

Somatic and germinal growth of the infaunal brittle stars *Amphiura filiformis* and *A. chiajei* in response to organic enrichment

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ABSTRACT: The effects of a short-term organic enrichment on growth of the 2 brittle stars *Amphiura filiformis* (O. F. Müller) and *Amphiura chiajei* (Forbes) were studied in undisturbed sediment cores obtained from a coastal and an offshore site of the Kattegat and Skagerrak, and then maintained in the laboratory as microcosms. Growth was measured as arm regeneration (somatic growth) and oocyte size (germinal growth). Macrofaunal abundance and biomass were higher in the offshore sediment compared to the coastal site. Organic enrichment was studied by adding concentrated phytoplankton (diatoms) to mimic the sedimentation pulse of a spring bloom in terms of quality and quantity. The experiment was run for 2.5 mo (April to June 1993). Somatic growth of *A. filiformis* increased in response to the phytoplankton addition in the coastal site sediment but not in the offshore site sediment. Arm regeneration in *A. chiajei* increased in both sediments in response to the food pulse. A positive correlation between regenerated arm length and oocyte size was obtained for both species. The results show that the brittle stars were capable of increasing their growth and gonad development in response to the short-term organic enrichment. The difference between sites also suggests negatively density dependent growth indicative of space or food competition in the densely populated offshore sediment.

KEYWORDS: Regeneration · Oocyte size · Echinodermata · Ophiuroidea · Eutrophication · Kattegat · Skagerrak · Microcosm

INTRODUCTION

The food supply to benthic organisms relies on the sedimentation of organic matter that is produced in the euphotic zone by primary producers. The seasonal cycle of phytoplankton production and sedimentation in the Kattegat-Skagerrak shows peaks in sedimentation of primary organic material during spring and autumn blooms and low inputs to the sediment during the rest of the year (Smetacek 1980). The most important energy input to the benthos occurs shortly after the spring bloom, when large amounts of diatom cells sediment out as aggregates (Smetacek & Hendrikson 1985, Kjørboe et al. 1994). Diatoms have a high nutritive value due to a well-balanced content of essential

fatty acids and amino acids (Tenore 1988). Sedimentation of the spring bloom takes only a few days creating a tight coupling between the pelagic and benthic systems (Graf et al. 1982, Davies & Payne 1984). The spring pulse of organic matter thus constitutes a labile, immediately available and highly nutritive food source for the benthos (Balzer 1984, Henrichs & Doyle 1986, Lopez & Levinton 1987, Marsh & Tenore 1990).

An essential question is how benthic organisms respond to the seasonal fluxes of organic matter sedimentation. Several field studies have focused on the importance for the benthos of this short-term organic enrichment. Following sedimentation events in the Kiel Bight, Graf et al. (1982, 1983) observed an increase in bacterial activity and meiofaunal biomass, but no convincing effects on macrofauna. In bivalves, an increase in somatic growth and gonad development has been directly related to seasonal fluctuations in

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food availability (Ansell 1974, Ankar 1980). Sedimentation from the spring bloom was found to be the main regulating factor for the somatic growth and development of reproductive tissue of benthic macrofauna in the northern Øresund (Christensen & Kanneworff 1985, Kanneworff & Christensen 1986, Nicolaisen & Christensen 1986).

Field observations of short-term sedimentation events and their benthic-pelagic coupling are complicated by the overwhelming role of the hydrography. Metabolic processes such as growth rate also depend on temperature; however, several studies have shown that food supply clearly dominated temperature effects on metabolism (Christensen & Kanneworff 1985, Graf 1992). In experimental studies with undisturbed sediments a known quantity and quality of organic matter is supplied to natural benthic assemblages and then the effects of the added organic matter can be evaluated without the influences of hydrography and temperature (Kelly & Nixon 1984, Grassle et al. 1985, Graf 1987, Duineveld et al. 1991, 1992, van Duyl et al. 1992, Webb & Montagna 1993, Osinga et al. 1995). Processes such as nutrient fluxes, oxygen consumption and microbial abundance have, however, been more studied than the effects on macrofaunal growth.

The Kattegat and the Skagerrak have during the last decades experienced increased anthropogenic inputs of nutrients (Rosenberg et al. 1990), and primary production is estimated to have increased from less than 100 to about 200 g C m⁻² yr⁻¹ since the 1950s (Richardson & Heilmann 1995). In areas where the water mixing is efficient, an increase of benthic macrofaunal abundance and biomass, especially of the dominant brittle star *Amphiura filiformis*, has been observed and correlated to this eutrophication (Pearson et al. 1985, Pearson & Rosenberg 1987, Josefson 1990, Josefson et al. 1993). *A. filiformis* populations are known to be stable over several years in density and size structure and to have low rates of recruitment (O'Connor et al. 1983, Duineveld & Noort 1986). However, it is not known how brittle star populations respond to short-term sedimentation events of highly nutritive organic matter, such as after the spring bloom. Increase in biomass of long-lived species can only be correlated to increased food input if food is a limiting resource and if no density-independent factors such as predation regulate population size and biomass. Harris (1989) showed theoretically that intraspecific injury models can lead to population stability provided that survival or reproduction is affected. Sub-lethal predation on *A. filiformis* is indeed high (Bowmer & Keegan 1983, Duineveld & Noort 1986, Munday 1993, Sköld & Rosenberg 1996), suggesting that changes in predation intensity may also be a factor contributing to the explanation of increased biomasses.

The aim of this study was to investigate if short-term organic enrichment, of the same magnitude and quality as a spring bloom, stimulates growth of the common infaunal brittle stars *Amphiura filiformis* (O. F. Müller) and *Amphiura chiajei* (Forbes), the latter being a strict deposit feeder, while *A. filiformis* can switch from deposit to suspension feeding (Buchanan 1964). We also compared the growth response of these brittle stars in undisturbed sediment cores from 2 sites of the Skagerrak-Kattegat differing in macrofaunal abundance and biomass.

MATERIAL AND METHODS

Site descriptions and sampling. Coastal site sediments were collected on the Swedish west coast (57°40.31' N, 11°41.39' E, Fig. 1) at 21 m depth on March 9, 1993. The offshore site sediments were collected in the south east Skagerrak (57°57.40' N, 11°09.00' E, Fig. 1), at a depth of 90 m on March 11, 1993. Eight sediment cores were taken from each site with a modified USNEL box-corer (0.25 m²), with a fitted internal plexiglass liner to provide undisturbed sediments according to the method of Berge et al. (1986). The box-cores were handled cautiously to minimise resuspension and aerated during transport

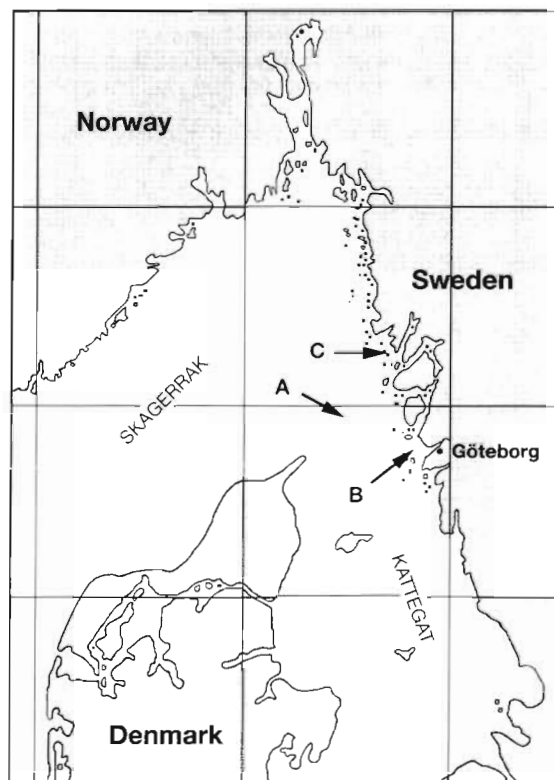


Fig. 1. Sampling sites: (A) Offshore site. (B) coastal site and (C) site where brittle stars added to the experiment were collected

to the Marine Research Station Solbergstrand, Norwegian Institute for Water Research, where the experiment was carried out.

Three box-cores from each site were collected as above to determine macrofaunal abundance and biomass. The samples were sieved (1 mm mesh size), the macrofauna preserved in 70% ethanol, and animals later sorted at 6 times magnification. Total biomass was determined as ethanol wet weight including shells.

In order to avoid genetic differences and pre-adaptation to the sediment conditions, the brittle stars measured in the experiment (*Amphiura filiformis* and *Amphiura chiajei*) were collected at an independent site west of Gullmarsfjorden (58° 17.80' N, 11° 23.70' E) at a depth of 25 m on March 10, 1993. The ophiuroids were sampled with a box-corer (30 × 30 cm), immediately collected from the sediment cores by gentle rinsing to avoid breaking of arms and transported to the laboratory in aerated thermo-tanks.

Sediment characteristics, contaminants and toxicity. Three sub-samples of the surface sediment (0 to 2 cm) of each box-core were taken at the start of the experiment and kept frozen (−20°C) for analyses of total organic carbon (TOC), contaminants and toxicity. After freeze-drying and removal of inorganic carbon according to Hedges & Stern (1983), TOC ($n = 8$) was analysed with a Carlo Erba NA 1500 C/N analyser. Two different standardised toxicity tests were run on sediment samples from each site. Toxicity testing with *Nitocra spinipes* (96 h LC_{50} at a highest concentration of 3.2 g wet wt diluted to 10 ml) was performed according to Swedish standard (SIS 1985) and Dave & Nilsson (1994) on 3 replicate sediment samples from each site with 5 parallel runs. A Microtox® test was performed according to Brouwer et al. (1990) on 3 replicate sediment samples, with 2 parallel runs at 1.0 g wet wt per 9 ml 2% NaCl solution. Contaminants were analyzed

by gas chromatography according to the method of Brorström-Lundén (1995) on 1 sample from each site.

Experimental set-up. The 16 box-cores were placed in a darkened room and connected in a flow-through style to a header tank receiving unfiltered 40 m deep fjord water. Temperature (6.2 to 7.6°C) and salinity (34 psu) of the incoming water were continuously registered during the experiment (Fig. 2). A semi-laminar flow over the surface of the box-cores was created by leading the water under 2 horizontal bars placed 2.5 cm above the sediment as in Skei et al. (1987). Flow was adjusted by observing the fate of a tracer (milk) and flow rate was regulated to ca 1.2 l min^{−1}, creating a bottom current of ca 0.17 cm s^{−1}. Photographs of the surface and the upper sediment profile of each box-core were taken regularly in order to survey animal activity and to estimate the evolution of the sediment redox potential discontinuity (RPD) layer (Fenchel & Riedl 1970).

Addition of experimental brittle stars. Brittle stars were defined as adults by having an oral width > 2.1 mm (Sköld et al. 1994). An ocular micrometer was used to measure oral width to a precision of 0.1 mm according to the method of O'Connor et al. (1983). Before addition, the first arm clockwise to the madreporite of each animal was cut between the seventh and eighth arm segment counted from the mouth. *Amphiura filiformis* ($n = 5$) were added on March 26 and *Amphiura chiajei* ($n = 4$) on April 6, and the experiment terminated between June 8 and 13.

Organic enrichment. Organic enrichment was provided to 4 box-cores from each site by adding weekly batches of concentrated phytoplankton on 4 occasions. The first 3 pulses were taken from a concentrated mono-culture of the marine diatom *Phaeodactylum tricornutum* grown in a 600 l tank filled with filtered deep water and growth medium. The culture was left to sediment out and aggregated algae were collected

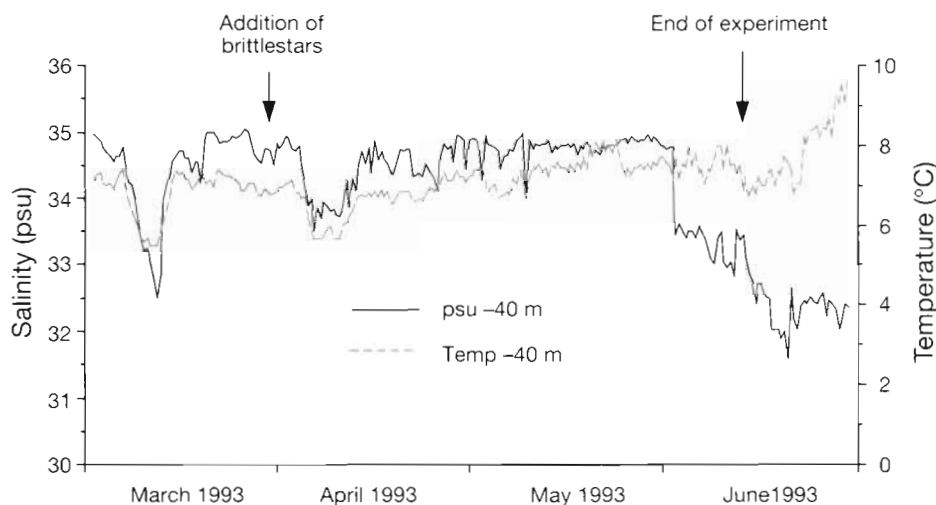


Fig. 2. Salinity (psu) and temperature (°C) of incoming Oslofjord water (−40 m) from March to June 1993

at the bottom. The fourth batch was collected in May by plankton haulings in the Oslofjord, mainly consisting of *Skeletonema costatum*, which were left to aggregate for 24 h. TOC and nitrogen content of each batch was determined by filtering 5 to 15 ml of the algae with GF/C filters. TOC and total nitrogen (TN) were measured as described above for the sediments. An average C:N ratio of 6.5 was obtained. The addition of organic material was performed by first stopping the water flow, then adding the concentrated suspension via the water inlet and letting it sediment out for 2 to 4 h. The amount of organic material leaving the box-cores when the water flow was reinstated was estimated by collecting 1 l of outflowing water in a time series (0, 3, 5, 8, 11 and 14 min) from each box-core. The water was filtered (GF/C filters) and particulate matter dry weights (held at 60°C till constant weight) were plotted against time. The time series integrated dry weights, with the normal dry weights of the outflowing water (sampled before organic addition) subtracted, were considered to represent the loss of organic material. Control levels of particulate matter of the incoming water were 1.01 (SD = 0.71) mg dry wt l⁻¹ and 0.36 (SD = 0.21) mg ash-free dry wt l⁻¹. Organic carbon was measured to ca 48% of the dry weight giving a mean loss of added organic material of 9.7%. The total concentration of organic material added to the treated box-cores (i.e. the sum of the 4 pulses) was estimated to be 16.8 g org C m⁻².

Measurements of arm regeneration and oocyte size.

At the end of the experiment, the macrofauna was sieved out (1 mm mesh size), all brittle stars were examined, and the 2 *Amphiura* species with a regenerated arm at the point of amputation were recorded alive by video and then preserved with 10% formalin buffered sea water. The experimental brittle stars were identified by size (i.e. oral width) and the position of the regenerated arm. Regenerated arm length and disk area were measured by image analysis (software package NIH Image 1.50). Oocyte area of *Amphiura filiformis* and *Amphiura chiajei* was measured by Confocal Laser Scanning Microscopy (CLSM) (Molecular Dynamics) with a Nikon Planapo 20/0.75 objective lens. Whole brittle stars were put in Herr's clearing fluid (Herr 1971, Fredriksson 1991) after being transferred through a gradient of alcohols (70, 95 and 99.5% ethanol). Herr's clearing fluid made the gonads transparent within 12 to 15 h. Gonads were then carefully dissected from the brittle stars, transferred to a cavity slide with some droplets of Herr's fluid, and a cover glass was added. Sex was determined and female gonads were sectioned 10 times in 5 µm steps. No staining was necessary since the oocytes displayed autofluorescence at a wavelength of 488 nm. Each oocyte was then examined and considered sectioned

through the middle when the maximum area, the nucleus and the nucleolus were displayed on the same section. Oocyte area was then measured by image analysis (software package Image Space by Molecular Dynamics) on 10 oocytes from 3 gonads of each individual. Stage of maturity was determined according to Fenaux (1970) and Bowmer (1982).

Statistics. Data were analysed by factorial analysis of variance (ANOVA) with regenerated arm length or disk area of the brittle star species as dependent variables. Site (coastal and offshore) and treatment (control and organic enrichment) were considered as fixed factors and were tested over the nested factor (aquarium) mean square. If the first order interaction was significant, multiple comparisons were performed using Student-Newman Keul's test (SNK-test) (Sokal & Rohlf 1995). To fulfill the criterion of homoscedasticity, disk area was corrected by $x^{1/2}$ transformations since the variance was approximately equal to the mean (Sokal & Rohlf 1995). All data were tested for presence of gross heterogeneity of variances with Cochran's C-test ($\alpha = 0.05$) (Snedecor & Cochran 1967).

As an unbalanced and small number of females of each species was recovered, no ANOVA was run on these data. Instead, correlations between mean oocyte diameter (calculated from the area) and arm length were performed on the females of *Amphiura filiformis* and *Amphiura chiajei*. Trends were considered significant when ($\alpha = 0.05$ for the product moment correlation coefficient r (Sokal & Rohlf 1995).

RESULTS

Sediment and macrofauna characteristics of the sampling sites

Macrofaunal densities and biomass were about 3 and 11 times higher in the offshore sediment in comparison to the coastal sediment, respectively. The most abundant species overall in both sediments was *Amphiura filiformis* with an exceptionally high density of about 3350 ind. m⁻² in the offshore compared to 360 ind. m⁻² in the coastal sediment (Table 1). Levels of contaminants and organic carbon did not vary considerably between sites. Neither the toxicity testing with *Nitocra spinipes* for the sediments nor the Microtox® test showed any significant toxicity or difference between sites.

Response to organic enrichment

Each addition of organic matter to the box-cores caused an immediate active response of the brittle stars. The organic material sedimented out into a fluffy layer

on the sediment surface, in which the brittle stars were seen sweeping and curling their arms around aggregates to bring them downwards to their mouth. After 3 to 4 h all algal material had disappeared from the surface of the offshore sediment, while brown patches of aggregates were still visible on the coastal sediment surface until the end of the experiment. Surface photographs of the box-cores revealed no differences in time, indicating that the benthic communities remained healthy throughout the experiment. No differences in RPD layer were observed from the photos of the aquaria walls, indicating that no deterioration in sediment oxidation potential due to organic enrichment or experimental conditions took place.

Difference between treatments and site

As significant interactions between treatment and site were found for arm length and disk size of *Amphiura filiformis* and disk size of *Amphiura chiajei* (Table 2a, b & d) comparisons were made on the sites separately. For the coastal site sediment both species showed an increase in disk area when dosed with organic enrichment compared to the control (SNK-test $p < 0.05$); however, the SNK test failed to detect any difference for arm length in *A. filiformis*, but the pattern was similar with an increased growth due to the treatment in the coastal site sediment (Fig. 3A). For the offshore site sediment no treatment effect was found either for disk size and arm length in *A. filiformis*, or for disk size in *A. chiajei*. Analyses of arm length of *A. chiajei* (Table 2c and Fig. 3C) showed increased arm length for the organic enrichment compared to the control, but no difference between sites.

Differences between *Amphiura filiformis* and *Amphiura chiajei*

Amphiura chiajei regenerated only ca 20% of the increase observed for *Amphiura filiformis* (Table 3). Maturation stage of oocyte development at the time of the experiment was also different between species. *A. filiformis* was in prematuration to shedding phases (Fig. 4A), while *A. chiajei* was in shedding to post shedding phases with 2 obvious size groups of eggs visible in most of the gonads (Fig. 4B). Because of the unbal-

Table 1. Compilation of biological and chemical characteristics at the coastal and offshore sites. Chemical units are based on sediment dry weight. Values within parentheses are standard deviation (SD)

Sediment variable	Coastal site	Offshore site	Unit
Biological			
Total abundance	1872 (169)	6576 (1091)	ind. m ⁻²
Polychaeta	469 (49)	832 (373)	
Mollusca	1019 (348)	2048 (331)	
Crustacea	11 (18)	37 (51)	
<i>Amphiura filiformis</i> abundance	357 (263)	3349 (507)	
<i>Amphiura chiajei</i> abundance	5 (9)	267 (196)	
Total biomass	44.9 (18.4)	517.9 (232.1)	g wet wt m ⁻²
Polychaeta	12.0 (12.8)	37.3 (16.1)	
Mollusca	3.4 (1.6)	18.8 (10.5)	
Crustacea	—	10.9 (18.8)	
<i>Amphiura filiformis</i> biomass	18.9 (14.0)	264.7 (109.7)	
<i>Amphiura chiajei</i> biomass	0.2 (0.3)	34.9 (21.3)	
Chemical			
Total PAH ^a	0.68	0.85	µg g ⁻¹
Total PCB ^b	4.2	3.5	ng g ⁻¹
HCB ^c	0.1	0.29	
p + p'-DDT + DDD + DDE ^d	0.34	1.1	
Methyl mercury	0.38	1.1	
Organic carbon	1.6	1.5	% dry wet
Sediment characteristics	Sandy clayey mud	Silty clayey mud	
Toxicity (<i>Nitocra spinipes</i>)	Not significant	Not significant	1/96 h LC ₅₀
Toxicity (Microtox [®])	No difference between sites		

^aPolycyclic aromatic hydrocarbons; ^bpolychlorinated biphenyls; ^chexachlor benzene; ^dsum of the pesticide DDT and its derivatives

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anced numbers of individual females of both species, and because of the 2 size classes found in *A. chiajei*, no ANOVA was performed on egg sizes. Instead trend analysis, with mean oocyte diameter (transformed from measured area) as the independent variable and regenerated arm length as the dependent variable, were performed for both species. Trends were positive ($n = 31$, $r = 0.53$, $p < 0.05$ for *A. filiformis*; $n = 26$, $r = 0.49$, $p < 0.05$ for *A. chiajei*) indicating that oocyte size had increased with organic enrichment.

DISCUSSION

Microcosms with undisturbed natural sediment cores offer an increased possibility in understanding how benthic fauna may react to environmental changes, including or excluding factors that can explain by themselves or in an interactive way biological variability. The generality of influences on growth by spatial variation in sediment type and quality was not tested in this experiment since the type of sites from which the sediments were collected was not randomly chosen and replicated. The factor treatment (control and organic enrichment) used here is easier to interpret since observed effects are dependent only on the phytoplankton addition.

Table 2. Factorial ANOVA with sites (coastal and offshore) and treatment (organic enrichment and control) as main effects and aquaria as nested factor; * $p < 0.05$; ** $p < 0.01$; ns: not significant

Source of variation	df	Sum of squares	Mean square	F	p	Error term
(a) Dependent: arm length of <i>Amphiura filiformis</i>						
Site	1	10.123	10.123	0.803	0.3877 ns	Aquarium
Treatment	1	7.275	7.275	0.577	0.4620 ns	Aquarium
Site \times Treatment	1	86.815	86.815	6.889	0.0222 *	Aquarium
Aquarium (Site, Treatment)	12	151.228	12.602	1.317	0.2529 ns	Residual
Residual	35	334.905	9.569			
(b) Dependent: $x^{1/2}$ transformed disk area of <i>Amphiura filiformis</i>						
Site	1	0.361	0.361	2.253	0.1592 ns	Aquarium
Treatment	1	0.851	0.851	5.303	0.0400 *	Aquarium
Site \times Treatment	1	2.046	2.046	12.757	0.0038 **	Aquarium
Aquarium (Site, Treatment)	12	1.925	0.16	0.633	0.8000 ns	Residual
Residual	35	8.874	0.254			
(c) Dependent: arm length of <i>Amphiura chiajei</i>						
Site	1	1.39	1.39	2.413	0.1463 ns	Aquarium
Treatment	1	5.603	5.603	9.727	0.0089 **	Aquarium
Site \times Treatment	1	0.004	0.004	0.007	0.9349 ns	Aquarium
Aquarium (Site, Treatment)	12	6.912	0.576	0.907	0.5475 ns	Residual
Residual	65	24.652	0.379			
(d) Dependent: $x^{1/2}$ transformed disk area of <i>Amphiura chiajei</i>						
Site	1	0.386	0.386	0.996	0.3379 ns	Aquarium
Treatment	1	1.722	1.722	4.446	0.0567 ns	Aquarium
Site \times Treatment	1	3.426	3.426	8.848	0.0116 *	Aquarium
Aquarium (Site, Treatment)	12	4.647	0.387	1.781	0.0836 ns	Residual
Residual	65	24.652	0.379			

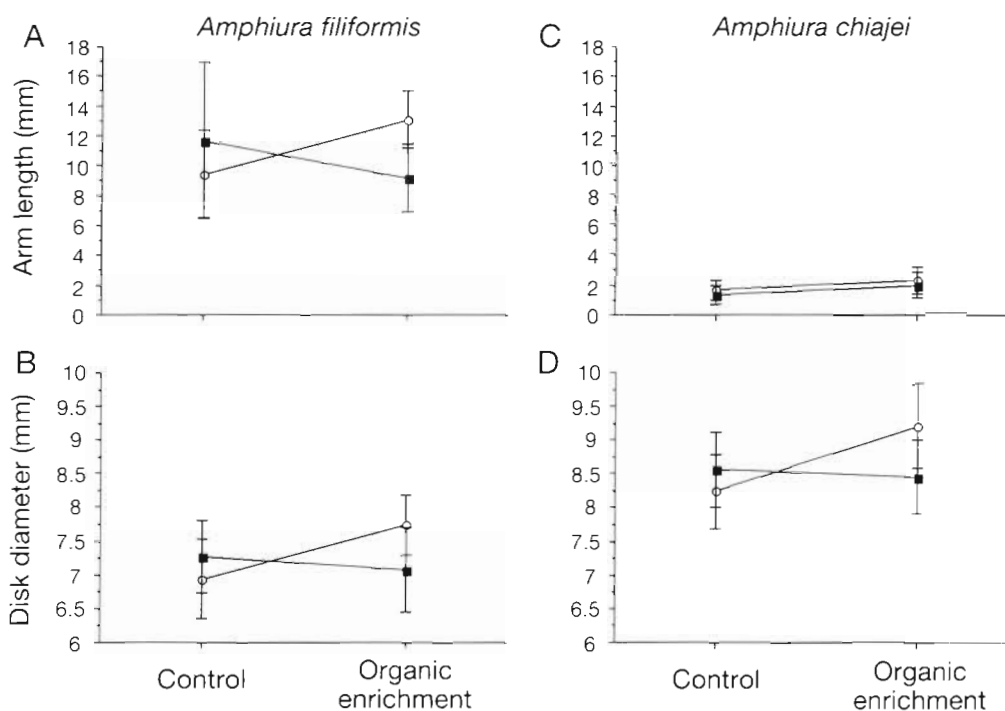
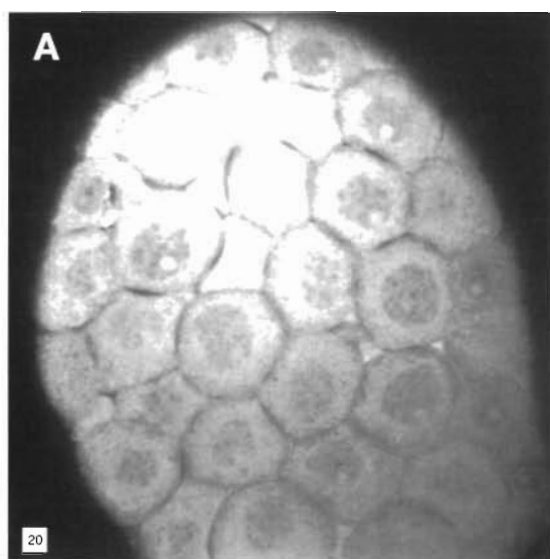


Fig. 3. Interaction plots of arm length and disk diameter (converted from measurements of disk area). (○) Coastal site; (■) offshore site. Error bars are standard deviation. (A) Arm length of *Amphiura filiformis*. (B) Disk diameter of *A. filiformis*. (C) Arm length of *A. chiajei*. (D) Disk diameter of *A. chiajei*.

Table 3. Mean regeneration and standard deviation (SD) in mm and dry weights. Dry weights (Y) are converted from arm length (X) by the biometrical relationship for regenerated *Amphiura filiformis* arms: $Y = 0.010564X^{1.2878}$ (Sköld et al. 1994). Regeneration rates are calculated by dividing regenerated arm lengths by 77 d for *A. filiformis* and 66 d for *Amphiura chiajei*. Organic enrichment: box-core treated with aggregated phytoplankton. Control: no organic enrichment

Site	Treatment	Number of individuals (n)	Mean regenerated arm length (mm)	SD (mm)	Regeneration rate (mm arm d ⁻¹)	Mean regenerated dry wt (g)
<i>Amphiura filiformis</i>						
Coastal	Control	15	9.4	2.9	0.12	0.19
Offshore	Control	11	11.7	1.9	0.15	0.25
Coastal	Organic enrichment	16	13.1	5.2	0.18	0.29
Offshore	Organic enrichment	9	9.2	2.3	0.12	0.18
<i>Amphiura chiajei</i>						
Coastal	Control	16	1.7	0.7	0.026	0.02
Offshore	Control	16	1.3	0.6	0.020	0.02
Coastal	Organic enrichment	11	2.3	0.9	0.035	0.03
Offshore	Organic enrichment	15	2.1	0.9	0.032	0.03



Differences between sites

The major difference between the 2 sediments was the striking difference in biomass and density of brittle stars. The dense population of *Amphiura filiformis* (3300 ind. m⁻²) at the offshore site is among the highest abundance observed in the Kattegat-Skagerrak. This site is situated on the western slope of a 90 m deep trench in the Skagerrak. Rosenberg (1995) has recently investigated this area and suggested that its extreme macrofaunal density is correlated to an important near-bottom particle transport, rich in organic matter, offering particularly favourable conditions for suspension feeders such as *A. filiformis*. The coastal site situated west of Göteborg is a shallower (21 m) estuarine site. Sediment toxicity and elevated levels of contaminants have been reported in this area (Dave & Dennegård 1994, Dave & Nilsson 1994). In this study, however, the contaminant content was not higher than at the offshore site and none of the toxicity tests performed showed any difference between the sites. In the controls, receiving only natural unfiltered sea water, both *Amphiura* species regenerated their arm, indicating that both species could either collect some food from the sediment and the overlying water, or allocate previous body reserves into arm regeneration. However, no difference in growth between sites was observed.

Fig. 4. (A) Confocal Laser Scanning Microscopy (CLSM) picture of female *Amphiura filiformis* gonad in prematuration stage. Section 5 μm thick. Scale quadrat in bottom left corner is 20 × 20 μm. (B) Female *Amphiura chiajei* gonad in post-shedding stage, with remaining large eggs from previous spawning. Section 5 μm thick. Scale quadrat in bottom left corner is 20 × 20 μm

Response to organic enrichment

The rate of food supply afforded by the external environment may limit growth. However, in excess of food, as during a spring bloom sedimentation, growth may still be rate-limited due to the benthic animals' food processing rate rather than supply (Jumars & Wheatcroft 1989). Increased growth was observed in the coastal site sediment in response to the organic enrichment. When dividing the organic matter input per *Amphiura filiformis*, the supply was ca 10 times higher in the coastal compared to the densely populated offshore sediment. The most probable explanation for the observed lower growth rate in the offshore site sediment when supplied with organic enrichment is a negatively density-dependent effect on growth indicative of strong competition for food in that sediment. A similar negatively density-dependent growth has also been observed in the facultative deposit feeding bivalve *Macoma balthica*, but not in the obligate suspension feeding *Cerastoderma edule* (Kamermans et al. 1992). Josefson (1995) estimated somatic growth rates of *A. filiformis*, from oral width, over a 143 d interval and found density-independent growth despite high densities at some of the sites investigated. However, as the density of natural populations of *A. filiformis* is likely structured by food supply (Pearson et al. 1985, Pearson & Rosenberg 1987, Josefson 1990, Josefson et al. 1993), the food supply per *A. filiformis* may not necessarily differ, which could explain why Josefson (1995) did not observe any density dependence.

Contrary to many previous organic enrichment experiments (e.g. Graf 1987, van Duyl et al. 1992, Osinga et al. 1995), the added organic matter was not frozen, crushed or heated but constituted of naturally aggregated diatoms. This was done to mimic natural conditions in which the spring phytoplankton bloom is often composed of diatoms, which settle rapidly as a pulse of aggregates onto the sediment surface (Hargrave 1980, Smetacek & Hendrikson 1985). This settling material has been shown to have a high nutritional value (Tenore 1988), reflected by a low C:N ratio, and to cause a rapid and short-lasting increase of benthic metabolic processes (Graf et al. 1983, Christensen & Kanneworff 1985, Graf 1987). Values of sedimenting organic carbon during a spring bloom in the Kattegat-Skagerrak are scarce. However, the amount of organic carbon added in the present study, 17 g C m^{-2} (68 d), is within the range of values of spring input from sediment trap and *in situ* measurements from 4.6 g C m^{-2} (25 d) in the northern North Sea (Davies & Payne 1984), 20 to 26 g C m^{-2} (55 d) in the southern Kattegat (Christensen & Kanneworff 1985), to 70 g C m^{-2} (50 d) in the western Kiel Bight (Graf et al. 1983). Benthic organisms have also been suggested to be nitrogen

limited rather than limited by the supply of organic carbon (Findlay 1982). The seasonal pattern of spring bursts in benthic metabolism may in fact be triggered by the quality of the organic supply, especially the presence of limiting micronutrients (essential amino acids and polyunsaturated fatty acids) in diatoms, rather than by the quantity of macronutrients (organic carbon and nitrogen) (Tenore 1988, Marsh & Tenore 1990). In this study, the response to the addition of diatoms was manifested in an increased activity of the 2 *Amphiura* species. The increased growth of arm length and disk size of *A. filiformis* and disk size of *A. chiajei* due to the organic enrichment in the coastal site (Fig. 3A, B and C) shows that this short-term organic enrichment can be rapidly converted into growth of the 2 *Amphiura* species. Oocyte size was also positively correlated with regenerated arm length for both species; this indicates that germinal growth followed changes in somatic growth and possibly also depended on food availability. The interactive growth patterns suggest density-dependent growth due to intraspecific competition with food as the limiting factor, which is also supported by the observed faster processing of the aggregated phytoplankton in the offshore sediment. Competition for space in the densely populated offshore site sediment may also have occurred; this in turn could have lead to sub-optimal feeding behaviour and possibly slower growth in this sediment.

Differences between species

Regenerated arm length of *Amphiura chiajei* increased in sediments from both sites when phytoplankton was given. The slower regeneration rate of *A. chiajei* compared to *A. filiformis* is supported by Buchanan (1964), who estimated growth rate, longevity and oxygen consumption of the 2 species. He found that *A. filiformis* exceeded *A. chiajei* in oxygen consumption by a factor of 5, and concluded that *A. filiformis* had a higher metabolic rate and food requirement than *A. chiajei*. As the added organic material in this study was allowed to sediment out and was available as deposited material for both species, the increase in growth rate in both species was due to efficient deposit feeding of the phytoplankton aggregates. However, suspension feeding may have contributed to maintenance metabolism in *A. filiformis*, as this species has the capacity of switching from deposit to suspension feeding (Buchanan 1964, Woodley 1975) and theoretically can balance growth and respiration by suspension feeding on available seston (Loo et al. 1996). The suspended matter in the unfiltered seawater in this experiment was probably available to *A. filiformis*, as raised arms into the water column, indica-

tive of filter feeding activity, were observed during the whole experiment in all treatments. The faster regeneration rate and metabolism of *A. filiformis* compared to *A. chiajei* may thus be attributed to a more efficient and competitive feeding strategy in *A. filiformis* compared to the obligate deposit feeding *A. chiajei*. Another possible explanation of the difference in regeneration rate between the 2 species could be that energy allocation is more important in early arm regeneration for *A. chiajei*, so that early regeneration may be independent of external food supply. This explanation is supported by Dobson et al. (1991), who, reporting about another amphiurid brittle star, found that internal reserves from the disk, oral frame region and distal arm regions of the body were used in early disk regeneration.

The regeneration rates of *Amphiura filiformis*, ranging from 2.9 to 3.8 $\mu\text{g dry wt arm}^{-1} \text{d}^{-1}$ in this experiment (Table 3) are within the range reported by Salzwedel (7.1; 1974), Andreasson (4.8; 1990), and Nilsson & Sköld (3.7; 1996), but lower than 82 $\mu\text{g dry wt arm}^{-1} \text{d}^{-1}$ reported by O'Connor et al. (1986).

Consequences of increased levels of organic matter to *Amphiura filiformis* populations

Amphiura filiformis is one of the dominant species below the halocline in the Kattegat where densities of around 100 to 400 ind. m^{-2} are common (Pearson et al. 1985). A doubling of primary production has occurred in the Kattegat since the 1950s (Richardson & Heilmann 1995). When food supply increases, *Amphiura* spp. populations can respond in alternative ways, e.g. by means of increased new recruits and growth; still the populations may be regulated by density independent factors such as predation. An increase in both somatic and germinal biomass was observed for both species in this study; however, the ultimate fate of this biomass increase on the population level is beyond the scope of this study. Nilsson & Sköld (1996) investigated effects of hypoxia on growth rates of *A. filiformis*. To estimate the yearly possible loss in production of regenerated arms due to the frequent autumnal hypoxia observed in the Kattegat they used a 3000 km^2 area, and 280 ind. m^{-2} as an approximate mean abundance of *A. filiformis*. Using similar figures of abundance and area, an estimate of the possible effect of an organic enrichment of 16.8 g C m^{-2} over 2.5 mo on arm growth of this hypothetical *A. filiformis* population in the Kattegat can be calculated. This gives an increased arm length of 39% compared to the controls in this experiment (calculated from Table 3). If the volume of an arm is considered to be proportional to its weight and to have the form of a cylinder, an increase in

length of the arm is directly proportional to the increase in volume and weight; thus the weight of the arm should increase by 39% due to the organic enrichment. Disk production, estimated by the disk volume increased about 12% in diameter (calculated from mean disk areas of *A. filiformis*). Analogously, the increase in volume of the *Amphiura* spp. disk, assuming it has the form of a cylinder, is directly proportional to the square of the diameter, thus 25%. Sköld et al. (1994) estimated total production to 9.46 g dry wt m^{-2} for a population of that density. Thirteen percent of that was due to arm regeneration and 69% due to disk growth. Combining these patterns of growth with effects on arm regeneration and disk growth due to the organic enrichment in this study increased growth in the Kattegat area (3000 km^2 ; 280 ind. m^{-2}) are estimated to 301 and 1041 metric tonnes due to arm regeneration and disk growth, respectively. A possible net result over a year of an increased spring bloom sedimentation (Richardson & Heilmann 1995), followed by autumn hypoxia (Rosenberg et al. 1992) in the Kattegat, can thus be estimated. Considering the increase due to arm regeneration (301 metric tonnes) and recalling the comparable loss estimated by Nilsson & Sköld (1996) (81 to 145 metric tonnes), increasing biomass of *A. filiformis* during spring bloom sedimentation probably stimulates secondary production more than autumn hypoxia (1.8 to 2.7 $\text{mg O}_2 \text{l}^{-1}$) reduces it, at least in terms of arm regeneration. Consequently, a positive correlation between primary production and adult biomasses of this species can be expected assuming other regulating factors, e.g. sub-lethal predation, to be constant. This is in accordance with the reports of increased growth patterns, abundance and biomass of *A. filiformis*, in some areas of the Kattegat and the Skagerrak (Pearson et al. 1985, Rosenberg et al. 1987, Josefson 1990, Josefson & Jensen 1991, 1992, Josefson et al. 1993). However, as evident from areas where mortality is high due to severe autumn hypoxia, this biostimulating effect of eutrophication may drastically switch to catastrophic events of high mortality if the community's increased O_2 demand is not compensated (Rosenberg & Loo 1988, Baden et al. 1990, Rosenberg et al. 1992).

CONCLUSION

This study indicates that a short-term organic enrichment, on the order of magnitude of and with the same quality as sedimenting matter during a spring bloom, significantly increases secondary production measured as arm regeneration, disk growth and oocyte development of dominant macrofauna, i.e. *Amphiura filiformis* and *Amphiura chiajei*. The amplitude of the

response of *A. filiformis* growth rate to an organic enrichment also seems to be correlated to abundance and biomass and suggests negatively density-dependent growth, indicative of food or space competition.

It was also found that the opportunistic feeding *Amphiura filiformis* has a higher regeneration rate compared to the strictly deposit feeding *Amphiura chiajei*.

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