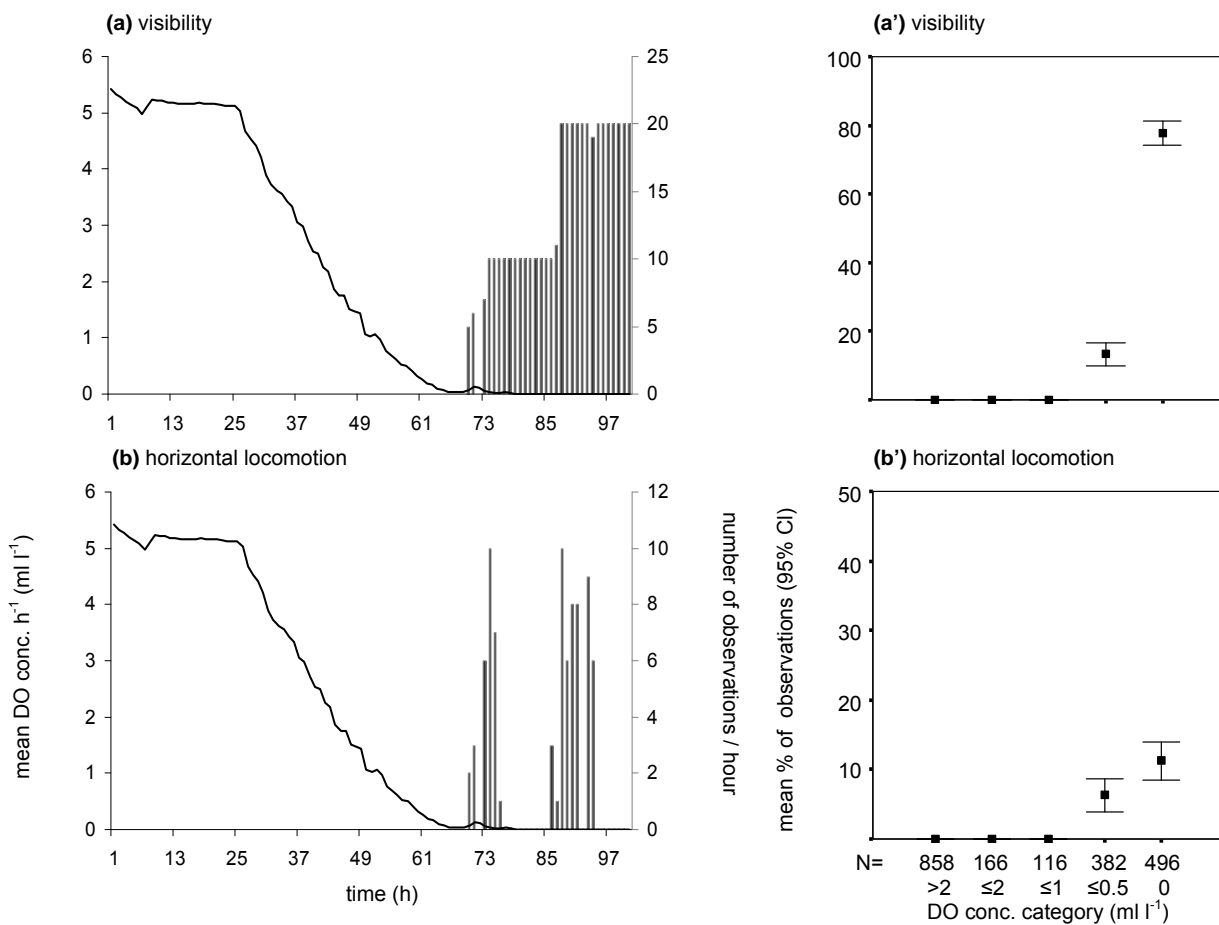
Figure 20a-d clearly show a succession of arm-posture correlated to decreasing oxygen in all evaluated individuals. The photographs on the right side illustrate the atypical changes in arm posture.



**Fig. 20:** Changes in the species-specific behaviour of *Ophiothrix quinquemaculata* during induced hypoxia and anoxia. The histograms (a-d) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis.

### 3.3.6 (ii) *Ophiura* spp.

The brittle star *Ophiura* spp. is an epifaunal subsurface suspension-feeder. The first individual emerged at severe hypoxia (h70), the second at anoxia (h88). Both remained visible until the end of the experiment (Fig. 21a) and showed horizontal movement after emergence from the sediment (Fig. 21b). The first peak (Fig. 21b) between h70-h76 reflects the locomotion of the first emerged individual; the second peak between h86-h94 describes the movement of the other one. The last arm movements were observed at h88 and 95 and mortality occurred.



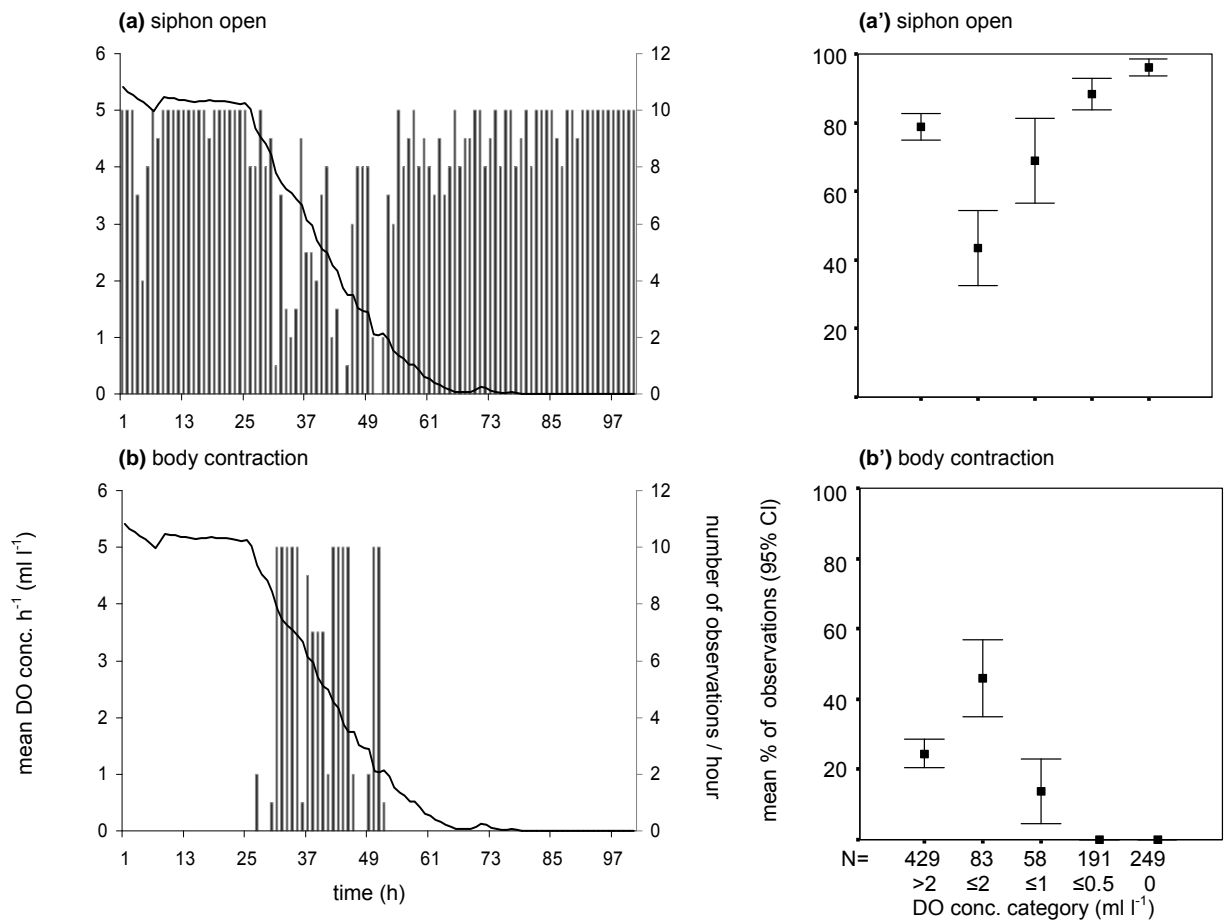
**Fig. 21:** Changes in the species-specific behaviour of *Ophiura* spp. during induced hypoxia and anoxia. The histograms (a-b) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). Note different scales on the second y-axis. The error bar diagrams (a'-b') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.



### 3.3.7 Ascidiacea

#### 3.3.7 (i) *Microcosmus sulcatus*

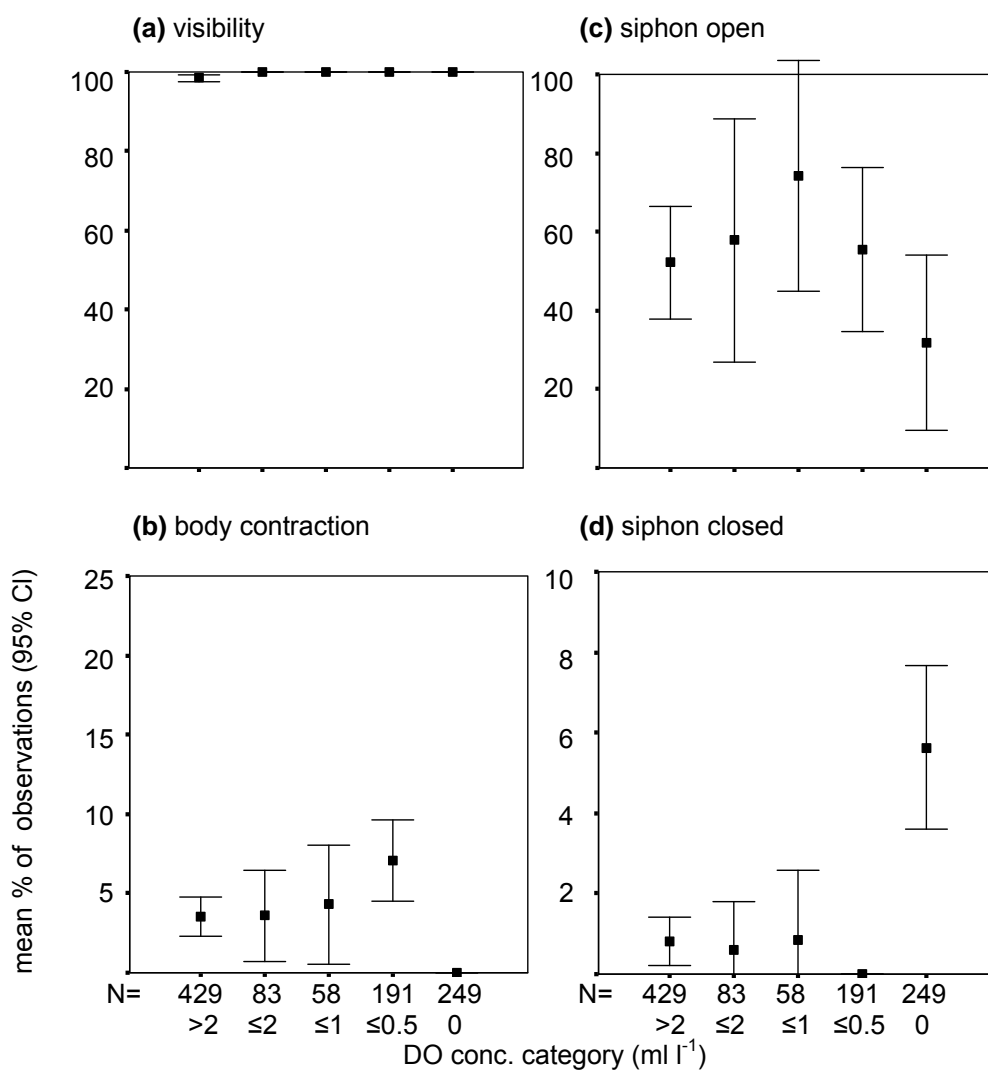
This ascidian individual was part of clump 2. During normoxia both siphons remained largely open (80 %, Fig. 22a'). With beginning hypoxia, however, the ascidians started to close both siphons simultaneously (Fig. 22a). This behaviour correlated with body contraction (the ascidian contracts its entire body), peaking at beginning hypoxia (nearly 50 %). At moderate hypoxia, siphons were open again but body contraction decreased until the end of the experiment (Fig. 22b+b').



**Fig. 22:** Changes in the species-specific behaviour of *Microcosmus sulcatus* during induced hypoxia and anoxia. The histograms (a-c) show the number of behaviours observed per hour in relation to oxygen curve (values averaged per hour). The error bar diagrams (a'-c') show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.3.7 (ii) *Phallusia mammilata*

The two individuals of the ascidian species *Phallusia mammilata* belong to clumps 2 and 3 and their siphons were always visible (Fig. 23a). They started to contract their body up until the time where anoxia was reached. Fig. 23b shows that the number of body contractions increased slightly until severe hypoxia and ceased at anoxia ( $p < 0.01$ ) observed in h75. While the individuals contract their body, the siphons were open up to 78 % of the time (Fig. 23c). During body contractions, closed siphons were rare (about 1.5 %). Afterwards, at anoxia, when there were no body contractions, the number of observed closed siphons increased to 5 % (Fig. 23d).



**Fig. 23:** Changes in the species-specific behaviour of *Phallusia mammilata* during induced hypoxia and anoxia. The error bar diagrams show course of selected behaviours during the five oxygen categories. N = number of photographs per oxygen category.

### 3.4 Mortality

Decreasing oxygen caused not only atypical behaviours, but also led to mortality. Except for the bivalve *Corbula gibba*, all evaluated organisms died at either severe hypoxia or anoxia. Mortality was defined by the last visible activity such as locomotion, arm- or body movement (in the species *Serpula vermicularis*, *Schizaster canaliferus* and *Paguristes eremita* plus two hours to account for a potential moribund phase; Table 3). Table 3 also lists the time of death (h), the oxygen value and the hydrogen sulphide value for each dead individual.

The evaluated brittle star *Ophiothrix quinquemaculata* individuals were the most sensitive to low dissolved oxygen concentration. This species not only showed atypical behaviour very early on, at beginning hypoxia, but were also among the first to die (between h66 and h77, DO 0.3, H<sub>2</sub>S absent). Due to the lack of hydrogen sulphide, mortality can be attributed solely to the low oxygen concentration.

As contrast, all evaluated hermit crabs *Paguristes eremita* died forewards the end of the experiment, approximately 24 h after the brittle stars (h101, DO 0, H<sub>2</sub>S 17.9).

In the infaunal sea urchin *Schizaster canaliferus* the two juveniles died earlier (h88 and h91) than the adult (h99).

In some species (*Chlamys varia*, *Psammechinus microtuberculatus*, *Phallusia mammilata*, *Microcosmus sulcatus* and sponges) it was not possible to determine the time of death due to the lack of clear signs of mortality (e.g. drooping in ascidians).

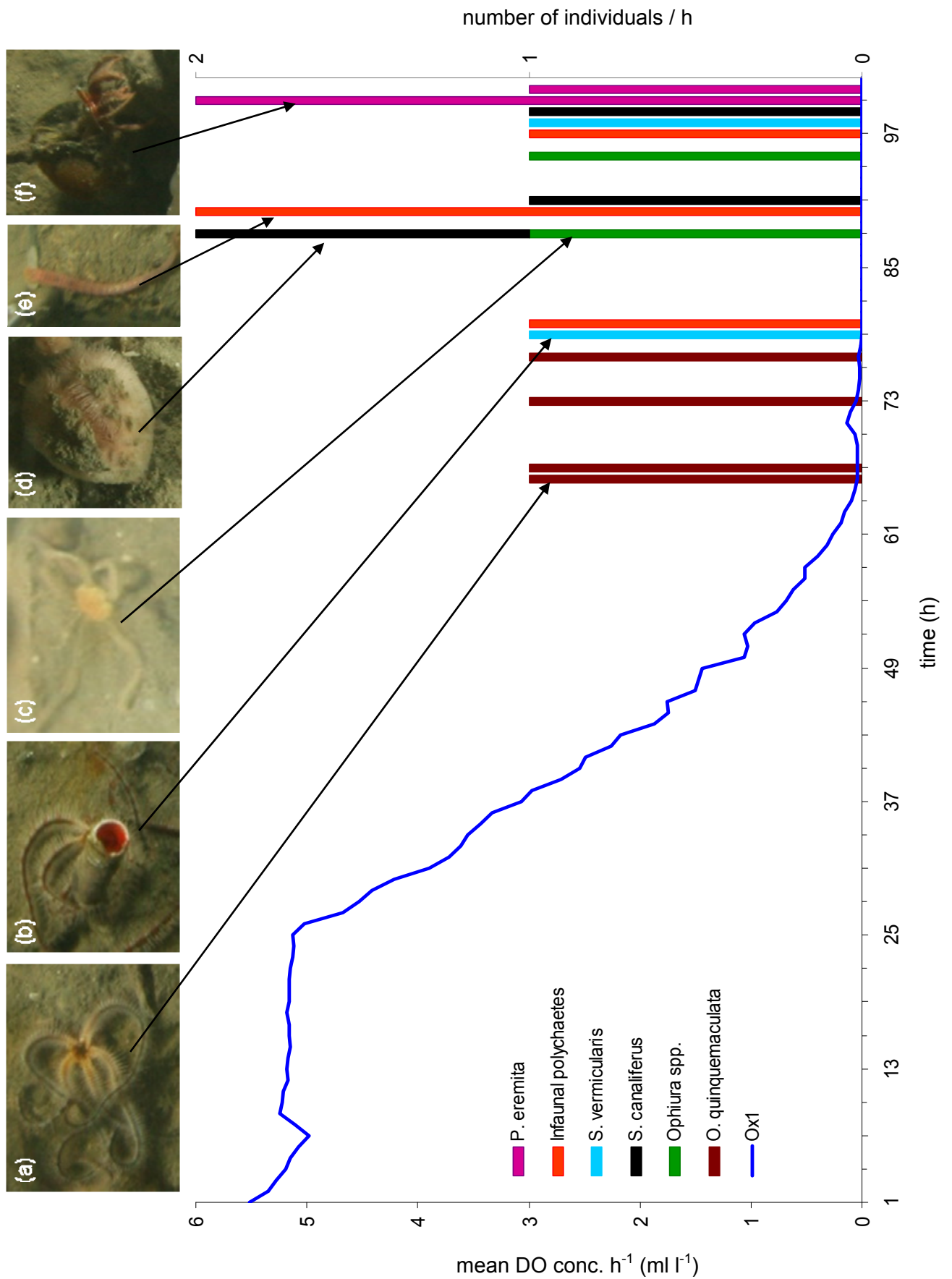
**Table 3:** Criteria and time of death.

Taxonomic group	N	Final activity	Time of death (h) no. Individuals	DO (ml l <sup>-1</sup> )	H <sub>2</sub> S (µM)
<i>O. quinquemaculata</i>	4	arm movement	66/67/73/77/-	0.3/0.3/0.3/0.3	0/0/0/0/-
<i>S. vermicularis</i>	2	movement + 2 h	79/98	0/0	0/12.8
Infaunal polychaetes (not identified)	9	body movement	80/90/90/97/-	0/0/0/0/-/-/-/-	0/9.0/9.0/11.6/-/-/-
<i>Ophiura</i> spp.	2	arm movement	88/95	0/0	5.15/10.27
<i>S. canaliferus</i>	3	locomotion + 2 h	88/91/99	0/0/0	5.2/10.3/14.1
<i>P. eremita</i>	5	body movement + 2 h	100/100/101/-	0/0/0/-	15.4/16.7/17.9/-
<i>C. varia</i>	1	-			
<i>P. microtuberculatus</i>	1	-			
Porifera	5	-/-/-/-/-			
<i>M. sulcatus</i>	1	-			
<i>P. mammilata</i>	2	-/-			
<i>C. gibba</i>	7	survivors			

- mortality in this deployment undiscernible.

A temporal succession of mortality is shown in Fig. 24. The purple bars mark the time of death of the brittle star *Ophiothrix quinquemaculata* at severe hypoxia or anoxia. Especially the infaunal polychaetes (orange bars) and the *Serpula vermicularis* (blue bars) show clear intraspecific differences, up to 19 h within one species.

Body movements of the hermit crab *Paguristes eremita* (pink bars) were still observed in the final photographs (h100 and 101); it is therefore possible that they survived longer.



**Fig. 24:** Sequence of mortality. a = *Ophiothrix quinquemaculata*, b = *Serpula vermicularis*, c = *Ophiura* spp., d = *Schizaster canaliferus*, e = infaunal polychaetes, and f = *Paguristes eremita*.

## 4. Discussion

The lack or absence of dissolved oxygen in coastal marine ecosystems influences a wide range of organisms and therefore impacts the system as a whole. Biological factors like respiration are the main drivers for decreasing oxygen and pH values (Hagerman 1998).

The organisms show special behaviours correlated to different oxygen thresholds. The experimental approach and the evaluation of one full deployment enabled me to define the sequence of *in situ* reactions of species, to correlate them to different oxygen concentrations and to determine a sequence of mortality.

### 4.1 Critical oxygen levels and behavioural reactions

Decreasing oxygen caused different types of basic reactions, reinforcing the results of Riedel et al. (2008):

- increase or decrease in normal activities
- initiation of atypical behaviours
- emergence of infaunal organisms
- colour changes of species and the sediment
- mortality

The experiment described in Riedel et al. (2008) provided an initial evaluation of the responses of the benthic community and provided a framework for the present study. It was subdivided by an unexpected oxygen peak into two declining phases. The organisms showed the same behaviours twice, once during each decline (with recovery in between), underlining the hypothesis that behavioural changes are correlated to different oxygen thresholds. There are considerable differences in tolerance to oxygen depletion from species to species due to their different physiological capacities and adaptabilities. Moreover, the presence of hydrogen sulphide reduces this tolerance due to its toxic effects (Hagerman 1998).

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

Hypoxic conditions affect the life and behaviour of many marine organisms (Nilsson and Sköld 1996; Stierhoff et al. 2006; Vismann 1990; Vistisen and Vismann 1997). Although most authors (Rosenberg et al. 1991; Diaz and Rosenberg 1995; Riedel et al. 2007; Stachowitsch et al. 2007; Diaz and Rosenberg 2008; Riedel et al. 2008) apply oxygen thresholds below  $2 \text{ ml l}^{-1} \text{ DO}$ , Vaquer-Sunyer and Duarte (2008) are of the opinion that this threshold is too low for the more sensitive taxa. In the present study, this also applied to the bivalve *Corbula gibba* and the ascidians *Microcosmus sulcatus* and *Phallusia mammilata*: they already showed initial aberrant behaviour at slightly below  $4 \text{ ml l}^{-1} \text{ DO}$ .

Nevertheless, most organisms in my experiment first initiated atypical behaviour at the generally accepted level of  $2 \text{ ml l}^{-1} \text{ DO}$ , which we termed beginning hypoxia. The hermit crabs *Paguristes eremita* left their hiding places below  $2 \text{ ml l}^{-1} \text{ DO}$ . The brittle stars *Ophiothrix quinquemaculata* started to stand on their arms and to cling to the multi-species clumps. The infaunal sea urchins *Schizaster canaliferus* started to approach the sediment surface.

A typical species of the transition zone between detrital and muddy bottoms is the highly abundant lamellibranch *Corbula gibba* (Crema et al. 1991). They showed an atypical behaviour by emerging from the sediment when the oxygen concentration was at  $4 \text{ ml l}^{-1} \text{ DO}$ , making them one of the first organisms to show a clear response.

The hermit crabs *Paguristes eremita* also showed very early atypical behaviour. Most of these crabs were initially not visible because they were hidden under multi-species clumps. They left their hiding places as oxygen values fell and then moved around within the EAGU frame during the whole deployment. Stachowitsch (1984) observed abnormal extension from shells already on "day 1" of a mass mortality event in 1983, but oxygen values were not measured at that time. In the present deployment this behaviour started at h 80.

A total of 20 individuals of the brittle star *Ophiothrix quinquemaculata* were in the EAGU frame ( $50 \times 50 \text{ cm}$ ). This is equivalent to  $80 \text{ individuals m}^{-2}$  and agrees with the observations in Fedra et al. (1976), who counted 50 to 250 specimens  $\text{m}^{-2}$  within the ORM-community. Under normoxia and beginning hypoxia, most individuals were positioned on multi-species clumps. That behaviour was also observed by Wurzian (1977), who related this to the recruitment of these brittle stars, and who reported an increase in the numbers and weight of *O. quinquemaculata* with increasing clump

size. Such high densities on one clump were also reported for *Ophiothrix fragilis* (Morgan and Jangoux 2004) and reduce the risk of the juveniles being isolated from the group. Moreover, this behaviour enables the juveniles to feed by cleaning off the adult spines with their own tube-feet. The fact that the juveniles were found only on adults or on other suspension-feeding organisms indicates that they are unable to suspension-feed themselves. The close relationship between the two age groups could also influence juvenile survival and thus the stability of the population (Morgan and Jangoux 2004). *O. quinquemaculata* showed its first atypical behaviour at 4 ml l<sup>-1</sup> DO: they started to wrap themselves around the multi-species clumps, as was also observed in a "natural" oxygen crisis in 1983 (Stachowitsch 1986). The major change in behaviour took place at slightly below 1.5 ml l<sup>-1</sup> DO, at which point they began to show "arm-tipping". One explanation for this behaviour is that there is typically more oxygen higher up in the water column. This is supported by Fig. 6, where the upper sensor shows higher oxygen values. Vertical movement is one of the simplest strategies to avoid hypoxia (Hagerman 1998).

Both ascidians *Microcosmus sulcatus* and *Phallusia mammilata* showed two simultaneous behaviours: they started to close both siphons and to contract their body. This agrees with the observations on the ascidian *Pyura praeputialis* in Australia (Evans and Huntington 1992). That species closed its siphons to avoid osmotic stress and increased its so-called squirting behaviour. Such coordinated contractions of the mantle wall and siphons cause an outflow of hypoxic water when the oxygen or salinity concentration was reduced, refilling their body with normoxic water. The infaunal sea urchins *Schizaster canaliferus* were in the initial phase of emergence. This was evident because the sediment above them began to bulge (i.e. the animals themselves were not yet visible).

#### 4.1.2 Moderate hypoxia ( $\leq 1$ ml l<sup>-1</sup> DO)

The infaunal sea urchins *Schizaster canaliferus* were still emerging, but not yet visible. Emerging sea urchins under hypoxic conditions were also described by Schinner et al. (1997). This probably positioned them in better oxygenated surroundings.

In this oxygen category the infaunal polychaetes emerged from the sediment. As shown for *Arenicola marina* and *Nereis diversicolor* (Schöttler 1990), polychaetes are able to survive anaerobic conditions for more than 4 d at 12 °C because they can



switch to anaerobic energy production or reduce their metabolic rate. Mangum (1973) described *Glycera dibranciata* as a species with a large pool of high-oxygen-affinity haemoglobin, which allows them to continue a low level of aerobic metabolism in the absence of oxygen uptake.

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

The last two thresholds are severe hypoxia and anoxia; both induced further atypical behaviours and mortality.

After beginning to emerge at moderate hypoxia, all individuals of the infaunal sea urchin *Schizaster canaliferus* were visible on the sediment surface. This observation agrees to those of Stachowitsch (1984), who saw many individuals lying on the sediment. In agreement with Shumway et al. (1983), both juveniles were the last to emerge from the sediment because they are more tolerant to low oxygen due to their lower respiratory demands. Schinner et al. (1997) described *S. canaliferus* as very sensitive to oxygen depletion and observed that they lose their spines within 4 days after emergence. Due to the short duration of the EAGU development, no spine losses were observed.

Under normoxia, epifaunal regular sea urchins like *Psammechinus microtuberculatus* are camouflaged with material from the surrounding environment (e.g. shell debris). Such an "antipredator adaption" should make them less visible to predators and pose a physical barrier that reduces predator success (Dumont et al. 2007). Moreover, those authors observed a significantly higher covering in exposure to direct sunlight. With decreasing oxygen levels, however, the sea urchins start to drop their camouflage and at severe hypoxia they lack it entirely. One potential explanation is that it is too cost-intensive to hold the material with the tube feet, and that under stress it is dropped.

*Ophiothrix quinquemaculata* no longer showed their arm-tipping behaviour but remained wrapped around clumps and lying on the sediment. From severe hypoxia on, an increasing number of animals lay overturned on the bottom. This condition was also reported during a hypoxic event in 1983 (Stachowitsch 1984, 1986). In the present EAGU deployment they died approximately 42 h after the onset of hypoxia, making them the first species to show mortality. As shown in Table 4 and Figure 25, they died in severe hypoxia, when some oxygen still remained ( $0.3 \text{ ml l}^{-1} \text{ DO}$ ).

The epifaunal brittle stars *Ophiura* spp. live in the subsurface of the sediment; in contrast to Riedel et al. (2008), where ophiroids emerged at 1.5 ml l<sup>-1</sup> DO, they were not observed in the present study until severe hypoxia has reached. The tolerance to low oxygen levels (without hydrogen sulphide) was high and agrees the results of Vistisen and Vismann (1997). Those authors also show that severe hypoxia has to be of long duration before the individuals are affected and that the presence of very small concentrations of sulphide decreases survival significantly. Nevertheless, *Ophiura* spp. are more vulnerable to oxygen depletion than infaunal brittle stars (e.g. *Amphiura filiformis*), which supports the hypothesis that infaunal species are more tolerant to hypoxia than epifaunal species (Hagerman 1998). Arm fragmentation in *Ophiura* spp. was observed in the 1983 event (Stachowitsch 1984) but not in the present deployment. Hypoxic conditions reduce arm regeneration significantly and thereby alter benthic production (Nilsson and Sköld 1996).

Stachowitsch (1984) observed that the multi-species clumps were covered with whitish grey threads of mucus-like matter at an early stage of the 1983 hypoxic event. This marine snow played an important role in that event but doesn't occur in the EAGU deployments. Furthermore, he reported sponges to be among the most sensitive organisms, based on colour changes. In the present experiment, however, no colour change from the normal light brown to dark grey was observed prior to anoxia.

*Phallusia mammilata* no longer showed body contractions, but closed its siphons. *P. mammilata* and *Microcosmus sulcatus* did not droop during the photographic documentation, and mortality could therefore not be determined. In contrast to *Corbula gibba*, which closes its valves, *Chlamys varia* opens them under anoxic conditions. The mantle tissue was swollen and the valves were gaping widely. The data on the ascidians and bivalves correlate to the observations in Stachowitsch (1984): he assumed mortality of ascidians and reported widely gaping *Chlamys varia* at day 4.

The tubeworms *Serpula vermicularis* are filter feeders and extend their tentacle crowns to filter the water. Although the arms of the brittle stars *Ophiothrix quinquemaculata* were near the tube aperture, the worms did not retract. Retraction is a typical response to predators (Dill et al. 1997). Those authors describe a decrease in food availability as a trigger for lengthier retraction times. However, reduced

oxygen levels in the water column require the worm to re-emerge in order to respire. This behaviour was also observed in the present deployment.

Riedel et al. (2008) also described certain behaviours that are not correlated to thresholds but increase gradually during the experiment. One example is the hermit crabs *Paguristes eremita*, which extended even further from their shell. This behaviour was also observed in 1983 (Stachowitsch 1984), although the oxygen value at the time of that behaviour was not known.

## 4.2 Mortality

Different authors (Diaz and Rosenberg 2008; Vaquer-Sunyer and Duarte 2008) have described mortalities of organisms due to hypoxia and anoxia. Mortalities due to "natural" oxygen crises, for example in the northern Adriatic Sea in 1983, are very rapid. Organisms making up more than 90 % of the total macroepifaunal biomass died in the first four days after the first indication of stress (Stachowitsch 1992). The tolerance of organisms is not only influenced by the absence of oxygen, but also by the appearance of hydrogen sulphide and differs from species to species (Theede et al. 1969).

In the experiment evaluated here, anoxia began at h78 and H<sub>2</sub>S reached 19.2 µM at the end of the photographic documentation. All atypical behaviours described above eventually end in mortality. Mortality of the four brittle stars *Ophiothrix quinquemaculata* was already observed at h66, 67, 73 and 77. All other organisms died later, when anoxia was reached and hydrogen sulphide began to increase: *Serpula vermicularis* (h79 and 98), infaunal polychaetes (h80 - 97), *Ophiura* spp. (h88 and 95), *Schizaster canaliferus* (h88 - 99) and *Paguristes eremita* (h100 - 101). For those species, the relative roles of oxygen deficiency or H<sub>2</sub>S evolution are less clear. These results confirm those of Vaquer-Sunyer and Duarte (2008), who described molluscs to be the organisms most tolerant to hypoxia (having the lowest LC<sub>50</sub>). In the present study the bivalve *Corbula gibba* was the only species that survived. This opportunistic bivalve is very common in unstable environments (e.g. polluted bays and harbours) and colonizes defaunated communities. They are very tolerant to anthropogenic and natural disturbances and also known as bioindicators of pollution in benthic communities. They survive hypoxic events by closing their valves

hermetically and switching between aerobic and anaerobic metabolism by increasing the lactate concentration in the tissue (reviewed by Hrs-Brenko 2006). In contrast to this bivalve, crustaceans have been repeatedly described as very sensitive taxa (Vaquer-Sunyer and Duarte 2008). The bioherm associated crustaceans in experiment 12 were treated in Haselmair (2008). Vaquer-Sunyer and Duarte (2008) reported that crustaceans showed the highest  $LC_{50}$  and the lowest  $LT_{50}$ , and Riedel et al. (2008) described certain species (*Hexaplex trunculus*, *Macropodia* sp.) leaving their hiding places already at beginning hypoxia.

Differences between the hypoxia tolerance of juveniles and adults are known for the infaunal sea urchin *Schizaster canaliferus* (Shumway et al. 1983). Although both juveniles emerged later on the sediment, they were the first to die. Generally, juveniles are more susceptible to sulphide due to the lower respiratory demand, but more tolerant to low oxygen (Shumway et al. 1983; Tyson and Pearson 1991).

As shown by Nilsson and Rosenberg (1994), the composition of benthic communities can change during hypoxia, and survival is significantly affected in the step between moderate and severe hypoxia. Sulphide production after anoxia leads to a reduced survival in macrobenthic communities of about 20 % in most species (Theede et al. 1969) because its toxicity reduces the tolerance to oxygen deficiency (Hagerman 1998).

Whereas death is very rapid, re-colonization of collapsed marine ecosystems in the northern Adriatic Sea is a long-term process that takes years or perhaps decades (Stachowitsch 1992; Stachowitsch and Fuchs 1995). After a mass mortality the original benthic composition is no longer present. Often, only shells of gastropods and bivalves or the tests of sea urchins remain on the sediment surface. These then serve as a nucleus for the settlement of new larvae (Stachowitsch 1991; Kollmann and Stachowitsch 2001). Three years after an oxygen crisis in the northern Adriatic, Kollmann and Stachowitsch (2001) observed that the biomass was 50% of previous levels and that it was dominated by sessile (serpulid polychaetes) and rapidly growing species (certain ascidians). Further community development was then interrupted by a series of other disturbances (fisheries) and by another mass mortality in 1988.

The suspension-feeding community in the northern Adriatic Sea is not a short-term phenomenon (Fedra et al. 1976). However, oxygen crises eliminate large and long-lived species, and the populations shift toward smaller species, which are more short-

lived (Diaz and Rosenberg 1995). In general these smaller species are less capable of bringing oxygen downward into the sediments. Thus, the change in community structure goes hand in hand with a change in function (Karlson et al. 2002). This makes the community more susceptible to renewed or persistent hypoxia (Conley et al. 2009a). If human inputs such as fertilizers etc. are not reduced, then the number of oxygen crises is expected to increase, especially in the northern and western parts of the Adriatic Sea (Justic et al. 1987).

First, EAGU successfully induced decreasing oxygen values and increasing hydrogen sulphide value after anoxia. Second, the responses of infaunal as well as epifaunal organisms to hypoxia and anoxia are similar to those in "natural" oxygen crises (Stachowitsch 1984, 1986 and 1992). Third, the sequence of atypical behaviours and mortality is correlated to decreasing oxygen and anoxia. This is an important step forward in compiling (1) a generally valid catalogue of behaviours, (2) a list of sensitive and tolerant species and (3) a range of community compositions. This will allow conclusions to be drawn on the status and stability *in situ* of marine ecosystems here and elsewhere.

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## 6. Abstract

Hypoxia and anoxia are key threats to shallow coastal ecosystems worldwide, and the northern Adriatic Sea is a case study for such sensitive seas. Benthic community collapse during low dissolved oxygen (DO) events is not a gradual process, but involves a series of sudden steps. Using a new underwater device, the Experimental Anoxia Generating Unit (EAGU), equipped with time-lapse camera and sensor equipment, we artificially induced anoxia in a sublittoral macrobenthic community in 24 m depth in the northern Adriatic Sea. The deployment shows that the oxygen levels beginning ( $\leq 2 \text{ ml l}^{-1}$  DO), moderate ( $\leq 1 \text{ ml l}^{-1}$  DO), severe hypoxia ( $\leq 0.5 \text{ ml l}^{-1}$  DO) and anoxia ( $0 \text{ ml l}^{-1}$  DO) cause a series of different atypical behaviours and lead to mortality. Under beginning hypoxia the hermit crabs *Paguristes eremita* left their hiding places under multi-species clumps and moved around actively. Moderate hypoxia caused the emergence from the sediment of polychaetes and the infaunal sea urchin *Schizaster canaliferus*. At severe hypoxia the epifaunal sea urchin *Psammechinus microtuberculatus* discarded its camouflage and the first mortality in the brittle star *Ophiothrix quinquemaculata* occurred. In the present deployment, anoxia caused mortality in all organisms except the bivalve *Corbula gibba*. Whereas some behaviours are reversible, mortalities lead to long-term shifts in the benthic community and thereby alter the whole ecosystem.

The observations in the evaluated deployment are a step forward in compiling a generally valid catalogue of behaviours, a list of sensitive and tolerant species, and a range of potential community compositions. This can help to determine the status and stability of such benthic ecosystems *in situ*.

## 7. Zusammenfassung

Hypoxien und Anoxien in seichten Küstengewässern stellen ein weltweites Problem dar (Diaz und Rosenberg 2008). Wenn der Anteil an gelöstem Sauerstoff im Wasser unter  $2 \text{ ml l}^{-1}$  (Hypoxie) sinkt oder ganz verschwindet (Anoxia) kommt es unter anderem zu Verhaltensveränderungen und physiologischen Anpassungen (Diaz und Rosenberg 1995; Vismann 1991, Mangum 1973), die es den Organismen erlauben kurzfristig Störungen zu überleben. Halten hypoxische und anoxische Bedingungen jedoch über einen längeren Zeitpunkt an, können großflächige Massensterben auftreten. Zwei Faktoren, die Schichtung der Wassersäule (Stratifizierung) sowie der Anstieg der Planktonbiomasse durch Eutrophierung, lassen die Anzahl der betroffenen Gebiete deutlich ansteigen. Über 400 so genannte "dead-zones" wurden bereits beschrieben. Ca. 50% davon sind einmal im Jahr hypoxisch, 17% weniger als einmal im Jahr, und 8% sind permanent hypoxisch (Diaz und Rosenberg 2008).

Die Nordadria ist wegen der geringen Tiefe ( $< 35 \text{ m}$ ), dem schlammigen Boden, dem hohen Süßwassereinstroms (v.a. durch den Fluss Po, Italien), der hohen Produktivität und der Schichtung der Wassersäule im Spätsommer, ein sehr empfindliches Ökosystem (Stachowitsch und Avcin 1987). Der Großteil der nördlichen Adria wird von macrobenthischen Bodengemeinschaften bedeckt. Im Golf von Trieste setzt sie sich Großteils aus dem Schwamm *Reniera* spp., dem Schlangensterne *Ophiothrix quinque maculata* und der Seescheide *Microcosmus sulcatus* zusammen, und wird deshalb auch die *Ophiothrix-Reniera-Microcosmus* Gemeinschaft (ORM-Gemeinschaft) genannt (Fedra et al. 1976). Diese filtrierende Gemeinschaft umfasst ca.  $370 \pm 73 \text{ g m}^{-2}$  Nassgewicht. Die filtrierenden Organismen können ca. 5% der pelagischen Biomasse pro Tag abbauen (Ott und Fedra 1977) und werden deshalb auch als Puffer oder "natürliche Eutrophierungskontrolle" bezeichnet (Officer et al. 1982).

Seit dem dramatischen Anstieg der Eutrophierung kommt es weltweit auch immer öfter zu solchen Sauerstoffkrisen (Gray et al. 2002). Die hohe Primärproduktion im Meer führt anfänglich zu einem Anstieg der Biomasse der Bodenorganismen. Im Frühling kommt es durch die Stratifizierung zu einer Trennung der oberen, sauerstoffreichen von der unteren, sauerstoffärmeren Wasserschicht. Die Bodenorganismen verbrauchen während des Sommers weiteren Sauerstoff und

verstärken so die hypoxischen Bedingungen. Im Winter kommt es dann zu einer Durchmischung der kompletten Wassersäule und die Bodenorganismen wachsen wieder. Eutrophierung verstärkt den Effekt der Stratifizierung und führt zur Bildung des so genannten Meeresschnees. Wenn sich dieser über der Sprungschicht ansammelt, wird er auch als "falscher Benthos" bezeichnet. Durch das Auflösen der Sprungschicht, sinkt dieser Meeresschnee zu Boden und bedeckt große Flächen der benthischen Lebensgemeinschaften, die dann aufgrund des Sauerstoffmangels sterben (Ott 1992; Ott und Stachowitsch 1992). Eine „natürliche“ Sauerstoffkrise im Jahr 1983 wurde von Stachowitsch (1984, 1986) photographisch dokumentiert.

Mit einem neuen Unterwassergerät, dem Experimental Anoxia Generating Unit (EAGU), das mit Foto- und Sensorausstattung ausgestattet ist, wurden in einer Tiefe von 24 m im Golf von Trieste *in situ* Hypoxien und Anoxien induziert. Mit den Fotos und den dazugehörigen Sauerstoffwerten war es möglich, das Auftreten atypischer Verhaltensweisen bestimmter Sauerstoffgrenzwerten (beginnende Hypoxie:  $\leq 2 \text{ ml l}^{-1}$  DO; moderate Hypoxie:  $\leq 1 \text{ ml l}^{-1}$  DO; schwere Hypoxie:  $\leq 0.5 \text{ ml l}^{-1}$  DO sowie Anoxie:  $0 \text{ ml l}^{-1}$  DO) zuzuordnen. Während beginnender Hypoxie kommt zum Beispiel die infaunale Muschel *Corbula gibba* aus dem Sediment heraus und Einsiedlerkrebse (*Paguristes eremita*) zeigen einen deutlichen Anstieg an Lokomotion. Der Schlangensterne *Ophiothrix quinquemaculata* reagiert mit einer veränderten Armposition und beginnt entweder sich auf die Arme zu stellen oder sich eng an das Substrat zu ziehen. Die Seescheide *Microcosmus sulcatus* beginnt mit Körperkontraktionen und schließt währenddessen die beiden Siphonöffnungen. Unter moderater Hypoxie verlassen infaunale Polychaeten das Sediment. Sedimentbewegungen darauf hin deutet, dass der infaunale Seeigel *Schizaster canaliferus* bald an der Sedimentoberfläche erscheint. Sichtbar wird er (unter anderem auch der Schlangensterne *Ophiura* spp.) erst in der Kategorie "schwerer Hypoxie". Weiters lässt der epifaunale, reguläre Seeigel *Psammechinus microtuberculatus* seine Tarnung (z.B. Muschelstücke) los. Erste Mortalitäten (alle ausgewerteten Individuen der Art *O. quinquemaculata*) traten unter schwerer Hypoxie auf. Mit Ausnahme von *C. gibba* starben alle anderen Tiere bis zum Ende des Experimentes (Anoxie nach ~ 48 Stunden; H<sub>2</sub>S 17.9  $\mu\text{M}$ ).

Diese Arbeit, eingebettet in ein vom FWF gefördertes Projekt, ist ein erster Schritt um (1) einen Verhaltenskatalog unterschiedlicher Arten in Bezug zu verschiedenen Sauerstoffgrenzwerten zu erstellen, und (2) empfindliche und tolerante Arten für die Nordadria zu bestimmen um zukünftig *in situ* rasch den Status und die Stabilität des Ökosystems zu bestimmen.

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## Curriculum Vitae

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### Education and training

- 05/2008 – 03/2009 M.Sc. thesis at the Department of Marine Biology, University of Vienna.  
Thesis title: Reactions of a macrobenthic community to anoxia: behaviour and mortality sequences in a time-lapse camera experiment in the Gulf of Trieste. Part of the FWF-project P17655-B03. Supervisor: Doz. Dr. M. Stachowitsch, Advisor: Dr. B. Riedel, Ao. Prof. Dr. M. Zuschin, Univ.-Prof. Dr. Jörg Ott
- since 02/2007 Studies in biology/ecology with special emphasis on marine ecology at the University of Vienna
- 10/2003 – 02/2007 Baccalaureate of Ecology and Biodiversity at the University of Salzburg, Austria
- 10/1995 – 05/2003 Grammer school in Braunau am Inn (Upper Austria)
- 10/1989 – 07/1995 Elementary school in Braunau am Inn (Upper Austria)

### Marine field courses

- 2005 Marine biological field course on the Mediterranean fauna and flora; Centre for Marine Research Rovinj, Croatia.
- 2006 Marine ecology field course, Institute of Marine Biology, Isola del Giglio, Italy.

- 2007 Marine ecology practical course; underwater fieldwork with S.C.U.B.A. included, in STARESO, Calvi, Corsica, France.
- 2007 Coral reef course; underwater fieldwork with S.C.U.B.A. included, in Dahab, Egypt.
- 2007 Practical course for protection of sea turtles (*Caretta caretta*) in the Mediterranean sea, in Fethiye, Turkey.

### **Conferences**

- 2009 ASLO Aquatic Sciences Meeting, Nice, France, 25-30 January (talk) - Marine benthic communities: threshold in a non-linear response to hypoxia and anoxia.
- 2009 Conferences north American Paleontological Convention 2009 (poster): Hypoxia and anoxia in a modern "Paleozoic" benthic community from the northern Adriatic Sea.

### **Awards**

- 2008 Rupert-Riedl-Award, Haus des Meeres - Aqua Terra Zoo, Vienna, Austria

### **Personal skills**

- **Languages**

Mother tongue: German

Other languages: English – reading, writing and verbal skills: good

- **Computer skills**

MS-Office, Adobe Photoshop, ArcGIS, PAST

- **Additional qualifications**

Electron microscopy (REM and TEM)

Microbial ecology course (with F.I.S.H.)

S.C.U.B.A. (PADI AOWD) underwater photography and fieldwork

### **Working experience**

- since 08/2008 Zoo Vienna - Tiergarten Schönbrunn - "commented feedings"
- 2006 Tutor in a marine biology school course in Rovinj, Croatia.