

NOTE

Phototrophic and heterotrophic pico- and nanoplankton in anoxic depths of the central Baltic Sea

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ABSTRACT: An unexpected increase of phototrophic and heterotrophic pico- and nanoplankton under anoxic conditions was encountered in the central Baltic Sea below 120 m depth in August 1991 and 135 m depth in July 1992, respectively. Cell abundances in anoxic layers were up to 10 times higher for chroococcoid cyanobacteria, 6 to 8 times for phototrophic nanoflagellates and 3 to 4 times for bacteria, as compared to the overlaying aphotic oxic layers. A characteristic ciliate community occurred at the oxic/anoxic interface. Phototrophic organisms of anoxic depths were capable of productivity when reoxygenated and returned into the light. Considerable grazing on bacteria (0.41 to 0.65 d^{-1}) and chroococcoid cyanobacteria (1.19 to 1.29 d^{-1}) was revealed by serial dilution experiments. Cyanobacteria showed gross growth rates of 0.85 to 1.33 d^{-1} under aphotic/anoxic conditions. The origin of deep-water phototrophic pico- and nanoplankton and the existence of a permanent anaerobic microbial food web are discussed.

A number of investigations have emphasized the occurrence of procaryotic and eucaryotic pico- and nanoplankton in the oxic deep-sea (Hamilton et al. 1968, Fournier 1970, 1971, Silver & Alldredge 1981, Platt et al. 1983, Davis et al. 1985, Silver et al. 1986, Lochte & Turley 1988). Little information exists, however, on pico- and nanoplankton in anoxic depths. For near-shore areas, recent investigations revealed distinct populations at the oxic/anoxic interface and in anoxic near-bottom layers (Fenchel et al. 1990). Anoxic conditions are typical for the deep basins of the Baltic Sea (Matthäus 1986). The present paper describes our observations of the occurrence of phototrophic and heterotrophic pico- and nanoplankton in deep anoxic water layers of the Gotland Basin, central Baltic Sea. We dis-

cuss whether these unique biotas are of overall significance for the interpretation of the Baltic ecosystem.

Material and methods. Data presented were collected during RV 'Alkor' cruises BAMBI '91 and BAMBI '92 (Baltic Microbial Biology Investigation) in August 1991 (8 d drift study) and July 1992 (9 d drift study) in the eastern part of the Gotland Sea, central Baltic (Fig. 1).

Water samples from the surface to the bottom were taken with black PVC Niskin-type water bottles each morning. Temperature was recorded by a ME CTD probe and salinity was derived from salinometry of water cast samples. In 1992, *in situ* fluorescence and turbidity were recorded by a Backscatt fluorescence sensor mounted on the CTD probe. Oxygen concentrations were estimated by Winkler titration and H_2S concentrations as described by Grasshoff et al. (1983).

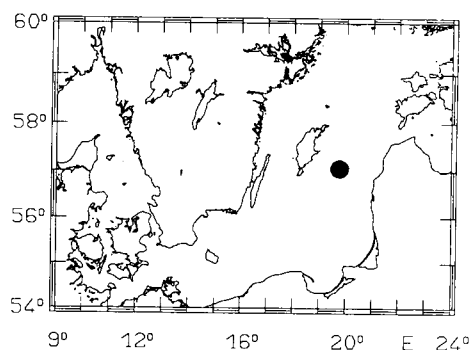


Fig 1. Location of drift studies during BAMBI '91 and BAMBI '92 in the central Baltic Sea at $57^{\circ}30'N$, $20^{\circ}19'E$

Abundances of pico- and nanoplankton were determined by epifluorescence and light microscopy. For epifluorescence microscopy, samples were fixed with glutaraldehyde (final concentration 0.3 %) and aliquots filtered onto Irgalan Black prestained Nuclepore filters of 0.2 μm pore size. Unstained chroococcoid cyanobacteria (Syn) and phototrophic flagellates (ANF) could easily be distinguished by their different autofluorescence, orange and red respectively, under blue-light excitation (450 to 490 nm). For bacteria, Acridine-orange staining (Zimmermann 1977, Porter & Feig 1980; 0.015 % solution) and for heterotrophic flagellates (HNF), Proflavine staining (Haas 1982) were applied. Filters were prepared within 6 h after sampling and stored frozen (-18°C). In order to prevent cell loss, stained preparates were counted within 3 wk (Turley & Hughes 1992). For phototrophic organisms and bacteria, about 200 cells were counted in a minimum of 20 fields, counting error thus being below 15 % (Venrick 1978). In the case of heterotrophic nanoflagellates, 30 to 100 cells were counted within 70 to 220 fields (counting error: 20 to 35 %). For the quantitative analysis of the abundance of ciliate species, samples were fixed in 5 % by volume Bouin's fixative and stained by the quantitative protargol method (Montagnes & Lynn 1987); 10 to 200 cells were counted.

To assess the physiological state of phototrophic pico- and nanoplankton in terms of productivity potential, the uptake of H^{14}CO_3 (Steeman Nielsen 1952) was measured in samples from anoxic depths (160 m on BAMBI '91, 170 m on BAMBI '92). Triplicate samples were incubated for 8 h in a deck incubator cooled by running surface seawater (18 to 20°C) under anoxic as well as oxygenated conditions achieved by 20 min air-bubbling prior to ^{14}C addition. Samples from 5 m depth or the surface were taken as references. After incubation, all samples were size-fractionated by filtering through 2.0 μm Nuclepore filters onto 0.2 μm membrane filters and measured in a Packard TriCarb liquid scintillation counter. Primary production of picoplankton was normalized to cellular production using cell numbers derived from epifluorescence microscopy. Measurements of CO_2 -dark fixation in the water column were performed during BAMBI '91. To follow the dark uptake of H^{14}CO_3 , 100 ml samples in duplicate and a formalin-killed sample which served as a blank were amended with 10 μCi $\text{NaH}^{14}\text{CO}_3$ and incubated at *in situ* temperature for 10 h. Contamination with air was avoided for samples from the oxic/anoxic interface.

To estimate the grazing impact on chroococcoid cyanobacteria and bacteria under oxic and anoxic conditions, serial dilution experiments (Landry & Hassett 1982) were conducted. Sample portions were 100, 75, 50, 33 and 20 %. In both years, samples were incubated for 24 h at *in situ* temperatures.

In 1991, samples were taken from 2 m and 160 m depth, prefiltered through a 100 μm net and incubated; no precautions were taken to avoid oxygen- and light-contamination during tapping in the case of the deep-water samples, which were incubated in the dark. Surface experiments were repeated 4 times with triplicate samples per dilution step, the deep experiment was performed once with duplicate samples. In 1992, 1 serial dilution experiment was performed under strictly anoxic conditions. Incubation bottles (100 ml) were sealed with silicon stoppers and flushed with nitrogen gas for 1 min. Prefiltered (0.2 μm) water from 170 m depth, dedicated for dilutions, was kept anoxic by addition of NaS as reductor and bubbling with nitrogen gas. Anoxia was monitored by addition of resazurine as oxygen indicator (colourless in the absence of oxygen; Süßmuth et al. 1987). Samples from 170 m depth were taken from water bottles by nitrogen-flushed glass syringes and injected into the anoxic dilution water through silicon stoppers, avoiding any contact with air. Cell numbers were estimated by epifluorescence microscopy before and after incubation.

Apparent growth rates ($\ln N_t/N_0$) were plotted versus dilution steps (not shown) and growth rates (μ) and grazing rates (g) were estimated by linear regression (Landry & Hassett 1982). The percentage of gross production grazed (% P_g) was calculated using the following equations:

$$\text{Gross production } P_g = \mu \times B^*; \quad (1)$$

$$\text{Mean biomass } B^* = (B_t - B_0) / \ln(B_t/B_0) \quad (2)$$

where B_0 = initial biomass; B_t = final biomass;

$$\text{Grazed biomass } G = g \times B^*; \quad (3)$$

$$\text{thus } \% P_g = G/P_g \times 100 = g/\mu \times 100 \quad (4)$$

Results and discussion. Typical profiles taken from 1 station, which were representative for all stations of each drift study, are shown in Figs. 2 & 3. In 1991, a strong thermocline occurred at 10 m depth (Fig. 2a). Below 60 m depth, a gradual increase in salinity was recorded. Below 120 m depth, no oxygen could be measured and H_2S concentration increased to ca 70 μM .

In the upper 50 m of the water column nutrient concentrations were low (Fig. 2b). Ammonia and phosphate concentrations increased markedly at the oxic/anoxic interface at 125 m depth (further also referred to as chemocline) whereas nitrate concentrations peaked between 80 and 100 m depth. Nitrite concentrations were low throughout the water column.

Pico- and nanoplankton consisted of chroococcoid cyanobacteria cf. *Synechococcus*, phototrophic flagellates, bacteria, heterotrophic nanoflagellates and ciliates. The increase of pico- and nanoplankton organ-

Fig. 2. Profiles of (a) temperature, salinity, O₂ and H₂S concentrations; (b) ammonia, phosphate, nitrate and nitrite concentrations; (c) abundances of *Synechococcus* (Syn.), photoautotrophic nanoflagellates (ANF) and bacteria (Bact.) and CO₂-dark fixation; (d) abundance of ciliates. All profiles are taken from the same station and are representative of all stations of the drift study 1991

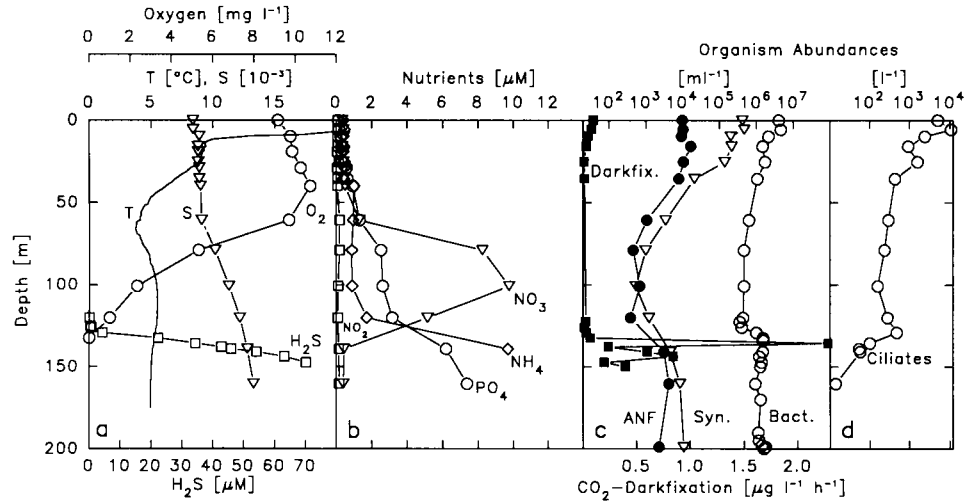
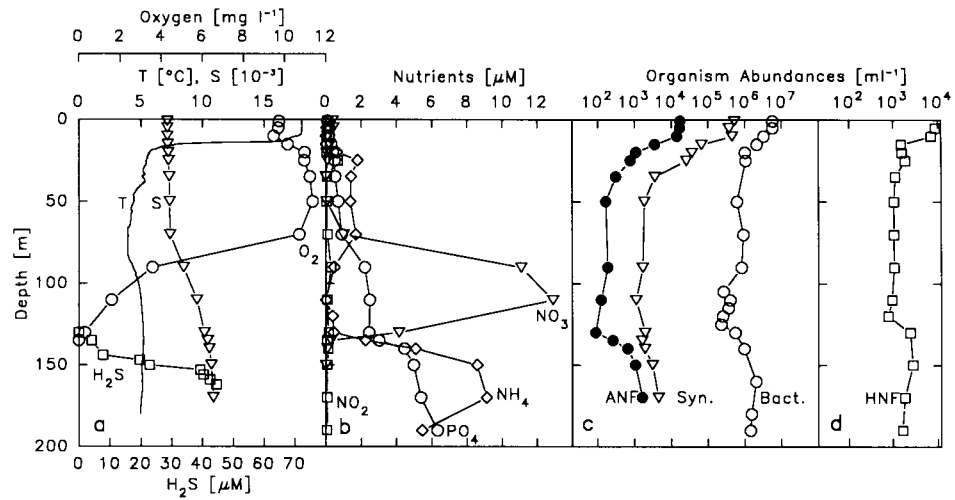


Fig. 3. Profiles of (a) temperature, salinity, O₂ and H₂S concentrations; (b) ammonia, phosphate, nitrate and nitrite concentrations; (c) abundances of *Synechococcus* (Syn.), photoautotrophic nanoflagellates (ANF) and bacteria (Bact.); (d) abundance of heterotrophic nanoflagellates (HNF). All profiles are taken from the same station and are representative of all stations of the drift study 1992



isms below 120 m depth was spatially related to the oxic/anoxic interface (Fig. 2c). Between 100 and 160 m depth, abundance of chroococcoid cyanobacteria increased from 1000 to 8000–10000 cells ml⁻¹, corresponding to 1 to 2 % of surface values. For phototrophic nanoflagellates, a similar increase from 500 to 3000–4000 cells ml⁻¹ was obvious, amounting to 50 % of values in upper layers. Bacterial numbers increased from 0.3 × 10⁶ to 1.3 × 10⁶ cells ml⁻¹, corresponding to 30 to 50 % of surface values. Below the chemocline, a shift in size structure was observed in favour of larger bacteria. Abundances of heterotrophic nanoflagellates amounted to 10 to 37 % of surface values (Trenkel 1992; data not shown). Ciliate abundance (Fig. 2d) increased from 160 to 480 cells l⁻¹, amounting to 10 % of surface values. Below 120 m depth, ciliate population structure completely differed from those in overlaying layers. As many as 18 to 20 species occurred in surface waters but only 4 to 5 different species were found at the oxic/anoxic interface, among them specimens of the genus *Metopus*.

CO₂-dark fixation (Fig. 2c) peaked sharply at the chemocline (2.2 μg C l⁻¹ h⁻¹). This corresponded to an average of 30 % of surface primary production. Values of CO₂-dark fixation below the chemocline were 700 % higher in anoxic than in oxic waters.

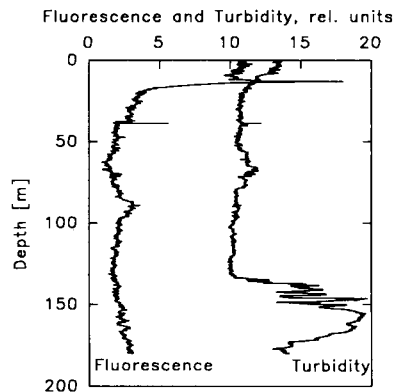


Fig. 4. Profiles of fluorescence and turbidity during cruise BAMBI '92. One profile which is representative for each station of the drift study 1992 is shown

Table 1. Primary production potential normalized to cell numbers of phototrophic picoplankton of samples from near-surface and anoxic depths under oxic and anoxic conditions. nd: not detectable

Depth (m)	Condition	Cellular primary production (fg C h ⁻¹ cell ⁻¹)
BAMBI '91 (incubated at 20 °C)		
5	Oxic	4.9
160	Oxic	39.5
160	Anoxic	nd
BAMBI '92 (incubated at 18 °C)		
1	Oxic	8.2
170	Oxic	24.5
170	Anoxic	nd

Hydrographic conditions during BAMBI '92 (Fig. 3a) were fairly similar to those in 1991. The chemocline was encountered at 135 m depth. The vertical distribution of nutrients (Fig. 3b) was comparable to that in 1991, as well. *In situ* fluorescence as an indicator of phototrophic biomass showed a slight increase in anoxic waters and turbidity peaked between 135 and 175 m depth (Fig. 4).

Typical profiles of abundances of *Synechococcus*, phototrophic nanoflagellates and bacteria (Fig. 3c) displayed both similar shapes and cell numbers as compared to the 1991 study. The increase of cell numbers in aphotic depths again coincided with the oxic/anoxic interface. Cell abundances in anoxic waters amounted to 1 % of surface values for *Synechococcus*, 10 % for phototrophic nanoflagellates, and 50 % for bacteria, thus comparable to the 1991 results. Abundance of heterotrophic flagellates increased within the chemocline, concentrations rose from 780 to 2400 cells ml⁻¹, amounting to 23 % of surface values. No data for abun-

dance of ciliates and CO₂-dark fixation are available for the 1992 study.

Primary productivity of phototrophic picoplankton was not detectable under anoxic conditions in both studies whereas reoxygenation of deep-water samples resulted in measurable activities (Table 1). Cellular primary production in reoxygenated deep water was higher than in oxic surface water. Keeping in mind that all experiments were conducted at similar temperature and light regimes, high cellular primary production of the deep-water samples was enhanced by the rise in temperature during incubation. Also higher phosphate and ammonia concentrations in the deep-water samples (Figs. 2b & 3b) might have stimulated primary production. These experiments proved that cells are still viable under anoxic conditions. The presence of H₂S seems to have inhibited biochemical pathways involved in photosynthesis. This inhibition was reversible by reoxygenation. Furthermore, it was possible to cultivate cells of the genus *Synechococcus* from deep-water samples under surface conditions in glass bottles (J. de Wall, Institut für Meereskunde, Kiel, pers. comm.).

Serial dilution experiments in 1991 showed considerable grazing rates on chroococcoid cyanobacteria and bacteria in anoxic waters, grazing rates being higher than growth rates (Table 2). Growth rates compared well to surface values but grazing rates were about 50 % higher in anoxic waters. Since no precautions were taken, contamination with oxygen may have enhanced growth and grazing rates of facultative anaerobic organisms. Therefore, strictly anoxic grazing experiments were performed in 1992, revealing again considerable grazing impact on cyanobacteria and heterotrophic bacteria. Nonetheless, unlike 1991, growth rates were higher than grazing rates. The results of both cruises point towards high growth rates of *Synechococcus* in aphotic anoxic depths, comparable to those in surface layers.

The unexpected increase in abundance of heterotrophic and especially phototrophic organisms of the microbial food web within or below the chemocline raises the questions as to whether this increase is due to vertical transport of these cells and subsequent passive accumulation in the absence of grazers or due to *in situ* reproduction of these organisms.

Considering Stoke's Law, cells of the picoplankton size fraction have neglectable sinking rates (Fournier 1971, Platt et al. 1983, Bienfang 1985). From this, Fournier (1971) inferred that cell aggregates formed in upper layers are able to sink to the deep sea and Lochte & Turley (1988)

Table 2. Growth and grazing rates as estimated by the serial dilution technique. Representative data from 1 out of 4 (2 m), 1 out of 1 (160 m) and 1 out of 2 (170 m) experiments

Organism group	Growth rate, μ (d ⁻¹)	Grazing rate, g (d ⁻¹)	% Gross production grazed, % P_g
BAMBI '91 (2 m)			
Bacteria	0.26	0.30	115
Cyanobacteria	0.88	0.72	82
BAMBI '91 (160 m)			
Bacteria	0.36	0.65	181
Cyanobacteria	0.85	1.29	152
BAMBI '92 (170 m)			
Bacteria	0.86	0.41	48
Cyanobacteria	1.33	1.19	89

reported that cyanobacteria and bacteria in Atlantic mid-waters sedimented as phytodetritus to greater depths. Silver et al. (1986) mentioned cells associated with 'rapidly sinking detritus'; from these organic particles, cells are delivered very rapidly to a 'pool of suspended cells' at greater depths. Another device of vertical transport of cells are fecal pellets, containing highly pigmented cyanobacteria and phototrophic cells (Silver & Alldredge 1981). In the 1991 study, aggregates were found in a sediment trap located in 200 m depth containing phototrophic nanoflagellates but no *Synechococcus* and only very few fecal pellets were observed (A. Stuhr, Institut für Meereskunde, Kiel, pers. comm.). These findings suggest a possible

vertical transport of aggregated pico- and nanoplankton to greater depths which nonetheless might have been too small to account for the increase below the chemocline. Under the epifluorescence microscope only unicellular autotrophic cells were observed at greater depths. A resuspension of aggregated cells could explain the occurrence of only unicellular cells at greater depths and the absence of *Synechococcus* in the aggregates observed in the sediment traps. However, it might have been also a combination of events, and a flux of aggregates prior to our investigation cannot be excluded.

Although Silver et al. (1986) calculated life expectancies of several weeks to years for cyanobacteria and eucaryotic nanoplankton in the deep sea, it still remains unclear how these originally oxyphototrophic organisms are maintaining survival and even growth under such uncomfortable conditions. The results of the serial dilution experiments pointed out that all organisms of the picoplankton size fraction were able to grow. The primary production experiments implied that cyanobacteria are not killed in the presence of H₂S but remain viable. The observed growth rates under anoxic conditions might result from heterotrophic activity. Heterotrophy is evidenced for at least some *Synechococcus* strains (Waterbury et al. 1986, Paerl 1991). In the case of phytoflagellates, heterotrophy (phagotrophy) is stated by Bird & Kalff (1989) and Antia et al. (1991), whereby these findings are restricted to oxic conditions so far. Nonetheless, there is no evidence that heterotrophy/mixotrophy of phytoplankton is impossible under anoxic conditions. Bacterial growth rates in anoxic depths were about 3 times higher than in surface layers. Chemoautotrophic and heterotrophic growth could not be distinguished for bacteria. CO₂-dark fixation values indicate that an important amount of the overall bacterial growth is attributed to chemoautotrophic bacteria. However, facultative and obligate anaerobic heterotrophic bacteria were also observed in anoxic depths in the Gotland Sea (Gast & Gocke 1988).

Setälä (1991) observed similar ciliate species within and below the chemocline of the Gotland Basin. The author supposed that the increasing size of bacteria in these depths seem to favour growth conditions for ciliates which are able to survive high H₂S concentrations. Furthermore, chroococcoid cyanobacteria could advance a survival of ciliates under these circumstances. Kuosa (1990) demonstrated grazing of ciliates on *Synechococcus* in the Baltic Sea. Moreover, the change in ciliate species composition related to anoxic conditions and the occurrence of *Metopus* spp. give evidence that, like in oxic waters, ciliates as grazers of microbial food web organisms are active at the oxic/anoxic interface and probably below. Several spe-

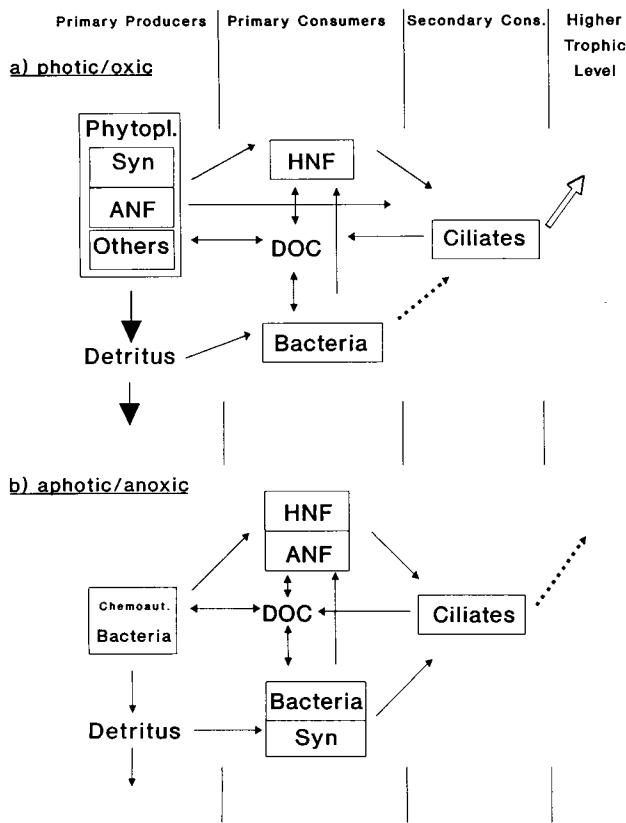


Fig. 5. Schematic model of the microbial food web in (a) photic/oxic and (b) aphotic/anoxic waters of the central Baltic Sea. Boxes on the left represent primary producers, boxes in the middle primary consumers of particulate and dissolved organic matter, respectively, and boxes on the right stand for secondary consumers. In the case of one-sided arrows, arrow heads point at grazers (except the connection between ciliates and DOC). For DOC, arrows show uptake and release of dissolved organic matter. Dotted arrows point out subordinate relations. For detritus and transport of organic matter to higher trophic levels, thickness of arrows indicates different amounts, which are representative for the whole food web. Syn: *Synechococcus*, ANF: photoautotrophic nanoflagellates (mixo-/phagotroph); HNF: heterotrophic nanoflagellates; DOC: dissolved organic carbon; Phytopl.: phytoplankton

cies of the genus *Metopus* are able to live in anoxic environments (Fenchel & Finlay 1990).

The presence of auto- and heterotrophic bacteria, viable cyanobacteria, flagellates and ciliates in anoxic depths of the central Baltic Sea gives evidence of an intact microbial food web. Although in both systems (photic/oxic and aphotic/anoxic) the same organism groups compose the food web, their role under oxic and anoxic conditions is different (Fig.5). Below the chemocline, photoautotrophic organisms are substituted by chemoautotrophic bacteria and possibly act as primary consumers of particulate (ANF) and dissolved (Syn) organic matter. Auto- and heterotrophic anoxic processes supply less energy than oxic processes. Therefore this system could be seen as a 'low level system', which functions properly but cannot build up as high production rates and biomasses as a photic/oxic system. For this reason, the transfer of organic matter to higher trophic levels should be low. This might explain low ciliate numbers in the anoxic microbial food web. These organisms possibly represent top predators within this system already.

The oxic/anoxic interface is an exception to the hypothesis presented here. Chemoclines in the Baltic Sea are known to be productive zones with rapid turnover of organic material, especially in the micro-aerobic layer (Brettar 1991, Giesenhagen & Hoppe 1991).

Further investigations should evaluate the structure, dynamics and importance of the postulated anoxic microbial food web, its seasonal variability and the physiological response of phototrophic organisms upon anoxic conditions.

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