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Food selection by calanoid copepods in the euphotic layer of the Gotland Sea (Baltic Proper) during mass occurrence of N_2-fixing cyanobacteria

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ABSTRACT: Food selection by 2 dominant calanoid copepods, *Acartia* sp. and *Temora longicornis*, was studied during mass occurrence of N_2-fixing cyanobacteria in June/July 1993 and 1994 in the Gotland Sea (Baltic Proper). The aim of this study was to assess the importance of N_2-fixing cyanobacteria in the diet of calanoid copepods. Two different methods were used: firstly the analysis of marker carotenoids by HPLC (high-performance liquid chromatography), and secondly the analysis of $\delta^{15}N$ signals of copepods by mass spectrometry. The first method provides a 'snapshot' of autotrophic material ingested; the second method summarises a longer period, and gives evidence that a certain food source is not only ingested but also assimilated. In 1994, mass occurrence of cyanobacteria showed a higher concentration in the euphotic layer than 1993 (97 µg C l^{-1} in 1994, 57 µg C l^{-1} in 1993), which was reflected in higher food uptake of N_2-fixing cyanobacteria in 1994. The average relative amount of myxoxanthophyll, the specific carotenoid of N_2-fixing cyanobacteria, in the copepod guts showed high values in 1994 (*Acartia* sp. 37%, *T. longicornis* 41%) and low values in 1993 (1% for both copepods). The low $\delta^{15}N$ values of both *Acartia* sp. and *T. longicornis* in 1994 (9‰) compared to those in 1993 (10.5‰) support the results of HPLC analyses, because N_2-fixing cyanobacteria have a lower $\delta^{15}N$ (average 0.7‰) than eukaryotic phytoplankton (average 12‰). The low $\delta^{15}N$ values in 1994 indicate that N_2-fixing cyanobacteria were not only ingested but also assimilated by the copepods to a higher extent in 1994 than 1993.

KEY WORDS: Selective feeding · N_2-fixing cyanobacteria · *Acartia* sp. · *Temora longicornis* · Baltic Sea

INTRODUCTION

So far little attention has been paid to the importance of N_2-fixing cyanobacteria as a food resource for zooplankton in the Baltic, despite their mass occurrence each summer. Most studies of the trophic relationship between copepods and cyanobacteria have focused on freshwater environments (e.g. Lampert 1981, Holm & Sharpio 1984, Fulton & Pearl 1987a,b, 1988, DeMott 1989, Ahlgren et al. 1990). These studies are based mainly on laboratory experiments and show that cyanobacteria are generally not preferred by copepods because of the bad manageability of the filaments, nutritional inadequacy or toxicity (e.g. Lampert 1987, Ahlgren et al. 1990). On the other hand field experiments in lakes demonstrate that copepods are capable of ingesting filamentous and colonial forms of cyanobacteria (DeMott & Moxter 1991, Schaffner et al. 1994).

In the marine environment only a few studies have examined the nutritional value of cyanobacteria for calanoid copepods and these have yielded contradictory results. Schmidt & Jónasdóttir (1997) reported that cyanobacteria in small quantities cannot necessarily be considered as poor food. Guo & Tester (1994) found

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that *Acartia tonsa* only ingest healthy intact cells of *Trichodesmium* sp. when no other food is available. Heerkloss et al. (1984) estimated assimilation efficiencies of 50 to 80% for *A. tonsa* when they fed on the cyanobacteria *Microcystis areuginosa*, while an assimilation efficiency of 35% was found when they fed on the cyanobacteria *Oscillatoria redeckii*. Field studies have shown low grazing on filamentous cyanobacteria by copepods and cladocerans during bloom conditions in temperate estuarine or brackish waters (Sellner et al. 1993, 1994, 1996). This result contrasts with that found for zooplankton feeding on cyanobacteria in freshwater environments (see above).

Therefore we conducted a more detailed survey on feeding behaviour of *Acartia* sp. and *Temora longicornis* in the Baltic Proper during mass occurrence of N_2-fixing cyanobacteria. We used 2 complementary methods: marker carotenoid analyses by high-performance liquid chromatography (HPLC) in copepod guts to estimate actual food uptake (Kleppel & Pieper 1984, Kleppel et al. 1988, Swadling & Marcus 1994), and $\delta^{15}N$ analyses of copepods by mass spectrometry that summarise feeding strategy of copepods over a longer period, giving evidence that a certain food source is not only ingested but also assimilated (Montoya 1994). The question we wanted to answer is: How important are N_2-fixing cyanobacteria as a food source for calanoid copepods during their bloom in the Baltic Proper?

MATERIALS AND METHODS

Description of the study area and sampling procedure. In the Baltic Sea during summer months blooms of N_2-fixing, filamentous cyanobacteria are a regular phenomenon (Kahru et al. 1994), thought to be caused by a shift from phosphorus to nitrogen limitation (Granéli et al. 1990). The major bloom forming species are *Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Anabaena* sp. (Hübel & Hübel 1995). In addition the non-N_2-fixing picocyanobacteria *Synechococcus* sp. occur in high cell numbers (10^6 cells ml^{-1}) in the Baltic Proper (Jochem 1990). These are, however, due to their small size (1 to 2 µm), considered unsuitable as food for copepods (Johnson et al. 1982).

Samples were taken from a water body marked with a drifting buoy (for details see Voß et al. 1997) during cruises of the RVs 'Professor Albrecht Penck' (June 28 to July 2, 1993) and 'Alexander v. Humboldt' (July 18 to July 22, 1994) east of Gotland (57° 17' N, 20° E) in the central Baltic Sea (Fig. 1). Sampling was done every day during a 5 d drifting period.

Sampling of copepods. Copepods were collected after sunset at the same stations as the water samples using a 200 µm mesh ring net (0.75 m diameter), towed vertically between 0 and 25 m depth. Immediately after capture, samples were screened onto a 200 µm gauze and frozen in liquid nitrogen for further species separation in the laboratory. For pigment analyses, adult *Acartia* sp. and *Temora longicornis*, the dominant copepods in the euphotic layer, were isolated under a binocular microscope equipped with a cold table under dim light and washed 3 times in cold water (0°C), to remove attached algae. Between 500 and 700 individuals of each species were collected on glass-fibre filters (Whatman GF/F) for pigment analyses and stored at –80°C for further analyses by HPLC.

Phytoplankton sampling. Water was collected from discrete depths (1 m only for pigment analyses, 5, 10, 15, 20 and 25 m) in both years using a rosette water sampler equipped with 12 l bottles. Subsamples were taken for estimation of phytoplankton biomass by microscope counts and flow cytometry, HPLC analyses of marker pigments, quantification of suspended particulate material (SPM), and $\delta^{15}N$ analyses of SPM and N_2-fixing cyanobacteria. The methods used are described in detail below.

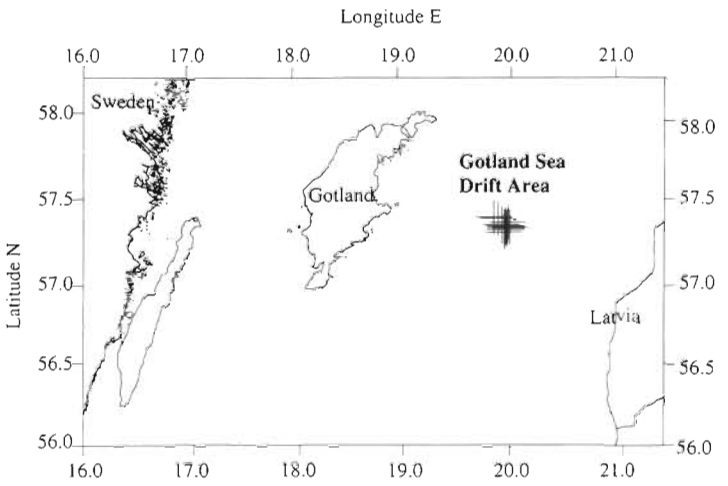

Fig. 1. Drift area in the Gotland Sea (Baltic Proper) studied in the periods June 28 to July 2, 1993, and July 18 to July 22, 1994

Estimation of phytoplankton biomass. Subsamples (0.5 l) were taken for analyses by light microscopy, epifluorescence microscopy (in 1993) and flow cytometry (in 1994).

Light microscopy: A 0.25 l subsample was fixed with 1% Lugol's solution and analysed under an inverted microscope at ×100 and ×400 magnification after settling (Utermöhl 1958). Algae >15 µm (see Table 1) present as filaments (N_2-fixing cyanobacteria), chains (e.g diatoms) and large unicellular algae (e.g *Dinophysis norvegica*) were counted by this

Table 1. Dominant filamentous, chain-forming and unicellular autotrophic species and their size range estimated during the study periods in 1993 and 1994. *Counted by light microscopy, **counted by epifluorescence microscopy (in 1993) and flow cytometry (in 1994)

Autotrophic group	Dominant species
Algae cells present in filaments and chains (>15 µm)	
N_2-fixing cyanobacteria	*Anabaena* sp.*
	*Aphanizomenon flos-aqua**
	*Nodularia spumigena**
Diatoms	*Thalassiosira baltica**
	*Skeletonema gracile**
	*Chaetoceros costatum**
Unicellular species >15 µm	
Dinoflagellates	*Dinophysis norvegica**
Unicellular species <15 µm	
Dinoflagellates	*Prorocentrum minimum***
	*Katodinium rotundatum***
	*Gymnodinium simplex***
Cryptophytes	*Rhodomonas* sp.**
Prasinophytes	Cf. *Pyramimonas* spp.**
Haptophytes	Cf. *Prymnesium parvum***
Cyanobacteria	*Synechococcus* spp.**

technique. The identified and counted algae were converted from biovolume to carbon values as described by Edler (1979).

Epifluorescence microscopy in 1993: For counting of unicellular autotrophic nano- and picoplankton (algae cells <15 µm, see Table 1) 2 to 5 ml of the samples were filtered onto black polycarbonate filters (pore size 0.2 µm), which were then transferred to microscope slides and stored at −20°C until they were counted in the laboratory under an ZEISS Axioskop epifluorescence microscope at ×400 magnification. The autotrophic cells were identified by their shape and bright red and orange autofluorescence under green excitation light (ZEISS filter set 48.79.00, excitation filters BP530-585, beam splitter FT600, long-pass emission filter LP615). A minimum of 400 cells were counted per sample and converted to biomass by using cell biovolumes calculated from individual cell size measurements, and applying the carbon conversion factors according to Verity et al. (1992).

Flow cytometry in 1994: A Partec PAS III flow cytometer was used to count unicellular autotrophic nano- and picoplankton (<15 µm, see Table 1). The instrument was equipped with a tuneable Argon laser (max. power 300 mW), adjusted to 488 nm. Emission light was detected as red (>630 nm, chlorophyll) and orange (560 to 590 nm, phycobilins) fluorescence light. Perpendicular light scatter was detected at the proper laser wavelength of 488 nm. Signals were recorded on a 3-decade logarithmic scale. Absolute sample volume was 700 µl.

For biomass estimation, an approximate size determination of nano- and picoplankton cells was carried out by size fractionation. Water samples were passed through meshes or polycarbonate filters of different pore sizes (2 to 15 µm). The respective filtrates were then measured in the flow cytometer The pore size retaining approximately 50% of a population (as measured in the cytometer) was taken as the approximate equivalent spherical cell diameter. Biomasses were calculated based on the cell numbers in the unfractionated samples and the approximate equivalent spherical diameters, using the carbon conversion factors according to Verity et al. (1992).

Marker pigment analysis by HPLC. Subsamples for HPLC (0.5 to 1 l) were filtered through Whatman GF/F filters (25 mm in diameter) and stored in liquid nitrogen for analysis in the laboratory. The frozen GF/F filters were extracted with 5 ml 100% methanol buffered with 2% ammonium acetate (3 ml for the copepod samples) and analysed by HPLC using the method described by Kraay et al. (1992). A detailed description of this method and the HPLC system is given by Meyer-Harms & Pollehne (1998).

Myxoxanthophyll served as a marker for N_2-fixing cyanobacteria. This pigment is absent in the non-N_2-fixing pico-cyanobacteria *Synechococcus* sp. (Zeitzschel pers. comm. and author's unpubl. data), which was abundant at our sampling sites during the study periods (Reckermann 1996). In both years, 6 pigments were selected as primary taxonomic markers to represent the major groups in the autotrophic community (Table 2).

Estimation of selective feeding behaviour. Food selection of specific phytoplankton groups was quantified using the selectivity index (SI) after Ivlev (1961): $SI = (r - p)/(r + p)$, where r is the percentage of its marker pigment in the gut and p is the percentage of its marker pigment in the euphotic layer. SI was calculated as follows: For the terms r and p of the eukaryotic phytoplankton, the sum of the frequency of all marker pigments that represent this group was used for each

Table 2. Diagnostic pigments for the characterisation of the different algal groups (Hirschberg & Chamovitz 1994, Jeffrey et al. 1997) in the euphotic zone during the study periods in 1993 and 1994 in the Gotland Sea

Autotrophic group	Marker carotenoids
N_2-fixing cyanobacteria	Myxoxanthophyll
Diatoms	Fucoxanthin
Dinoflagellates	Peridinin
Cryptophytes	Alloxanthin
Prasinophytes	Prasinoxanthin
Haptophytes	19'-hexanoyloxyfucoxanthin

depth. A mean value over the whole depth range was calculated for both groups (eukaryotic phytoplankton, cyanobacteria), because copepod samples were taken from 25 m depth to the surface. SI varies theoretically between −1 and +1, with SI = 0 indicating non-selective feeding on an algal group, $0 \leq SI \leq +1$ indicating an increasing preference for a phytoplankton group and $0 \geq SI \geq -1$ indicating an increasing discrimination against an algal group. SI indices were calculated for each sampling day during the 5 d drifting period in both years, followed by a calculation of an average of the results from the 5 d sampling period.

Analysis of nitrogen (N) and $\delta^{15}N$. For $\delta^{15}N$ analyses of adult *Acartia* sp. and *Temora longicornis*, 90 to 120 adult individuals of each species were collected from the frozen 200 µm gauze and transferred to a pre-combusted GF/F filter. For the determination of N and the $\delta^{15}N$ of SPM, water samples (0.5 to 1 l) were filtered onto GF/F filters. For the analysis of N and $\delta^{15}N$ of N_2-fixing cyanobacteria, seawater samples were passed through a 100 µm gauze and transferred into filtered seawater for further separation. Trichomes were isolated under a binocular microscope and transferred onto a GF/F filter with an inoculation loop.

All filters prepared for N and $\delta^{15}N$ analyses were stored at −20°C. Before analysis in a Carlo Erba CE 1108 elemental analyser coupled to a Finnigan MAT Delta S isotope ratio mass spectrometer the filters were dried at 60°C for 12 h and pelletised. For details see Voß et al. (1997). All isotope abundances were measured relative to a working standard of ultra high purity N_2 ($\delta^{15}N = -8.09$‰) and expressed as per mil (‰) deviation from the isotopic composition of atmospheric N_2 using the $\delta^{15}N$ convention: $\delta^{15}N$ (‰) = $[(R_{sample}/R_{standard}) - 1] \times 1000$; $R = (^{15}N/^{14}N)$.

Isotopically characterised organic standards (peptone, Merck Chemical) were analysed along with each batch of samples. The reproducibility of the values reported in this study was ± 0.1‰. Both the reference gas and the organic standards have been intercalibrated with IAEA Standard substances.

The concentration of $\delta^{15}N$ of the eukaryotic phytoplankton plus detritus was calculated by considering the respective contribution of the N_2-fixing cyanobacteria and the SPM as follows:

$$\delta^{15}N_{EPD} = [(\delta^{15}N_{SPM} \times N_{SPM}) - (\delta^{15}N_{CB} \times N_{CB})]/N_{EP}$$

where EPD stands for the eukaryotic phytoplankton plus detritus, SPM stands for the suspended particulate material and N_{SPM} the N content of SPM, CB stands for the N_2-fixing cyanobacteria and N_{CB} the N content of CB, and N_{EP} is the nitrogen content of eukaryotic phytoplankton (EP).

N_{EP} in Table 5 was calculated by converting the phytoplankton carbon (C) values estimated in this study and shown in Table 3 by using a C:N ratio of 9.0. This ratio is based on the investigation of Shaffer (1986) in the Baltic Sea. The other parameters used in the equation for calculating $\delta^{15}N_{EPD}$ are given in Table 5.

RESULTS

Phytoplankton distribution

The dominant autotrophic species found in the Gotland Sea during 1993 and 1994 are listed in Table 1. In both years the autotrophic community was similar. The N_2-fixing cyanobacteria were dominated by *Anabaena* sp., *Aphanizomenon flos-aquae* and *Nodularia spumigena*. Their biomass decreased with depth in both years (Table 3) from >100 µg C l^{-1} at 5 m depth to 1 (1993) and 40 µg C l^{-1} (1994) at 25 m depth, while the biomass of eukaryotic phytoplankton in the euphotic layer did not vary much between years (94 to 120 µg C l^{-1}). An exception was observed in 1994, when a high eukaryotic algae biomass of 239 µg C l^{-1} was found at 15 m depth, resulting from an accumulation of the large dinoflagellate *Dinophysis norvegica*. From 5 to 25 m depth the cyanobacteria biomass was 1.1- to 40-fold higher in 1994 than in 1993 (Table 3).

In 1993, the pigment myxoxanthophyll, the biomarker of N_2-fixing cyanobacteria, dominated the pigment composition (Fig. 2) from 1 to 5 m (mean 62%); in 1994 it dominated from 1 to 10 m (mean 65%). The relative amount of marker carotenoids of the different eukaryotic phytoplankton groups increased from 15 to 25 m depth in 1993 and represented more than 80% of all pigments detected, while in 1994 the values were <71% (Fig. 2). The estimated pigment distributions of the marker carotenoids for N_2-fixing cyanobacteria and eukaryotic phytoplankton in the euphotic layer are consistent with the distribution pattern for both groups estimated by cell counting (see Table 3 & Fig. 2).

Table 3. Biomass of autotrophic organisms (N_2-fixing cyanobacteria and eukaryotic phytoplankton) in the euphotic layer during the study periods in 1993 and 1994 in the Gotland Sea (n = 5, ±SD)

Depth (m)	N_2-fixing cyanobacteria (µg C l^{-1})		Eukaryotic phytoplankton (µg C l^{-1})	
	1993	1994	1993	1994
5	120 ± 52	134 ± 54	104 ± 24	95 ± 55
10	118 ± 39	163 ± 61	109 ± 15	94 ± 39
15	41 ± 18	118 ± 28	110 ± 42	239 ± 107
20	7 ± 2.0	31 ± 14	99 ± 8	115 ± 53
25	1 ± 0.3	40 ± 23	120 ± 17	99 ± 51

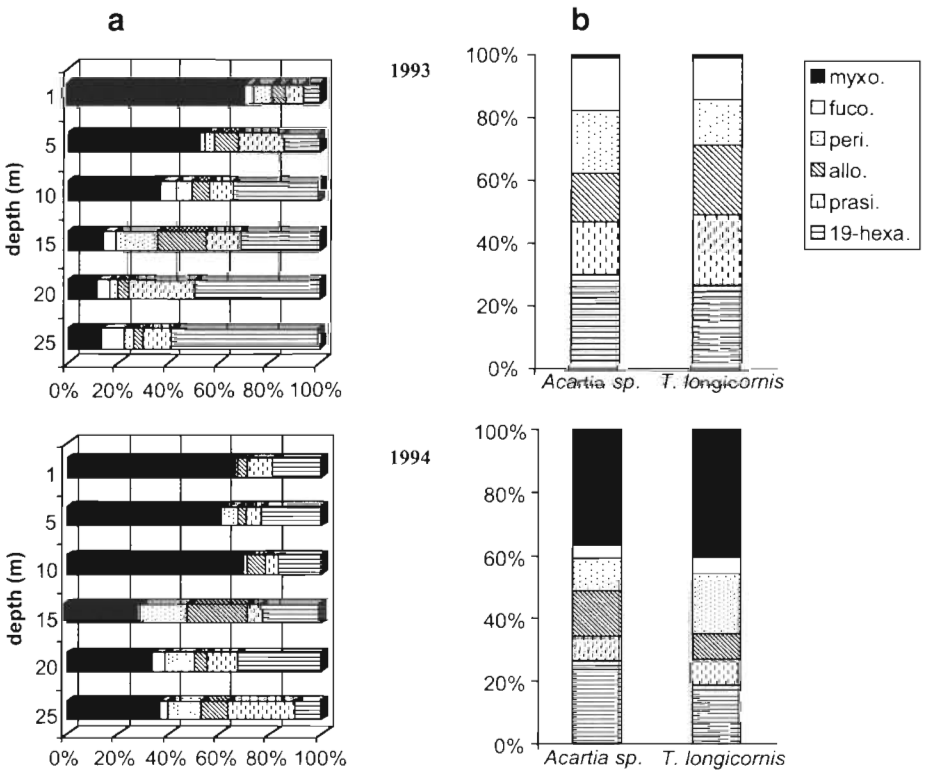

Fig. 2. Average distribution (in %) of marker carotenoids in 1993 and 1994 in (a) the euphotic layer (in 1993, n = 9, SD < 35% and in 1994, n = 7, SD < 41% for each depth) and (b) the guts of copepods *Acartia* sp. and *Temora longicornis* (in 1993, n = 20, SD < 10% and in 1994, n = 15, SD < 17%). Pigments: myxo. = myxoxanthophyll, fuco. = fucoxanthin, peri. = peridinin, allo. = alloxanthin, prasi. = prasinoxanthin, 19'-hexa. = 19'-hexanoyloxyfucoxanthin. The relations of the marker pigments to the algal classes are given in Table 2

Grazing activity of calanoid copepods

The higher concentration of cyanobacteria in the water body in 1994 compared to in 1993 was reflected by a higher relative amount of the corresponding pigment myxoxanthophyll in the copepod guts (1993: *Acartia* sp. 1.1% and *Temora longicornis* 1.2%, 1994: *Acartia* sp. 37% and *T. longicornis* 40%, Fig. 2). In 1993, the calculated selectivity index (SI) showed a selection of eukaryotic phytoplankton by both copepods, whereas in 1994 a more opportunistic feeding behaviour on both cyanobacteria and eukaryotic phytoplankton was observed (Table 4).

The $\delta^{15}N$ of the nutritional sources, N_2-fixing cyanobacteria ($\delta^{15}N_{CB}$) and eukaryotic phytoplankton plus detritus ($\delta^{15}N_{EPD}$) increased with depth in the euphotic layer (Table 5). The $\delta^{15}N_{CB}$ isolated from the field ranged from 0.2 to 1.2‰ in 1993 and 0.5 to 1.4‰ in 1994. The calculated $\delta^{15}N_{EPD}$ showed values from 8.0 to 16.5‰ (average 11.9‰) and 5.8 to 18‰ (average 11.6‰) in 1993 and 1994, respectively. The isotope values of the 2 food sources estimated in the present study are comparable with those found in the literature for each group (Michener & Schell 1990, Wada & Hattori 1991). The increase of $\delta^{15}N$ of the 2 different nutritional sources with depth found in this study may be explained by the utilisation of heavier nitrogen sources from the underlying water (Voß et al. 1997), which stems from remineralisation and denitrification processes.

The calanoid copepods *Acartia* sp. and *Temora longicornis* had average $\delta^{15}N$ signals of 11‰ in 1993 and 9‰ in 1994 (Fig. 3). Regarding the differences in $\delta^{15}N$ of the prospective food sources (see above) the $\delta^{15}N$ of the copepods should decrease with increasing amount of ingested N_2-fixing cyanobacteria. In fact the relative low $\delta^{15}N$ values of the copepods in 1994 correspond to a relatively high amount of the marker carotenoid of N_2-fixing cyanobacteria (myxoxanthophyll) in the copepod guts. However the high standard deviation of the $\delta^{15}N$ values means that the differences are not statistically significant. The reported range of

Table 4. Selectivity indices (SI) of *Acartia* sp. and *Temora longicornis* on the autotrophic material in the euphotic zone in 1993 and 1994 (n = 5) calculated according to Ivlev (1961). SI = 0: non-selective feeding on an algal group; $0 \leq SI \leq +1$: increasing preference for a phytoplankton group; and $0 \geq SI \geq -1$: increasing discrimination against an algal group

Nutritional source	1993		1994	
	Acartia sp.	*T. longicornis*	*Acartia* sp.	*T. longicornis*
N_2-fixing cyanobacteria	−0.95 ± 0.03	−0.95 ± 0.05	−0.14 ± 0.05	−0.10 ± 0.1
Eukaryotic phytoplankton	0.29 ± 0.11	0.29 ± 0.17	0.11 ± 0.05	0.08 ± 0.06

Table 5. $\delta^{15}N$ and µmol N of possible food sources for *Acartia* sp. and *Temora longicornis* during the investigations in 1993 and 1994 in the Gotland Sea (n = 5, ±SD). SPM: suspended particulate material, CB: isolated N_2-fixing cyanobacteria, EPD: eukaryotic phytoplankton plus detritus and EP: eukaryotic phytoplankton. The µmol N_{EPD} were calculated by using the mean values shown in Table 3 and a C:N ratio of 9.0 (Shaffer 1986), where $\delta^{15}N_{EPD}$ values were calculated by using the mean values of the other food sources shown here. For details see 'Materials and methods'

Depth (m)	$\delta^{15}N_{SPM‰}$ 1993	$\delta^{15}N_{SPM‰}$ 1994	µmol N_{SPM} (l^{-1}) 1993	µmol N_{SPM} (l^{-1}) 1994	$\delta^{15}N_{CB‰}$ 1993	$\delta^{15}N_{CB‰}$ 1994	µmol N_{CB} (l^{-1}) 1993	µmol N_{CB} (l^{-1}) 1994	$\delta^{15}N_{EPD‰}$ 1993	$\delta^{15}N_{EPD‰}$ 1994	µmol N_{EP} (l^{-1}) 1993	µmol N_{EP} (l^{-1}) 1994
5	1.9 ± 0.1	1.4 ± 0.2	5.0 ± 1.4	5.7 ± 1.4	0.2 ± 0.01	0.5 ± 0.02	1.1 ± 0.06	1.3 ± 0.04	9.3	8.1	1.0	0.9
10	1.3 ± 0.1	1.8 ± 0.1	6.4 ± 1.4	6.4 ± 1.1	0.2 ± 0.01	0.5 ± 0.01	1.5 ± 0.03	1.8 ± 0.04	8.0	11.8	1.0	0.9
15	3.4 ± 0.3	2.8 ± 0.1	3.6 ± 0.7	5.0 ± 0.7	0.2 ± 0.02	0.9 ± 0.04	0.5 ± 0.01	1.3 ± 0.05	12.1	5.8	1.0	2.2
20	5.4 ± 0.5	6.1 ± 0.6	2.4 ± 0.1	2.9 ± 0.7	0.7 ± 0.04	1.4 ± 0.06	0.8 ± 0.02	1.3 ± 0.08	13.8	14.4	0.9	1.1
25	6.8 ± 0.8	6.0 ± 0.4	2.9 ± 0.1	2.9 ± 0.8	1.2 ±0.08	0.8 ± 0.02	1.3 ±0.09	1.5 ± 0.12	16.5	18.0	1.1	0.9

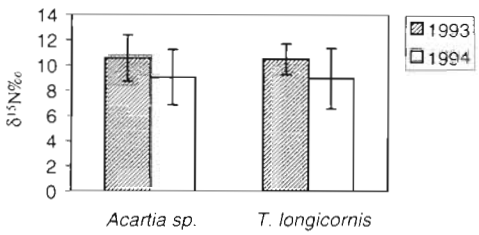

Fig. 3. Mean $\delta^{15}N$ values of *Acartia* sp. and *Temora longicornis* in the euphotic layer during the study period 1993/1994 in the Gotland Sea (n = 20, ±SD)

$\delta^{15}N$ of zooplankton measured in the Baltic Sea (8 to 14‰, Hansson et al. 1997) is consistent with the data presented in our study and can therefore be explained by different feeding strategies.

DISCUSSION

The present study has shown that cyanobacteria were utilised by calanoid copepods, a finding which is consistent with previous results. Schmidt & Jónasdóttir (1997) found from laboratory experiments that small additions of a cyanobacterium *Microcystis* sp. to a diatom diet led to a greater rate of egg production than on a pure diatom diet. This was suggested to result from the biochemical composition of cyanobacteria, which complements the nutrition derived from diatoms. Turner et al. (1998) reported from Kingston Harbour, Jamaica, that during bloom conditions of filamentous cyanobacteria, marine copepods and cladocerans showed a variable and inconsistent ingestion pattern on natural assemblages of phytoplankton and cyanobacteria without presenting any explanation for this phenomenon. The authors observed that copepods and cladocerans not only ingested entire cyanobacterial filaments, but also appear to bite off portions of filaments. Such 'filament clipping' on cyanobacteria has been reported for several freshwater zooplankton organisms (Schaffner et al. 1994 and references therein). Estimation of cyanobacteria uptake by zooplankton based on counting filaments only is therefore questionable.

The 2 methods applied in this study examine the food uptake during different phases of the metabolic process. Marker carotenoid analyses in copepod guts provide 'snapshot' information of autotrophic material ingested, while $\delta^{15}N$ signals of the copepods summarise feeding strategy over a longer period and provide evidence that a certain food source is not only ingested but also assimilated (e.g. Montoya 1994).

According to the marker pigment method *Acartia* sp. and *Temora longicornis* grazed more on eukaryotic phytoplankton than on N_2-fixing cyanobacteria. However, the distribution of myxoxanthophyll, the marker pigment of N_2-fixing cyanobacteria, in the copepod guts differed considerably between sampling periods in the Gotland Sea. Higher values were found in 1994 than in 1993. These findings are supported by $\delta^{15}N$ measurements. According to the average $\delta^{15}N$ values of the autotrophic food sources in the euphotic layer (N_2-fixing cyanobacteria, eukaryotic phytoplankton) an ingestion of both food sources but with a preference for eukaryotic phytoplankton can be assumed. Nevertheless the lower mean $\delta^{15}N$ value of the copepods in 1994 compared to 1993 shows that N_2-fixing cyanobacteria were ingested and assimilated to a greater extent in 1994. This suggestion thus contrasts with that of Pfannkuche & Lochte (1993) that cyanobacteria are well protected against digestion.

The present study provides the first investigation using nitrogen isotope signatures to analyse copepod grazing on N_2-fixing cyanobacteria in the Baltic Proper. $\delta^{15}N$ analyses seem a useful tool for differentiating between N_2-fixing cyanobacteria and other nutritional sources of calanoid copepods. Isotope fractionation is influenced by assimilation and excretion processes (Montoya 1994), but it is unclear how these processes are related to each other. By answering these open questions, $\delta^{15}N$ analyses might be a useful

tool for quantifying trophic relationships in the aquatic environment and may contribute to a better understanding of the feeding behaviour of zooplankton.

The different feeding behaviour found for *Acartia* sp. and *Temora longicornis* on cyanobacteria in 1994 compared to 1993 may be explained by 2 hypotheses: (1) a higher cyanobacteria biomass existed in 1994 than in 1993, especially in the deeper part of the euphotic zone; and (2) there was a later phase of the cyanobacteria bloom in 1994 compared to 1993.

In support of Hypothesis 1 a 50-fold higher amount of myxoxanthophyll was found in the 20 to 25 m depth layer in 1994 compared to 1993 (see Table 3), which is reflected in a 33-fold higher amount of myxoxanthophyll in the copepod guts in 1994. Therefore, it is possible that the higher amount of myxoxanthophyll in the copepod guts in 1994 was a result of the opportunistic feeding behaviour of both copepods on cyanobacteria caused by the higher cyanobacterial biomass at 20 to 25 m depth during that year. This explanation is supported by the calculated SI, which indicates that the 2 copepods fed on N_2-fixing cyanobacteria in 1994 according to their abundance (see Table 4).

Hypothesis 2 is supported by results of Hoppe (1981) and Repka et al. (1998). Hoppe (1981) reported a fauna of microzooplankton (e.g. ciliates, flagellates, rotifers) to be associated with aged agglomerates of N_2-fixing cyanobacteria. Thus cyanobacteria in a late phase of a bloom may be a more tasty and nutritious food for copepods. Repka et al. (1998) found in feeding experiments with *Daphnia galeatha* that detritus derived from the filamentous N_2-fixing cyanobacteria *Oscillatoria limnetica* was of higher food quality than live filaments and supported growth and reproduction of *D. galeatha*. Therefore differences in the feeding behaviour of the calanoid copepods on cyanobacteria in 1993 and 1994 might be explained by ageing processes of cells during bloom development, which increased their attractiveness as a food source.

This study shows that N_2-fixing cyanobacteria in the Baltic Proper are ingested and probably assimilated to some degree by calanoid copepods. Open questions remain on the change of their nutritional values with ageing processes and changes of feeding strategies of copepods related to their nutritional status. These studies are worthwhile, because, as shown here, the role of cyanobacteria as food for calanoid copepods seems to be so far underestimated.

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