



## Arm regeneration and spawning in the brittle star *Amphiura filiformis* (O.F. Müller) during hypoxia

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### Abstract

Arm regeneration and spawning in the brittle star *Amphiura filiformis* were studied in normoxic oxygen concentration  $> 8.5 \text{ mg O}_2 \text{ l}^{-1}$  (control) and in two levels of hypoxic oxygen concentrations (treatments),  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  and  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (29 and 19% oxygen saturation) in 'undisturbed' sediments. The treatments were chosen to simulate short term hypoxia (17 days) induced over a 2- month period. This study demonstrated reduced arm growth and disturbance of spawning in both hypoxic treatments compared with normoxia. Slowest regeneration of arms was observed at  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (65% arm regeneration compared to normoxia control). No difference in brittle star behaviour between treatments was observed by eye or in relation to redox potentials in the sediment. The results are discussed in relation to *Amphiura filiformis* production in Scandinavian waters, where seasonal oxygen depletion occurs in the bottom water in some areas. Estimates of possible biomass losses are calculated.

**Keywords:** Benthic production; Echinodermata; Eutrophication; Kattegat; Ophiuroidea; Oxygen depletion

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### 1. Introduction

Over the last decades increased input of nutrients to the pelagic system has altered the basis of the benthic ecosystem in large areas of the Skagerrak and the Kattegat, western Sweden (Baden et al., 1990a; Rosenberg et al., 1990). Large-scale reduction in benthic macrofauna abundance and biomass during seasonally dependent hypoxia or anoxia have been reported in the south-east part of Kattegat and the fjord systems of east Skagerrak (Josefson and Rosenberg, 1988; Rosenberg and Loo, 1988). Field studies in these areas and experimental studies from the Skagerrak have shown that oxygen concentrations less

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than  $1.0 \text{ mg O}_2 \text{ l}^{-1}$  prevailing for days to weeks, could significantly affect the structure of benthic communities (Rosenberg et al., 1992; Nilsson and Rosenberg, 1994). However, relatively recently, increased abundance and biomass of benthic macrofauna, in particular of the brittle star *Amphiura filiformis*, has been reported from well-ventilated sites of the Skagerrak. It has been suggested that this increase is related to eutrophication (Rosenberg et al., 1987; Josefson, 1990; Josefson and Jensen, 1992).

*Amphiura filiformis* is one of the dominant benthic species in the Kattegat-Skagerrak (Petersen and Boysen-Jensen, 1911; Josefson and Jensen, 1992). Arm regeneration contributes significantly to production of this ophiuroid (Sköld et al., 1994) and *Amphiura* spp. arms are important as food for demersal fishes (Duineveld and Noort, 1986; Mattson, 1992; Pihl, 1994) as well as for Norway lobsters *Nephrops norvegicus* (Baden et al., 1990b). A long-term change in the diet of these major large predators in the south-east Kattegat from hypoxia sensitive crustaceans towards more tolerant species, including *A. filiformis*, is described by Baden et al. (1990b) and Pihl (1994).

Arm regeneration in *A. filiformis* is described from the west coast of Ireland by Bowmer and Keegan (1983) where the mean annual regenerated arm biomass could be 25% of the total body weight. Regeneration rates in ophiuroids are sensitive to changing environmental conditions such as reduced salinity (Donachy and Watabe, 1986) and pollutants (Walsh et al., 1986). The amount of tissue being regenerated (e.g., number of arms) has also been shown to affect regeneration rates and energy allocation from the disk, including the gonads, towards regeneration of tissue (Salzwedel, 1974; Dobson et al., 1991). Somatic growth, measured as arm regeneration, seems therefore to be a relevant variable to study because of its sensitivity to stress, its coupling to higher trophic levels by arm nipping and its significant contribution to *A. filiformis* production.

In this experiment, we studied the effects of moderately lowered oxygen concentrations on arm regeneration, spawning and behaviour of *A. filiformis* in natural 'undisturbed' sediments. Hypoxic treatments ( $2.7 \text{ mg O}_2 \text{ l}^{-1}$  and  $1.8 \text{ mg O}_2 \text{ l}^{-1}$ ) and time of exposure were chosen to replicate the interval of late summer–autumn hypoxia which has been frequently recorded in the water below the halocline in the Kattegat since 1980 (Rosenberg and Loo, 1988).

## 2. Material and methods

### 2.1. Sampling

27 samples with relatively 'undisturbed' (i.e., not mixed due to sampling) sediment communities were collected on 9 May 1994 at a depth of 49 m with a modified frame-supported bottom HAPS corer ( $0.012 \text{ m}^2$ ; Kannevorff and Nicolaisen, 1973) at the mouth of the Gullmarsfjord, west Sweden. The HAPS cores were randomly divided into nine polyvinyl chloride (PVC) aquaria ( $56 \times 36 \times 40 \text{ cm}$ ), three in each. At time of collection, oxygen, temperature ( $T$ ) and salinity ( $S$ ) in the bottom water were  $8.5 \text{ mg O}_2 \text{ l}^{-1}$ ,  $5.6^\circ\text{C}$  and 33 psu, respectively. All PVC aquaria were placed randomly in a temperature controlled room and connected in a flow-through mode to unfiltered sea water ( $T = 10^\circ\text{C}$ ,  $S = 33 \text{ psu}$ ). The temperature was chosen to simulate autumn

conditions in the Kattegat where hypoxic conditions have been reported (Rosenberg et al., 1992).

*A. filiformis* specimens were collected with a box-core (0.1 m<sup>2</sup>) at a nearby station 2 days after the HAPS cores were taken. The brittle stars were gently rinsed with sea-water and picked out from the sediment avoiding the breakage of arms. Five adult specimens of *A. filiformis* (oral-width > 2.1 mm; Sköld et al., 1994) were added to each of the 27 HAPS cores. This density corresponds to 420 ind. m<sup>-2</sup> which falls within the range of densities of this species in the Kattegat (Petersen and Boysen-Jensen, 1911; Rosenberg and Loo, 1988). Before the brittle stars were added to the sediment in the HAPS cores, the first arm clockwise (oral side) to the madreporite was amputated between the 7–8 arm segment.

## 2.2. Experimental set-up

Three PVC aquaria were randomly selected for each of the three oxygen treatments consisting of > 8.5 mg O<sub>2</sub> l<sup>-1</sup>, 2.7 mg O<sub>2</sub> l<sup>-1</sup> and 1.8 mg O<sub>2</sub> l<sup>-1</sup>, referred to as normoxia, mild hypoxia and moderate hypoxia (Table 1). The added *A. filiformis* were acclimatized in the sediment for 7 days before oxygen concentrations were slowly lowered over 6 days to experimental conditions. The hypoxic treatments were chosen to simulate relatively slow decreasing oxygen levels and 17 days of hypoxia, followed by an exchange of water resulting in relatively rapid increase in oxygen levels. During the treatment period (17 days) the water was recirculated at 10 l min<sup>-1</sup> between the PVC aquaria and a separate header tank (110 l water volume; Fig. 1). 20% of the water was changed every week in all aquaria. After treatments, the experiment was continued in normoxic condition for 26 days, with the aquaria connected to the flow-through water system before termination. All brittle stars retrieved from the sediment in the cores were videotaped on the same day the experiment was terminated. Macrofaunal behaviour was observed every second day, and in more detail at least weekly. Dead, larger specimens of heart urchins *Echinocardium cordatum* Pennant and *Brissopsis lyrifera* Forbes were removed to avoid localised increased hydrogen sulphide concentrations in the aquaria.

Oxygen concentrations in all hypoxic aquaria were continuously controlled by oxygen

Table 1  
Treatment and experimental conditions during experiment Day 0 (17 May) to Day 58 (14 July)

Day	Flow system	Oxygen concentration		
		Normoxia	Mild hypoxia	Moderate hypoxia
0–7	Flow through	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>
8–13	Recirculation	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>	Decreasing	Decreasing
14–30	Recirculation	> 8.5 mg O <sub>2</sub> l <sup>-1</sup> 90% > 19 kPa > 0.27 mM	2.7 ± 0.4 mg O <sub>2</sub> l <sup>-1</sup> 29% 6.1 kPa 0.084 mM	1.8 ± 0.2 mg O <sub>2</sub> l <sup>-1</sup> 19% 4.1 kPa 0.056 mM
31–58	Flow through	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>	> 8.5 mg O <sub>2</sub> l <sup>-1</sup>

Mean oxygen concentrations during treatment time Day 14 to Day 30 in mg O<sub>2</sub> l<sup>-1</sup> ± SD (*n* = 17), air saturation, oxygen partial pressure and mM.

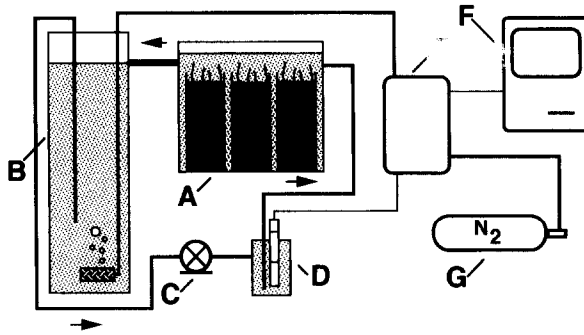


Fig. 1. Flow chart of experimental set-up of one aquarium during treatment (recirculation) period. Arrows indicate direction of water flow. (A) PVC aquaria with three HAPS cores, (B) header tank with bubbling stone, (C) water pump (Eheim 1048,  $10 \text{ l min}^{-1}$ ), (D) oxygen probe (Jenaway model 910) with measuring house, (E) box with magnetic valves and interface, (F) computer, (G) Nitrogen gas bottle.

meters (Jenaway, model 910) and logged every 15 min. Regulation and logging of oxygen concentrations were performed by a multi-channel computer system (LabView 2.2 for Macintosh computers) connected to oxygen meters and magnetic valves controlling bubbling of nitrogen in header tanks (Fig. 1). The oxygen meters were Winkler calibrated every second day (SIS, 1975). Header tanks in normoxic aquaria were continuously bubbled with air. The water surface in all aquaria was covered with a floating plastic cover (PVC aquaria) and plastic spheres (header tanks) to minimize diffusion of oxygen.

### 2.3. Measurements

The brittle stars were videotaped under a dissection microscope (Nikon SMZ-U) and regenerated arms (arm area and arm length) were measured by image analysis, using the software NIH Image 1.52 (Fig. 2). Individual gonad state was visually classified from

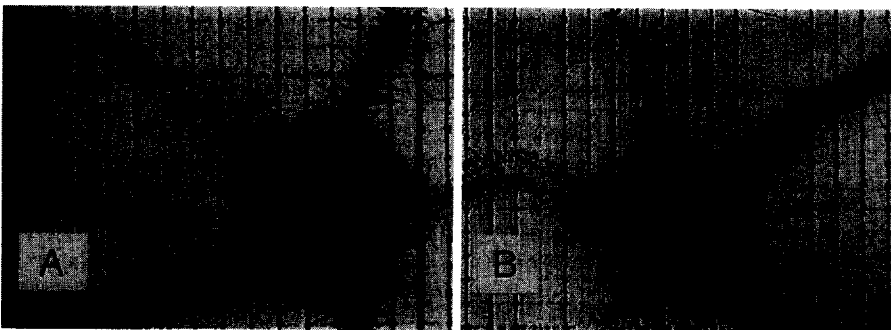


Fig. 2. (A) The video image of the oral side of an *Amphiura filiformis* with a regenerated arm (upper arrow) between arm segments 7 and 8 where the arm was cut at the start of the experiment. Example of specimen classified as having gonads in the analysis. The gonads are visible through the epidermis as white sacs (lower arrow). (B) The video image of the oral side of an *Amphiura filiformis* with a regenerated arm. Example of specimen classified as spent in the analysis. No gonads are visible and the disc appears hollow.

the videotapes of whole specimens under the dissection microscope. The experimental treatment was unknown at classification. If gonads were visible in the specimen, they were classified as having gonads (Fig. 2A). Individuals were considered to have spent their gonads (spawned) if their oral side appeared hollow and no gonads were visible (Fig. 2B). Spawning behaviour, however, was not observed. From this simple classification of spawning, recommended by Grant and Tyler (1983), the number of spent individuals from each aquarium was counted.

On three occasions (Days 0, 29 and 50), redox potentials were measured in six steps with a platinum electrode ( $\varnothing$  1.5 mm, Micro electrodes Inc. MI-800-412) from 0.5 cm above sediment surface down to 8 cm depth of the sediment. Calculations of redox potential (Eh) and 'area of redox profile' follow those outlined in Fenchel (1969) and Nilsson and Rosenberg (1994).

#### 2.4. Statistics

Analyses for differences in regenerated arm area and in 'area of redox profile' were performed with a nested one-way analysis of variance (ANOVA). Pooling of nested and treatment terms was done after the preliminary test if the nested factor was not significant ( $P > 0.25$ ) as recommended in Underwood (1981). Since not all brittle stars were retrieved in all cores with a regenerated arm tip intact, the number of brittle stars in the statistical analysis was balanced to  $n = 12$  in each aquaria. Differences in the number of spent animals in each aquarium were analyzed using a one-way ANOVA. Multiple comparisons of means were made with Student-Newman-Keuls test (SNK). Homogeneity of variances was examined using Cochran's  $C$  test (Snedecor and Cochran, 1967).

### 3. Results

#### 3.1. Redox potential

No significant differences in 'area of redox profile' was observed between any treatment or over time between reading 1 (DAY 0, pre-hypoxia) and reading 2 (Day 29, post-hypoxia) (ANOVA,  $P > 0.05$ , degrees of freedom ( $df$ ) = 2, 24). Fig. 3 shows the mean redox potential + SE from 0.5 cm above the sediment surface down to 8.0 cm depth for all recordings. The redox potential was stable at all depths in all treatments between all recordings.

#### 3.2. Arm regeneration

The two hypoxic treatments differed significantly (ANOVA SNK-test,  $P < 0.05$ ) from the normoxic treatment and between each other (SNK-test,  $P < 0.05$ ) (Table 2). Table 3 shows the mean regenerated arm area and arm length after 58 days in the three treatments. In moderate hypoxia ( $1.8 \text{ mg O}_2 \text{ l}^{-1}$ ) the mean regenerated arm was reduced by 25% in length and 36% in area and in mild hypoxia ( $2.7 \text{ mg O}_2 \text{ l}^{-1}$ ) the mean

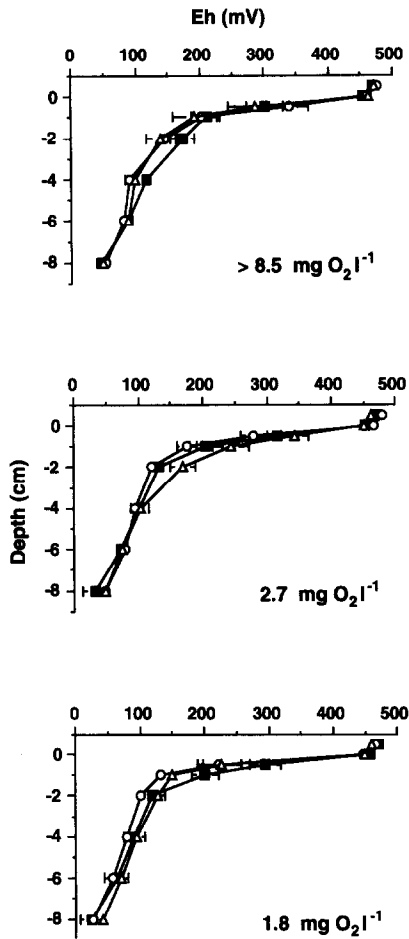


Fig. 3. Variation in mean redox potential  $\pm$  SE ( $n = 9$ ) with depth in the three treatments,  $> 8.5 \text{ mg O}_2 \text{ l}^{-1}$  (normoxia),  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  (moderate hypoxia) and  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (hypoxia). Three recordings are shown in each graph, Day 0 ( $\Delta$ ), Day 29 ( $\circ$ ) and Day 57 ( $\blacksquare$ ).

Table 2  
Statistical comparison of treatment effects on regenerated arm area

Source	<i>df</i>	Sum of squares	Mean square	<i>F</i> value	<i>P</i> value
One-factor nested ANOVA					
Treatment	2	17.855	8.927	9.775	0.0130
Aquaria (treatment)	6	5.480	0.913	1.244	0.2907
Residual	99	72.693	0.734		
One-factor pooled ANOVA					
Treatment	2	17.855	8.927	11.991	0.0001
Residual	105	78.173	0.745		

Table 3

Mean regenerated arm area  $\pm$  SD ( $n = 36$ ) and mean regenerated arm length  $\pm$  SD ( $n = 36$ ) after 58 days in the three treatments,  $>8.5 \text{ mg O}_2 \text{ l}^{-1}$  (normoxia),  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  (mild hypoxia),  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (moderate hypoxia)

Treatment	Arm area $\pm$ SD ( $\text{mm}^2$ )	Arm length $\pm$ SD (mm)
Normoxia	$2.8 \pm 1.0$	$10.4 \pm 2.8$
Mild hypoxia	$2.3 \pm 0.9$	$8.9 \pm 2.5$
Moderate hypoxia	$1.8 \pm 0.6$	$7.8 \pm 2.1$

regenerated arm was reduced by 14% in length and 21% in area, compared with the normoxic control.

### 3.3. Spawning

Treatment effects for the number of *A. filiformis* with no visible gonads (spent) at the end of the experiment are shown in Table 4. Multiple comparison (SNK-test), however failed to find differences because of overlap between groups. Fig. 4 shows the mean number of spent brittle stars in the treatments on 12 July, 58 days after the start of the experiment. In the normoxic treatment 42% had no visible gonads, in mild hypoxia (2.7

Table 4

Statistical comparison of treatment effects on 'spawning', defined as number of *Amphiura filiformis* without visible gonads in each aquarium

Source	df	Sum of squares	Mean square	F value	P value
One-factor ANOVA					
Treatment	2	0.103	0.052	5.742	0.0404
Residual	6	0.054	0.009		

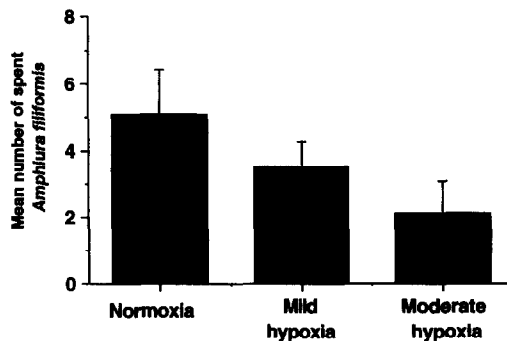


Fig. 4. Mean number of spent *Amphiura filiformis* + SD ( $n = 3$ ) of 12 analysed individuals in each aquarium at experimental Day 58 in the three treatments,  $>8.5 \text{ mg O}_2 \text{ l}^{-1}$  (normoxia),  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  (mild hypoxia) and  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (moderate hypoxia).

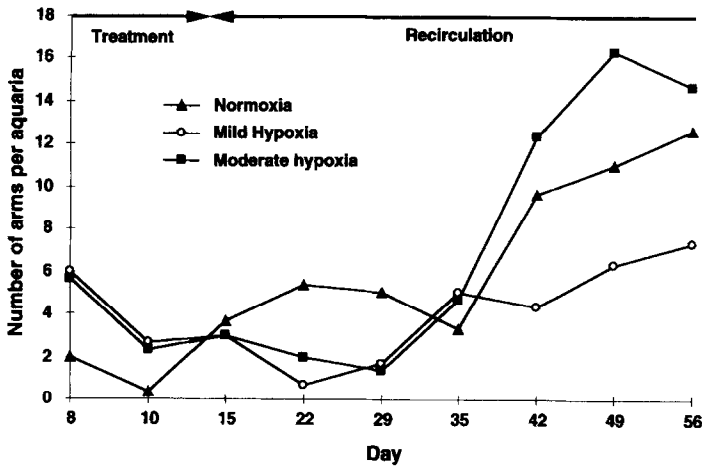


Fig. 5. Mean number of filter feeding arms (defined as arms raised up in the water column) in the three treatments,  $> 8.5 \text{ mg O}_2 \text{ l}^{-1}$  (normoxia),  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  (mild hypoxia) and  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  (moderate hypoxia) during the experiment.

$\text{mg O}_2 \text{ l}^{-1}$ ) 29%, and in moderate hypoxia ( $1.8 \text{ mg O}_2 \text{ l}^{-1}$ ) only 18% of the brittle stars had no visible gonads.

### 3.4. Macrofaunal activity

No mortality or escape behaviour of *A. filiformis* (i.e., disks above the sediment surface) was observed in either the normoxic or the hypoxic treatments. Filter feeding activity, defined as number of arms raised into the water column, increased in all treatments from a mean of 5 arms in each aquarium on Day 9, to 12 arms in each aquarium at the end of the experiment (Fig. 5). No significant differences were observed between the treatments.

With regard to the natural fauna in the HAPS cores, escape behaviour and mortality at the sediment surface were observed in a few individuals of the burrowing sea cucumber *Labidoplax buskii* McIntosh, and the heart urchins *Brissopsis lyrifera* and *Echinocardium cordatum* in both hypoxic treatments. In normoxia no escape behaviour or mortality was observed in any of these species.

## 4. Discussion

In earlier studies, threshold escape responses of *A. filiformis* have been recorded at oxygen concentrations just below  $1.0 \text{ mg O}_2 \text{ l}^{-1}$  with most of the specimens leaving the sediment during a few days of exposure (Rosenberg et al., 1991; Nilsson and Rosenberg, 1994). The present results confirm these studies in that no behavioural responses were observed in the hypoxic treatments ( $1.8$  and  $2.7 \text{ mg O}_2 \text{ l}^{-1}$ ) either as escape or altered

filter feeding. However, this study shows that these moderate hypoxic conditions do affect somatic growth and spawning.

The resolution between treatment effects and the accuracy in the analysis of experiments is related to the variation of the dependent variable and to repeatability. In arm regeneration studies of ophiuroids both weight and length measurements have been used (Salzwedel, 1974; Bourgoïn and Guillou, 1994). It is suggested that measuring regenerated arm areas by image analysis is a better method than weighing the regenerated arm, because it is not necessary to separate the regenerated arm. Separation of the regenerated arm can introduce a weight artifact due to the difficulty of amputating the arm at the precise point of regeneration. Since the regenerated arm is almost cylindrical or conical, the difference compared with weighing the regenerated part should be variation in density due to variation in  $\text{CaCO}_3$  and organic content as indicated by Dobson et al. (1991). However, in controlled experimental studies with no differences in organic input between treatments, this is not likely to be a determining factor. Regenerated arm area was decreased by a mean of 35% in moderate hypoxia compared with normoxia in this study, but regenerated arm length was only reduced by a mean of 25%. This indicates a secondary growth in arm thickness independent of arm length, and further suggests that regenerated arm area is a more relevant variable than arm length when studying treatment effects on arm regeneration. This suggestion is also supported by Salzwedel (1974) observations of arm regeneration in *A. filiformis*, where the arm initially grew in length and later increased in width.

Replicated microcosm experiments have lately been used in several studies and have been suggested as a bridge between field studies and experimental investigations (Bloesch, 1988). Since the effects of hypoxia on benthic infauna are strongly related to sediment chemistry and the three dimensional sediment habitat, the results of such studies are obviously related to the sediment condition and to the experimental design regarding temperature and water flow. Experimental studies (Nilsson and Rosenberg, 1994) and field studies (Rosenberg, 1977; Jørgensen, 1980) have shown a decreased redox potential in the sediments during hypoxia. In those studies, infauna have been observed escaping from the sediment during the hypoxic period, or were collected at lowered abundances after high mortality during the hypoxic event. Since all *A. filiformis* and almost all individuals of the resident fauna (except some Echinoidea and Holothuroidea) of the cores stayed in the sediment during the experiment in both hypoxic and normoxic aquaria, bioturbation and irrigation, measured as redox potential, varied only slightly, and are not considered to have had any major effects on the results. Sediment particle size, temperature, pH, bioturbation, irrigation, oxygen concentration in bottom water and organic content of sediment have been suggested to determine the redox potential in sediments (Fenchel, 1969; Vismann, 1991). Fenchel (1969) correlated redox measurements (Eh) to sulphide concentrations in both sandy and clay sediments and registered only very low levels of sulphide in sediments with redox potentials (Eh) above 0 mV. Since the redox potential was above +100 mV (Eh) down to 8 cm depth of the sediment, sulphide is not likely to be responsible for effects on brittle star arm regeneration and spawning disturbance in this experiment. The results of this study thus indicates that mild to moderate hypoxia ( $> 1.8 \text{ mg O}_2 \text{ l}^{-1}$ ) is not a sufficient factor to alter redox potentials in this sediment.

The capacity for anaerobic metabolism varies between species, but internal tissues of echinoderms rely to some extent on anaerobic respiration even under normoxic conditions (Shick, 1983). Whether oxygen deficiency in the near-bottom water leads to effects on the animals energy budget depends on whether or not they are capable of taking up oxygen more efficiently when levels are low (i.e., if they are regulators or conformers (Henze, 1910)), and their capacity for anaerobic metabolism. A shift from aerobic to anaerobic respiration based on observations of an alteration in sediment-water  $\text{CO}_2$  and  $\text{O}_2$  fluxes, has been suggested to occur in benthic communities during prolonged conditions at 20–30% oxygen saturation (Forster et al., 1995). Also, temperature alters critical oxygen levels since oxygen consumption is temperature dependent in most echinoderms (Lawrence, 1987). Fritzsche and von Oertzen (1995) observed an 1.5–2-fold increase of metabolic activity in two species of polychaetes (*Hediste diversicolor* and *Marenzelleria viridis*) when temperature was increased from 10 to 20°C. In the same paper they report a considerable decrease in survival during anoxia due to increased temperature. Decreased arm growth and disturbance in spawning in the present study occurred at 10°C, which is approximately the temperature when hypoxia is recorded in the Kattegat in late summer (Rosenberg et al., 1992). If the animals' growth is limited by food supply (i.e., their intake of energy) their growth also depends on oxygen availability since both regulating oxygen uptake and anaerobic respiration lead to higher energy costs (de Zwaan and Putzer, 1985; Baden et al., 1994).

Arm regeneration is a significant part of brittle star production, and regeneration rates are dependent on the number of body parts regenerating, indicating energy dependence (Salzwedel, 1974; Dobson et al., 1991). Regeneration rates for *A. filiformis* with only one arm amputated (0.31 mm day<sup>-1</sup>), Salzwedel, 1974 are similar to the controls (normoxia) of this study (0.28 mm day<sup>-1</sup>). In explaining decreased somatic growth, altered feeding behaviour seems less important for *A. filiformis* since no difference in the number of arms raised into the water column (assumed to indicate filter feeding activity) was observed between treatments. Possible explanations for the overall increase in the number of arms above the sediment surface over the experiment might be that feeding was reduced during the spawning phase as suggested by Thorson (1958), or that the brittle stars were not adjusted to the sediment conditions at the beginning of the experiment. However, a lack of, or reduced feeding activity and intake, in addition to lowered metabolic efficiency, may explain decreased somatic growth. Dobson et al. (1991) suggest for another amphiuroid brittle star that internal body reserves are used during early disk regeneration, which also indicates that energy allocation might be important early in arm regeneration. Reduced growth has also been found in hypoxic conditions (1.5 mg  $\text{O}_2 \text{l}^{-1}$ ) for juvenile oysters (Baker and Mann, 1992) and for polychaetes, *Capitella* sp. (Forbes and Lopez, 1990). The latter authors concluded that large worms would experience the greatest decline in growth rate due to preferential action of food or  $\text{O}_2$  depletion (< 25% oxygen saturation).

In moderate hypoxia (19%) a larger number of brittle stars had visible gonads at the end of the experiment (not spawned) compared with normoxia, which might be explained by delayed gonad development, due to effects on the metabolism, energy allocation to arm regeneration (see above), as well as disturbed spawning behaviour. Eriksson and Weeks (1994) investigated effects of hypoxia and copper on populations of

the amphipod *Corophium volutator*. They found higher mortalities in 29% and 19% oxygen saturation after 14 days treatment compared with the controls, but no difference in percentage egg bearing amphipods of survivors. *C. volutator* thus seems more acutely sensitive to low oxygen levels resulting in mortality, while *A. filiformis* survived, but showed sub-lethal effects when exposed to similar hypoxic conditions.

#### 4.1. Consequences for *Amphiura filiformis* populations and benthic communities exposed to low oxygen levels

Using estimates of reduced arm growth from this study and production estimates from Sköld et al. (1994), a rough estimation of the impact on arm regeneration of moderate hypoxia in the Kattegat can be made. Sköld et al. (1994) estimated total production to be  $9.46 \text{ (g DW) m}^{-2} \text{ year}^{-1}$  in *A. filiformis* at a site with a density of  $280 \text{ ind. m}^{-2}$  in the Skagerrak. Thirteen percent of this production was due to arm regeneration. In the Kattegat, large areas were exposed to oxygen concentrations below  $2.9 \text{ mg O}_2 \text{ l}^{-1}$  for 3 to 11 weeks yearly between 1980 and 1991 (Rosenberg and Loo, 1988; Rosenberg et al., 1992; Fig. 6). In September 1988 the hypoxic ( $< 2.9 \text{ mg O}_2 \text{ l}^{-1}$ ) area covered about  $5000 \text{ km}^2$  of the Kattegat, and in the central area, which covered about  $2000 \text{ km}^2$ , high mortality and loss of macrofaunal biomass including *A. filiformis* was observed at oxygen concentrations below  $1.4 \text{ mg O}_2 \text{ l}^{-1}$ . The estimated loss of *A. filiformis* biomass due to decreased arm regeneration measured as arm length, in the remaining moderately hypoxic area ( $3000 \text{ km}^2$ ;  $< 2.9 \text{ mg O}_2 \text{ l}^{-1} > 1.4 \text{ mg O}_2 \text{ l}^{-1}$ ) of the Kattegat in 1988 is calculated to be 81 or 145 metric tons dry weight assuming a density of  $280 \text{ ind. m}^{-2}$ . This is based on the results of this experiment, with effects on arm regeneration due to the mild  $2.7 \text{ mg O}_2 \text{ l}^{-1}$  and moderate  $1.8 \text{ mg O}_2 \text{ l}^{-1}$  hypoxic exposure of 17 days. Total loss of production may be much higher since gonad production, representing 69% of total production (Sköld et al., 1994), may also be reduced.

## 5. Conclusions

This study showed that arm regeneration is very sensitive to slightly lowered oxygen levels in the near bottom water. A generalized summary of the sensitivity of *A. filiformis* to hypoxia, based on previous studies by Rosenberg et al. (1991), Nilsson and Rosenberg (1994) and this study, is compiled in Fig. 7. These critical values of oxygen concentrations vary according to time of exposure, temperature and sediment conditions. Both hypoxic treatments in this study could be considered as very gentle treatments compared with earlier hypoxia studies in terms both of oxygen concentrations and the time of recovery after the treatment period (Rosenberg et al., 1991; Nilsson and Rosenberg, 1994). The decreased arm regeneration in this experiment suggests that periods of 2–3 weeks in moderate hypoxia can significantly alter benthic production. This effect of decreased benthic production can hardly be observed in traditional long-term field studies, and thus indicates the usefulness of such an experimental approach in complementing field studies.

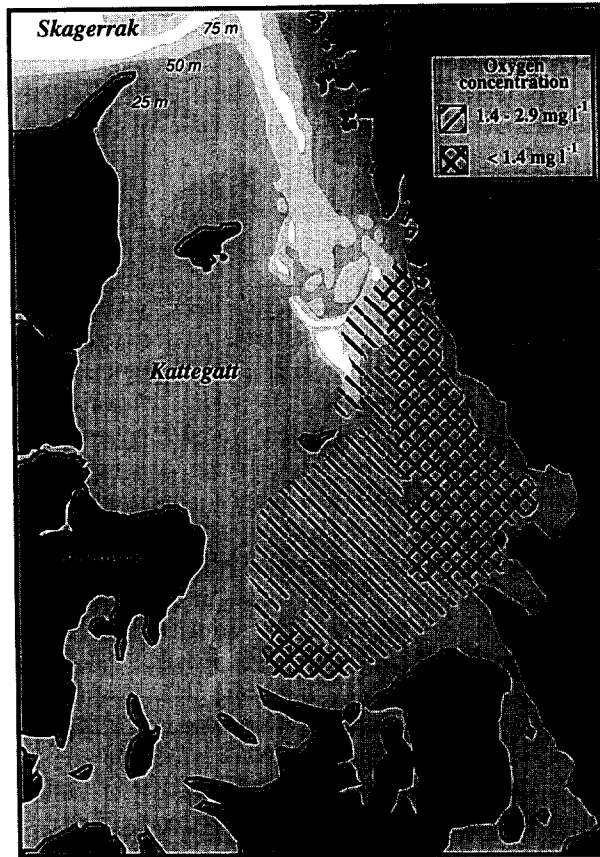


Fig. 6. Bottom water oxygen concentrations in the Kattegat in 1988 modified from Rosenberg et al. (1992).

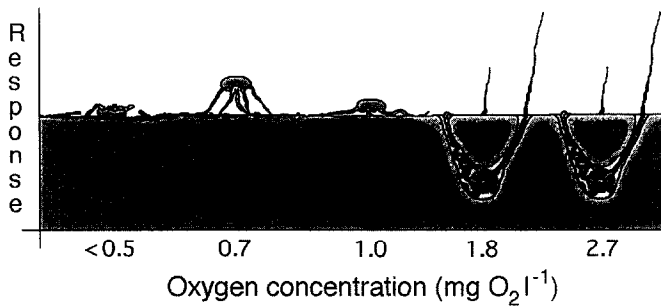


Fig. 7. Summary of *Amphiura filiformis* response to decreasing bottom water oxygen concentrations. 2.7 mg O<sub>2</sub> l<sup>-1</sup>, decreased somatic growth; 1.8 mg O<sub>2</sub> l<sup>-1</sup>, decreased somatic growth and spawning disturbed; 1.0 mg O<sub>2</sub> l<sup>-1</sup>, emerging from burrow; 0.7 mg O<sub>2</sub> l<sup>-1</sup>, standing on arm-tips; < 0.5 mg O<sub>2</sub> l<sup>-1</sup>, fragmentation of arms, increased mortality (Rosenberg et al., 1991; Nilsson and Rosenberg, 1994; this study).

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