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Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of size-fractionated plankton at a coastal station in the northern Baltic proper

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ABSTRACT: Seasonal cycles of $\delta^{13}C$ and $\delta^{15}N$ in dissolved organic carbon and size-fractionated plankton, ranging from bacteria to the jellyfish *Aurelia aurita*, were studied during a 1 yr cycle at a coastal station in the Baltic Sea. The observed isotopic changes were found with time lags in all size-fractions of plankton. The $\delta^{13}C$ showed a bimodal cycle with 2 local maxima, the first coinciding with the spring bloom and the second with the autumn bloom. In $\delta^{15}N$, the annual cycle was trimodal with 3 local maxima. The first occurred in connection with the spring bloom, the second in mid-summer and the third was a broad autumn-to-winter maximum. The causes of these patterns are discussed in relation to measured oceanographic variables. In the summer, a depleted nitrogen isotopic signal was propagated through all size-classes of plankton, indicating direct or secondary utilisation of fixed nitrogen from cyanobacteria. The strength of the signal indicated that nitrogen-fixing cyanobacteria are more ecologically important as instantaneous nitrogen sources in the Baltic than previously assumed. Enrichment of $\delta^{15}N$ in size-classes of plankton was found to be a linear function of logarithmic organism size from 20 to 500 µm, reflecting size-related consumption patterns of marine plankton food-webs. The explanatory power of the linear regression and the enrichment per unit size were stronger in spring and autumn than in the summer, reflecting time lags and diversity in the zooplankton community. The size-specific approach was found to be a simpler and more appropriate way of analysing trophic isotope enrichment in plankton food-webs than the assumption of a general enrichment factor per trophic level.

KEY WORDS: Stable isotopes · Food web · Baltic · Cyanobacteria · Seasonal cycle · Nitrogen fixation · Trophic enrichment

INTRODUCTION

Seasonal variability in stable isotopes of plankton

Stable-isotope methods are increasingly used as tracers to study material transport in food-webs (Owens 1987, Peterson & Fry 1987). The isotopic composition of both carbon and nitrogen show interannual variations in the plankton of temperate fresh and marine waters. In the Baltic Sea, the seasonal variation in production is very pronounced, and causes substantial fluctuations in the isotopic composition of plankton organisms. Measurements of isotopic ratios in plankton from different seasons are reported in several studies, but only a few describe a production cycle for both carbon and nitrogen stable-isotopes. In the marine environment, Wainright & Fry (1994) reported a bimodal distribution of $\delta^{13}C$, with a summer and autumn maximum enrichment for size-fractionated plankton in Woods Hole Harbor and Georges Bank during 2 production cycles. For $\delta^{15}N$ they found a steady enrichment during the productive season at Woods Hole, but no pronounced seasonal cycle at Georges Bank. In 2 Alaskan embayments, Goering et al. (1990) found enrichment of both $\delta^{13}C$ and $\delta^{15}N$ in phyto- and zooplankton with the progression of the spring bloom. Yoshioka et al. (1994) presented 2 yr data of isotopic variation in limnic phyto- and zooplankton. The data suggested a bimodal cycle in $\delta^{15}N$

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of phytoplankton, but the pattern was variable between years. Zohary et al. (1994) found, in a long-term study of plankton in Lake Kinneret (1971 to 1992), that $\delta^{13}C$ was most depleted during winter in both phyto- and zooplankton. The available information suggests a characteristic seasonal cycle, but sample variation has often been difficult to separate from seasonal variation. If the seasonal isotopic cycle is not properly described for organisms at a studied site, time lags and memory effects may interfere with attempts to analyse food-web dynamics and material use.

Marine plankton have been found to feed opportunistically in unstructured food webs, with particle size as the most important factor (Isaacs 1972, 1973, Platt & Denman 1977, Sheldon et al. 1977, Platt 1985). Size fractions of plankton are therefore likely to be representative of trophic groups in the marine plankton community. The present study investigated size-dependent changes and an annual cycle of $\delta^{13}C$ and $\delta^{15}N$ in size-fractionated Baltic plankton, ranging from <1 µm to the jellyfish *Aurelia aurita* (40 mm). The ANOVA design permitted separation of actual seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ from sample variation.

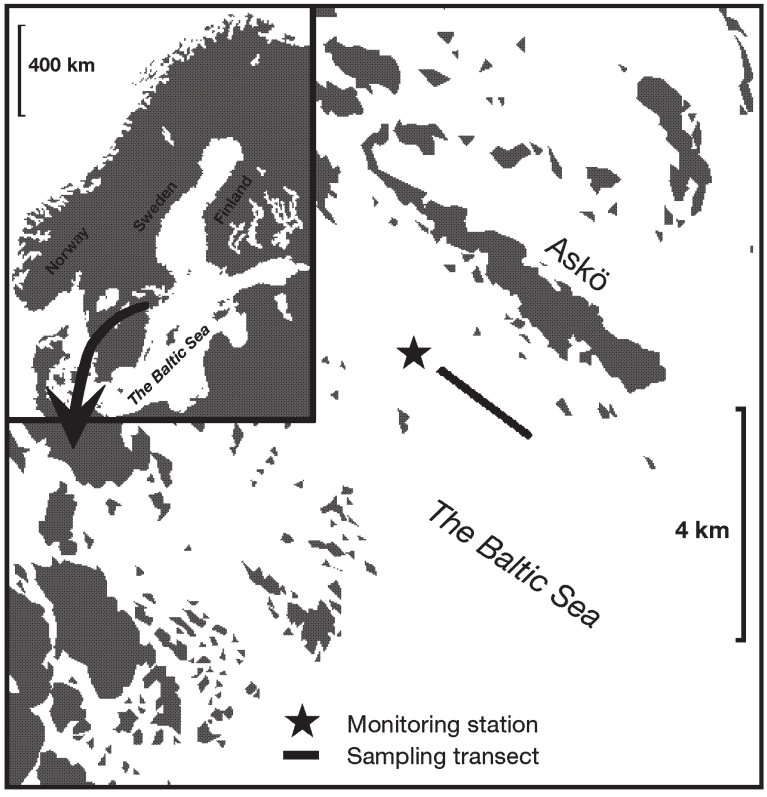


Fig. 1. Map of sampling area, monitoring station and sampling transect

Characteristics and plankton community of study site

The study was made in 1994 to 1995 at a coastal station in the Baltic, close to the Askö Laboratory (Fig. 1). Sampling was performed along a transect, ~1.5 km long, in a south-easterly direction from a monitoring station of the Swedish Environmental Protection Agency. Along the transect the sea floor is even and the depth ~40 m. Nutrients, primary production, chlorophyll *a* and several other oceanographic variables are continuously monitored at the station at monthly to weekly intervals, and unpublished monitoring data were kindly provided by Ulf Larsson, Department of Systems Ecology. The station is located in the outer archipelago facing the open sea, is generally covered by ice from December to March, and water temperature and production show pronounced seasonal variation. From mid-May to mid-September a thermocline developed at 20 m. The surface salinity at the station varied between 6.2 and 7, with the minimum in the summer months.

The characteristic seasonal phytoplankton succession was described by Hobro (1979). The spring bloom starts in early March, depending on the ice situation, and is initially characterised by a mixture of diatoms and dinoflagellates from the genera *Thalassiosira*, *Skeletonema*, *Chaetoceros*, *Achnanthes*, *Peridinella*, and *Gymnodinium*. When the nutrient supply is exhausted in mid-April, the post-spring-bloom stage is characterised by dinoflagellates (the genera *Dinophysis*, *Gonyaulax*, *Protoperidinium*, and *Gymnodinium*) and chrysophyceans (*Dinobryon* spp.). The summer production minimum is dominated by small flagellates (e.g. the genera *Dinophysis*, *Heterocapsa*), and in mid-summer a cyanobacterial bloom occurs (*Aphanizomenon* sp. and *Nodularia spumigena*). When the thermal stratification is disturbed by storms in September, regenerated nutrients may produce an early autumn bloom dominated by diatoms and small flagellates (the genera *Thalassiosira*, *Chaetoceros*, and *Heterocapsa*). Winter is characterised by very low production. The species composition varies between years, but diatoms and cyanobacteria generally dominate. Integrated annual primary production is ~140 g C m^{-2} yr^{-1} (U. Larsson pers. comm.).

The zooplankton production from 1977 through 1988 was described by Johansson (1992). In the late stage of the phytoplankton spring bloom (April to early May) Tintinnids and rotifers from the genera *Synchaeta* and

Keratella dominate the zooplankton community. In early summer a cladoceran population develops, consisting mainly of the genera *Bosmina*, *Podon* and *Evadne*. The cladoceran population reaches its maximum in July and then declines. Copepods of the genera *Acartia*, *Eurytemora*, *Temora* and *Pseudocalanus* then dominate the zooplankton community. The increase in the copepod population starts during the late phytoplankton spring bloom and the population peaks in August. On a biomass basis, the copepods totally dominate the zooplankton. For the years 1977 to 1988 Johansson estimated the average annual mesozooplankton production at ~6 g C m^{-2} yr^{-1}.

METHODS

Samples and sampling methods. The program included 12 different sample types and 9 sampling occasions (Tables 1 & 2). Since all organisms did not occur throughout the annual cycle, some sample types were not available on all sampling occasions (Table 2). Adult copepods and large copepodites (PL_6) were not found in early summer. Mysids *Mysis mixta* (MM) and *Neomysis integer* (NI) could only be sampled on dark nights in autumn, since they otherwise remain near the bottom. Adults of the jellyfish *Aurelia aurita* (AA) were found only in late summer. Cyanobacteria (CB) were sampled on 2 occasions in mid-summer, during a cyanobacterial bloom (*Aphanizomenon* sp. and *Nodularia spumigena*). The wind speeds were generally modest on the sampling occasions and in preceding days, and the amount of amorphous material in the samples was low (Tables 2 & 3).

The smallest size-class (GFF) of plankton and particulate organic material was collected by valve-free under-pressure suction. Ten litres of water were collected from 5 m through a stiff polyethylene tube into a polypropylene vacuum container. The water was gently pre-filtered through a 5 µm (3 dm^{-2}) nylon mesh and then filtered by underpressure (~200 hPa) through 47 mm Whatman GF/F glass-fibre filters (precombusted 400°C, 4 h) fitted in Nuclepore filter holders. Filtration was continued until the water flow almost ceased (6 to 8 l, <1 ml min^{-1}). The real cut-off of the filter was therefore considerably smaller than the nom-

Table 1. Codes of sample types, characteristics of samples and levels of replication. CB: consisted of *Aphanizomenon* sp. and *Nodularia spumigena*

Code	Size-class	Replicates	Dominating organism group	Method of isolation	Plankton class
AA	~400 mm	5	*Aurelia aurita* (Scyphozoa)	Hand-held dip-net	Macro
MM	~150 mm	5	*Mysis mixta* (Mysidacea)	Net/sieve/tweezers	Meso
NI	~150 mm	5	*Neomysis integer* (Mysidacea)	Net/sieve/tweezers	Meso
PL_6	~1.5 mm	5	Adult copepods	Net/sieve	Meso
PL_5	200–500 µm	5	Copepodites, cladocerans, rotifers	Net/sieve	Meso
PL_4	100–200 µm	5	Ciliates, nauplii, rotifers, diatoms	Net/sieve	Micro
PL_3	50–100 µm	5	Diatoms, phytoflagellates, ciliates	Net/suction/filt bottle/sieve	Micro
PL_2	20–50 µm	5	Phytoflagellates	Net/suction/filt bottle/sieve	Micro
PL_1	5–20 µm	5	Phytoflagellates	Net/suction/filt bottle/sieve	Nano
GFF	5 µm-GF/F filter	3	Flagellates, bacteria, cyanobacteria	5 µm sieve, GF/F-filtration	Pico/nano
DOC	<GF/F filter	3	Dissolved matter and femtoplankton	Filtration/evaporation	Femto
CB	Colonies	5	Filamentous cyanobacteria	Flotation	–

Table 2. Sampling dates, wind conditions and sample types obtained

Sampling occasion	Date (d.mo.yr)	Wind conditions during 36 h preceding sampling: Wind direction	Avg speed (m s^{-1})	Max. speed (m s^{-1})	Sample types obtained
1	30.03.1994	W-S	12.7	19	DOC, GFF, PL_1-PL_6
2	21.04.1994	S	4.6	6	DOC, GFF, PL_1-PL_5
3	04.05.1994	S	3.4	5	DOC, GFF, PL_1-PL_5
4	25.05.1994	S	4.4	8	DOC, GFF, PL_1-PL_5
5	29.06.1994	W	5.1	9	DOC, GFF, PL_1-PL_6
6	27.07.1994	S	2.5	4	DOC, GFF, PL_1-PL_6, CB
7	31.08.1994	W-NW	5.3	9	DOC, GFF, PL_1-PL_6, CB, MM, NI, AA
8	05.10.1994	W-NW	7.18	16	DOC, GFF, PL_1-PL_6, MM, NI
9	25.01.1995	SW	6.0	10	DOC, GFF, PL_1-PL_6, MM, NI

inal pore size (0.7 µm) and most particles down to bacterial size are likely to have been retained (Hickel 1984). Each filtration was ended by passing 5 ml Milli-Q water (with p.a. NaCl added to match ambient salinity) through the filter to remove inorganic carbon. The filters were wrapped in ethanol pre-washed aluminium foil and stored in glass vessels. DOC (with remaining micro-particles) was sampled by collecting the last 500 ml of filtrate in ethanol and acid-washed dark glass bottles. All samples were immediately frozen at –25°C.

The phytoplankton-dominated size-classes PL_1 to PL_3 (5 to 100 µm) were sampled by towing a buoyed and weighted 20 µm plankton net (WP2, UNESCO 1968) 1 tow along the sampling transect at 0.25 to 0.5 m s^{-1} at 5 to 10 m depth (Fig. 1). This concentrated the smallest size-classes of plankton sufficiently for analysis, while minimising contamination from the ship. The plankton suspension was continuously sucked from the cup of the net through a 35 m polythene tube (i.d. 15 mm). It passed six 4 l polypropylene vacuum Nalgene bottles with internal cylindrical mesh filters of decreasing mesh size (500, 200, 100, 50, 20 and 5 µm). The first 3 filters removed large particles, and the last 3 retained size-classes PL_3 to PL_1. This gentle continuous separation minimised induced inter-size-class grazing and predation during concentration and prevented fragments of larger organisms from contaminating the smaller size-classes. Between samplings, bottles were emptied, mesh filters rinsed, and ~50 l seawater pumped from the net (details in Rolff & Elmgren 2000). To avoid biases from diurnal vertical migration, the zooplankton-dominated size-classes PL_4 to PL_6 (100 to 1500 µm) were collected by tows from surface to near bottom with a 90 µm zooplankton net (WP2). The samples were successively, gently sieved through 500, 200 and 100 µm sieves giving size-classes PL_6 to PL_4. Samples PL_1 to PL_6 were stored on ship in 1 l polythene bottles in darkness at +1°C for up to 6 h and then further purified by repeated gentle manual sieving. During the cyanobacterial bloom, the PL_2 to PL_6 samples were cleansed from filamentous cyanobacteria (*Aphanizomenon* sp. and *Nodularia spumigena*) by repeated flotation. Mysids were collected by towing a 500 µm buoyed plankton net (WP2, with a weight on a 2 m line) parallel to the sea floor. Adults of the 15 mm size-class were pooled, 5 ind. $sample^{-1}$. Jellyfish (*Aurelia aurita*), of 40 mm diam. were collected in August from a small boat with a dip-net, pooled 5 ind. $sample^{-1}$, and wrapped in pre-washed aluminium foil. Filamentous cyanobacteria were sampled on 2 occasions (July and August) by filtering ~50 l of water through a 200 µm mesh. Collected filaments were cleansed from zooplankton by repetitive flotation in sea water. In the July samples the filaments were dark green, crisp, and looked healthy, whereas the August samples were yellow and under the microscope showed signs of decay. All samples were rinsed in isotonic Milli-Q water and immediately frozen in glass vessels at –25°C. Formalin-preserved sub-samples were studied under the microscope, and the taxonomic composition of samples PL_1 to PL_6 was estimated on a 3-level semi-quantitative scale of abundance (Table 3).

A sediment trap, with 2 collection tubes (105 × 505 mm) separated by 1 m (Larsson et al. 1986) was deployed at 20 m depth (below the thermocline) after the break-up of the ice, and was maintained from April 1994 to May 1995. Samples were collected biweekly to monthly, depending on season. Considering the short intervals between collections, the trap was unpreserved, to avoid the preservative from affecting the isotopic ratios. No smell of hydrogen sulphide was ever detected in the seston.

Sample preparation and stable-isotope analysis

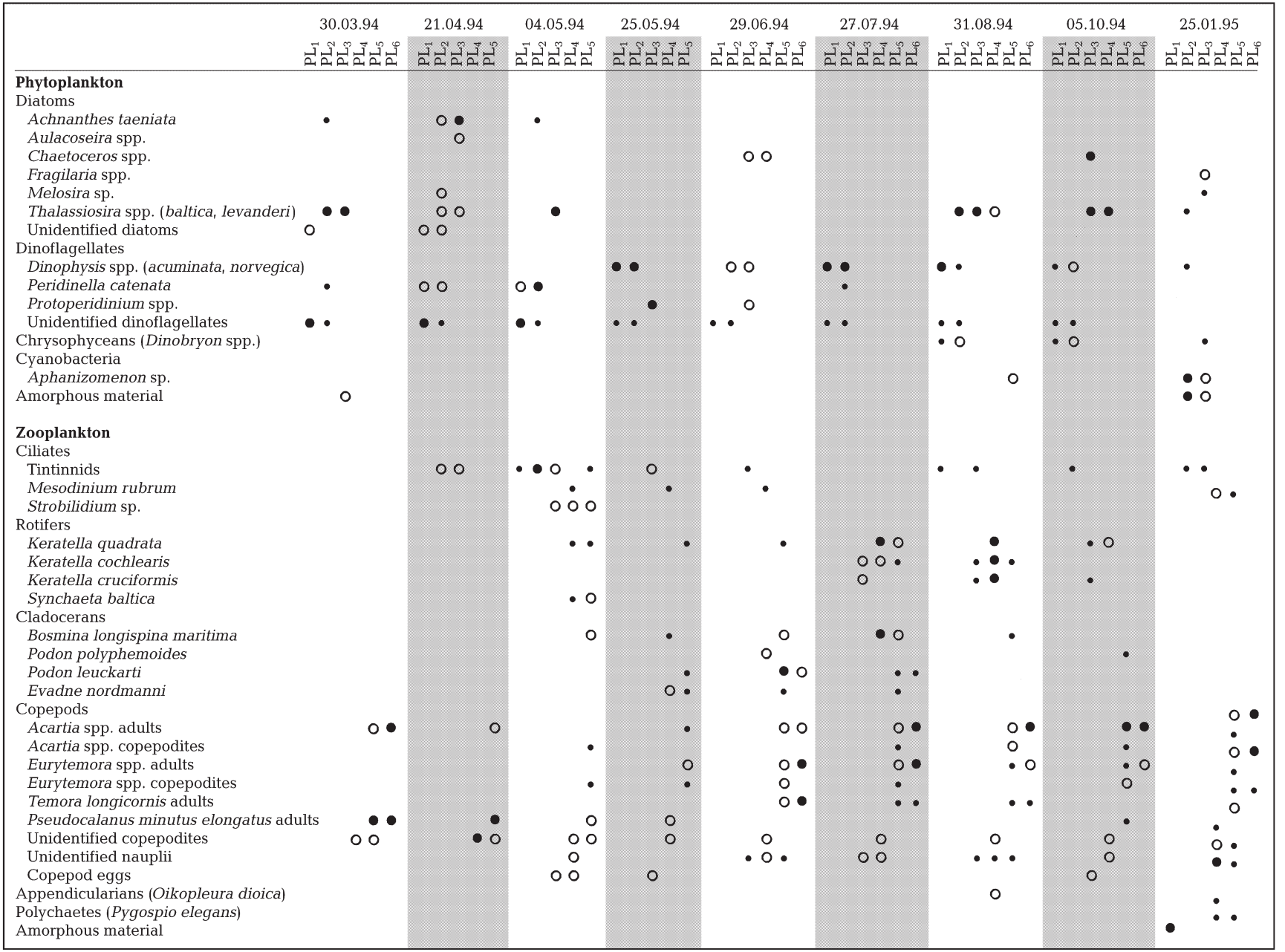
The plankton samples PL_1 to PL_6, mysids, cyanobacteria, jellyfish and sediment-trap samples were dried for 24 h at 60°C. They were not acidified, since calciferous plankton are rare in the northern Baltic Sea and since acidification has been found to change $\delta^{15}N$, but not $\delta^{13}C$, in such samples (Rolff unpubl. data), in agreement with Bunn et al. (1995). For analysis of DOC, 200 ml of filtrate was concentrated to 5 ml by vacuum evaporation at 50°C (~4 h), using water-driven ejector pumps to preclude compressor oil contamination (Fry et al. 1993). The samples were then acidified to pH 3 with HCl to remove carbonates, and dried to salt crusts at 60°C. The plankton, sediment-trap material and salt crusts were ground in an agate mortar and analysed in tin capsules. The GF/F filters were acidified with 0.01 N HCl to remove any remaining carbonates, and dried for 24 h at 60°C; one-eighth of the filter surface was analysed.

The samples were analysed on a Carlo Erba elemental analyser (E1108 CHNS-O) connected to a Fison Optima isotope-ratio mass spectrometer. Samples were run in continuous flow with a standard deviation of ±0.2‰ among replicate standard samples for both carbon and nitrogen. Analytical blanks were included and 3 working standards were run every 15 samples. Carbon and nitrogen isotopic composition are denoted by δ defined by the standard equation (1):

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000 \qquad (1)$$

where R is the ratio of heavy to light isotope (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$). Isotopic ratios are expressed relative to VPDB for $\delta^{13}C$ and to atmospheric nitrogen for $\delta^{15}N$. Analytical standards for carbon were NBS-19 (δ = +1.95‰ relative to VPDB) and IAEA-CH6 (δ =

Table 3. Semi-quantitative estimates of dominant species in samples. Abundances estimated on 3-level scale: present (•); common (○); dominant (●). Dates given as d.mo.yr



–10.43‰), and for nitrogen were IAEA-N1 (δ = +0.43‰ relative to atmospheric nitrogen) and IAEA-N2 (δ = +20.41‰). The terms 'lighter' or 'depleted' will be used for lower δ, and 'heavier' or 'enriched' for higher δ, while Δ represents the difference between the δ-values of 2 samples.

When analysing dried, salt-encrusted DOC samples, the catalytic incineration tube had to be changed every 30 samples to prevent analytical instability due to crystal formation in the tube. The method was tested on 4 analytical replicates each of 4 true-sample replicates, and gave an estimated $\delta^{13}C$ reproducible within ±0.3‰ between analytical replicates. Variability between the true replicates was no greater than in other sample types. The carbon content of the salt crusts was between 0.38 and 0.53‰ and the standard deviations of true replicates were between 0.01 and 0.04‰, indicating reproducible recovery of material. Method blanks were included and replicates were not concentrated or analysed simultaneously. The $\delta^{13}C$ values found were similar to those of comparable studies (Fry et al. 1993, 1996, Peterson et al. 1994, Guo et al. 1996, Rolff & Elmgren 2000). Since all sample processing is done in pure glass, stainless steel or agate, the method does not alter the composition of DOC (as with ultrafiltration) and does not expose the solution to large, potentially contaminating surfaces. The low salinity and high DOC concentration of Baltic water makes the method more likely to be successful in Baltic than in fully marine waters.

Statistical analyses

Differences between samples were analysed by ANOVA of raw data or data exponentially transformed by Box-Cox procedures (Sokal & Rohlf 1981) to induce normality and variance homogeneity. Values of $\delta^{13}C$ were all multiplied by –1 before ANOVA, to avoid problems in transforming negative variables. Since no positive values of $\delta^{13}C$ occurred in the original data this had

Table 4. Tukey post hoc tests of between-date differences in $\delta^{13}C$ and $\delta^{15}N$ per sample type. Box-Cox transformations indicated when used. (***p < 0.001; **p < 0.01; *p < 0.05; ns: p > 0.05). δ significantly different from preceding date is indicated by 'Y' for both carbon and nitrogen (equivalent to p < 0.05 in matrix diagonals). –: no sample

Sample, date (d.mo.yr)	$\delta^{13}C$									$\delta^{15}N$								
	$\delta_t \neq \delta_{t-1}$	30.03	21.04	04.05	25.05	29.06	27.07	31.08	05.10	$\delta_t \neq \delta_{t-1}$	30.03	21.04	04.05	25.05	29.06	27.07	31.08	05.10
DOC $(-\delta^{13}C)^{-4.6}$																		
21.04.1994	Y	*																
04.05.1994		ns	ns															
25.05.1994		ns	ns	ns														
29.06.1994		**	ns	*	ns													
27.07.1994		**	ns	*	ns	ns												
31.08.1994		***	ns	**	ns	ns	ns											
05.10.1994		***	*	***	***	ns	ns	ns										
25.01.1995		***	**	***	***	*	*	ns	ns									
GFF																		
21.04.1994											ns							
04.05.1994	Y	***									ns	ns						
25.05.1994	Y	ns		***							ns	**	ns					
29.06.1994		ns		***	ns					Y	ns	ns	ns	**				
27.07.1994	Y	***		***	***	***				Y	***	***	***	***	***			
31.08.1994	Y	***		ns	***	***	***			Y	ns	ns	ns	ns	ns	***		
05.10.1994	Y	***		***	ns	***	***	**		Y	***	ns	**	***	ns	***	**	
25.01.1995	Y	***		ns	***	***	***	ns	***	Y	ns	ns	ns	*	ns	***	ns	*
PL_1																		
21.04.1994	Y	***									ns							
04.05.1994	Y	***	***							Y	*	***						
25.05.1994	Y	***	ns	***							***	***	ns					
29.06.1994	Y	ns	***	***	***					Y	**	ns	***	***				
27.07.1994	Y	***	***	***	ns	***				Y	***	***	***	**	***			
31.08.1994		***	ns	***	ns	***	ns			Y	***	***	***	***	***	**		
05.10.1994	Y	***	ns	***	**	**	***	**		Y	***	ns	***	***	ns	***	***	
25.01.1995		***	ns	***	**	**	***	**	ns	Y	ns	ns	*	***	**	***	***	**
PL_2 $\delta^{15}N^{0.9}$																		
21.04.1994	Y	***								Y	***							
04.05.1994	Y	***	***							Y	ns	***						
25.05.1994	Y	***	***	***						Y	***	***	***					
29.06.1994	Y	*	ns	***	***					Y	ns	**	ns	***				
27.07.1994	Y	***	***	ns	***	***				Y	***	***	***	ns	***			
31.08.1994	Y	***	***	*	***	***	**			Y	***	***	***	***	***	***		
05.10.1994	Y	***	***	***	ns	***	***	***		Y	*	*	ns	***	ns	***	***	
25.01.1995	Y	***	ns	***	***	**	***	***	***		ns	***	ns	***	ns	***	***	ns
PL_3																		
21.04.1994		ns								Y	***							
04.05.1994	Y	**	**							Y	ns	***						
25.05.1994	Y	***	***	*							***	ns	ns					
29.06.1994	Y	ns	ns	***	***						***	ns	**	ns				
27.07.1994	Y	***	***	ns	ns	***				Y	***	***	***	***	***			
31.08.1994	Y	***	***	***	***	***	***			Y	*	**	ns	ns	*	***		
05.10.1994	Y	***	***	ns	ns	***	ns	***		Y	***	**	***	***	***	***	***	
25.01.1995		***	***	ns	ns	***	ns	***	ns	Y	ns	***	ns	***	***	***	*	***
PL_4 $\delta^{15}N^{0.5}$																		
21.04.1994	Y	**									ns							
04.05.1994	Y	***	***							Y	***	***						
25.05.1994	Y	***	***	***						Y	ns	ns	*					
29.06.1994	Y	***	ns	***	***						ns	ns	**	ns				
27.07.1994	Y	***	***	ns	***	***				Y	***	***	***	***	***			
31.08.1994		***	***	ns	***	***	ns			Y	***	***	***	***	***	***		
05.10.1994		***	***	*	***	***	ns	ns		Y	ns	ns	***	ns	ns	***	***	
25.01.1995	Y	***	***	ns	***	***	ns	**	**		ns	ns	***	ns	ns	***	***	ns
PL_5 $(-\delta^{13}C)^{-2.5}$, $\delta^{15}N^{1.5}$																		
21.04.1994		ns									ns							
04.05.1994	Y	***	***							Y	***	***						
25.05.1994	Y	***	***	***							***	***	ns					
29.06.1994	Y	***	**	***	***						***	***	**	ns				
27.07.1994	Y	***	***	***	ns	***				Y	***	***	***	***	***			
31.08.1994		***	***	***	ns	***	ns			Y	***	***	***	***	***	***		
05.10.1994	Y	***	***	ns	***	***	***	***		Y	ns	ns	***	***	***	***	***	
25.01.1995		***	***	ns	***	***	***	***	ns	Y	***	***	***	***	***	***	***	***
PL_6																		
29.06.1994		ns	–	–	–						ns	–	–	–				
27.07.1994	Y	***	–	–	–	***					ns	–	–	–	ns			
31.08.1994		***	–	–	–	***	ns				ns	–	–	–	ns	ns		
05.10.1994	Y	***	–	–	–	***	***	**		Y	***	–	–	–	ns	***	***	
25.01.1995	Y	***	–	–	–	***	ns	ns	***		***	–	–	–	ns	***	**	ns

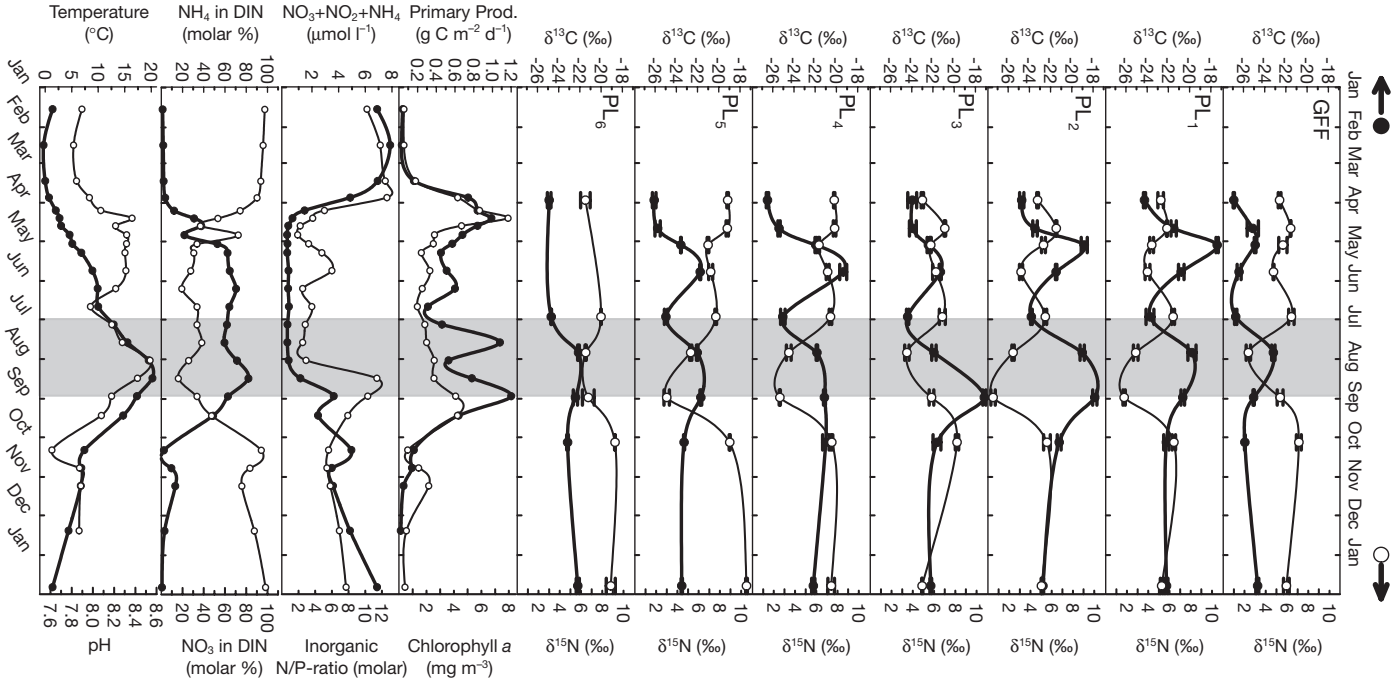


Fig. 2. Seasonal averages (±SE) of $\delta^{13}C$ and $\delta^{15}N$ of plankton in size-classes GFF to PL_6 (see Table 1 for size-class codes) and oceanographic background data (primary production, chlorophyll *a*, DIN, N/P-ratio, NH_4 and NO_3 in DIN, temperature, pH) from monitoring station. Monitoring data are integrated for top 20 m. Note that PL_6 size-class was not present at the station on some sampling occasions. Scale marks on abscissas indicate beginning of relevant months (1994 to 1995). (●) Left ordinate, (○) right ordinate. A spline function has been fitted to all variables (see 'Results'). Summer cyanobacterial bloom is indicated by grey shading

no effect on *F*-ratios. Tukey's HSD test was used for balanced post hoc tests, and the generalisation of this test (Spjøtvoll-Stoline) was used for unbalanced post hoc tests. Variance homogeneity was tested with Cochran's test, and the approximate normal distribution of the within-cell deviations from the respective cell means was tested graphically by normal probability plots. Complete ANOVA post hoc tests of differences between sampling dates are given in Table 4. For GFF, the $\delta^{13}C$ estimates of the sampling date 21 April were excluded from the ANOVA since no transformation could bring it to meet the assumption of variance homogeneity. Standard Type I linear regression was used to estimate food-chain enrichment of $\delta^{15}N$. In regressions of $\delta^{15}N$ on logarithmic size, the linearity of the regression model was tested by the *F*-ratio of MS about regression to MS within groups (Dixon & Massey 1969). Statistica for Windows 5.1, release '97 (StatSoft 1997) was used for all statistical analyses.

RESULTS

Taxonomic composition of samples

In 1994, spring was late and ice conditions severe. The spring bloom commenced in mid-March when ice was still present, but strong winds precluded sampling until 30 March, leaving the earliest stage of the spring bloom unsampled (Fig. 2). Population biomass estimates, based on cell counting, were kindly provided from 1994 monitoring data by S. Hajdu. In early spring, diatoms of the genera *Chaetoceros*, *Achnanthes* and *Thalassiosira* generally dominated (~30 to 60 mg C m^{-3}), whereas dinoflagellates (mainly *Peridinella* spp.) dominated the late-spring (~10 to 60 mg C m^{-3}) and *Dinophysis* spp. the early summer (~10 mg C m^{-3}). The characteristic Baltic summer bloom of filamentous nitrogen-fixing cyanobacteria, (*Nodularia spumigena* and *Aphanizomenon* sp. ~20 mg C m^{-3}) developed in July to August, and was succeeded by a bloom of small centric diatoms in September consisting mainly of the genera *Thalassiosira* and *Chaetoceros* (~20 mg C m^{-3}). In the early spring, the zooplankton (PL_4 to PL_6) was dominated by surviving adult copepods from the previous season. With the progression of the spring bloom, ciliates and rotifers became numerous. In early summer cladocerans dominated, and in mid-summer also rotifers. Copepod nauplii and copepodites dominated the larger size-classes during the entire productive season. In autumn the community was completely dominated by different developmental stages of copepods. In August there was some contamination of the PL_5 sample by cyanobacteria since the flotation

method was less effective for the decaying filaments. Due to the sampling method, the semi-quantitative estimates of abundance reflect the population abundance only within sample types (Table 3).

Seasonal isotopic cycle in size-fractionated plankton and DOC

A general pattern of seasonal isotopic fluctuation was found in the size-classes GFF to PL_5 (Fig. 2). The spline fitted to the averages is merely an aid to identify seasonal patterns and values between actually measured points, and should not be interpreted. Therefore, statements always refer to the nearest actual sampling date.

Carbon

The seasonal range of $\delta^{13}C$ in the size-classes GFF to PL_6, is considerable (2.8 to 7.2‰: Table 5). In the plankton samples (GFF to PL_5), a common and distinctive bimodal temporal pattern with 2 local maxima was found in $\delta^{13}C$, 1 in spring and 1 in late-summer to autumn (Fig. 2). The first was characterised by a strong enrichment of ^{13}C accompanying the progression of the spring-bloom. In the samples GFF to PL_2, carbon

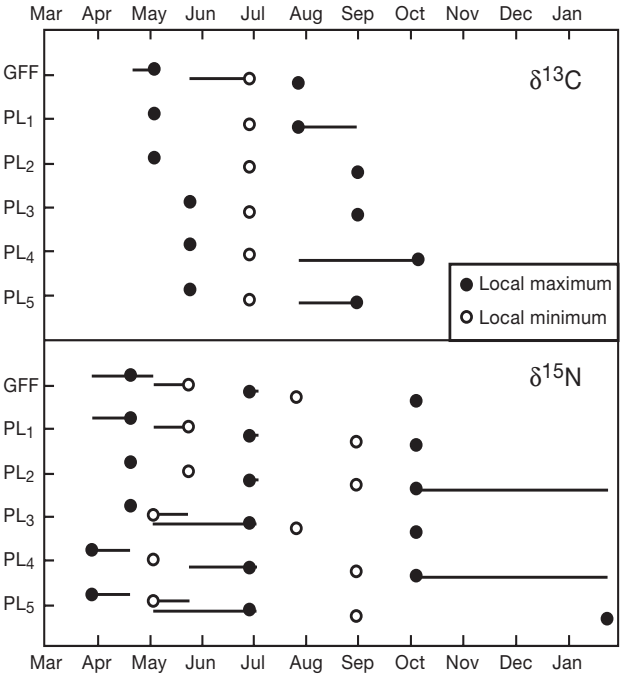


Fig. 3. Position of local minima and maxima in the annual isotopic cycle of samples GFF to PL_5 (Fig. 2). Where local extreme is not separable from 1 or several precedent or antecedent sampling occasions this is indicated by bar stretching across non-separable sampling occasions (see Table 4). Scale marks on abscissas indicate beginning of relevant months

Table 5. Means (SD) of $\delta^{13}C$ and $\delta^{15}N$ as a function of sampling occasion (d.mo.yr.) and sample type. Range: max.–min. average for each sample type. –: no sample

	30.03.1994	21.04.1994	04.05.1994	25.05.1994	29.06.1994	27.07.1994	31.08.1994	05.10.1994	25.01.1995	Range
$\delta^{13}C$ (‰)										
AA	–	–	–	–	–	–	–20.0 (0.40)	–	–	–
MM	–	–	–	–	–	–	–21.2 (0.29)	–20.7 (0.17)	–21.2 (0.21)	0.5
NI	–	–	–	–	–	–	–19.4 (0.40)	–19.5 (1.10)	–20.5 (0.18)	1.1
PL_6	–24.9 (0.37)	–	–	–	–24.7 (0.01)	–22.1 (0.17)	–22.4 (0.44)	–23.2 (0.17)	–22.2 (0.34)	2.8
PL_5	–26.1 (0.38)	–25.8 (0.55)	–23.6 (0.07)	–21.8 (0.10)	–25.0 (0.32)	–22.0 (0.15)	–21.7 (0.20)	–23.3 (0.11)	–23.5 (0.15)	4.4
PL_4	–26.5 (0.24)	–25.4 (0.25)	–21.9 (0.18)	–19.4 (0.75)	–25.1 (0.46)	–21.8 (0.16)	–21.1 (0.20)	–21.0 (0.67)	–22.2 (0.33)	7.1
PL_3	–24.0 (0.94)	–23.9 (0.72)	–22.4 (0.26)	–21.3 (0.19)	–24.4 (0.25)	–21.9 (0.47)	–17.2 (0.33)	–21.6 (0.83)	–22.2 (0.19)	7.2
PL_2	–24.7 (0.45)	–23.5 (0.47)	–18.8 (0.64)	–21.5 (0.17)	–23.8 (0.17)	–19.0 (0.45)	–17.8 (0.46)	–21.2 (0.41)	–22.7 (0.14)	6.9
PL_1	–24.2 (0.22)	–21.5 (0.55)	–17.4 (0.24)	–20.8 (0.58)	–23.7 (0.82)	–19.8 (0.85)	–20.6 (0.38)	–22.2 (0.56)	–22.2 (0.31)	6.8
GFF	–26.9 (0.11)	–25.1 (0.91)	–24.9 (0.15)	–26.4 (0.32)	–26.7 (0.19)	–23.2 (0.27)	–25.0 (0.11)	–25.9 (0.06)	–24.7 (0.18)	3.7
DOC	–28.1 (1.57)	–26.0 (0.53)	–27.3 (0.17)	–26.9 (1.07)	–25.6 (0.16)	–25.5 (0.08)	–25.3 (0.05)	–24.5 (0.11)	–24.3 (0.04)	3.8
CB	–	–	–	–	–	–25.3 (0.24)	–22.9 (0.18)	–	–	2.4
$\delta^{15}N$ (‰)										
AA	–	–	–	–	–	–	5.8 (0.85)	–	–	–
MM	–	–	–	–	–	–	10.1 (0.22)	8.8 (0.25)	8.4 (0.33)	1.7
NI	–	–	–	–	–	–	8.7 (0.38)	8.1 (0.29)	9.0 (0.24)	0.9
PL_6	6.4 (1.01)	–	–	–	7.9 (0.01)	6.4 (0.14)	6.7 (1.23)	9.2 (0.08)	8.8 (0.94)	2.8
PL_5	8.7 (0.16)	8.7 (0.25)	6.9 (0.17)	7.1 (0.41)	7.6 (0.18)	5.3 (0.22)	3.0 (0.55)	8.9 (0.11)	10.5 (0.17)	7.5
PL_4	7.7 (0.13)	7.7 (0.26)	6.3 (0.10)	7.1 (0.32)	7.3 (0.27)	3.4 (0.48)	2.6 (0.14)	7.5 (0.19)	7.4 (0.70)	5.1
PL_3	4.9 (0.24)	7.0 (0.19)	5.7 (0.11)	6.2 (0.70)	6.8 (0.63)	3.4 (0.46)	5.8 (0.48)	8.2 (0.36)	4.9 (0.07)	4.8
PL_2	4.7 (0.18)	6.4 (0.14)	5.2 (0.41)	3.1 (0.32)	5.4 (0.30)	2.4 (0.24)	0.5 (0.48)	5.5 (0.68)	5.0 (0.06)	5.9
PL_1	5.2 (0.59)	5.8 (0.11)	4.3 (0.39)	3.9 (0.52)	6.3 (0.29)	2.8 (0.50)	1.7 (0.24)	6.4 (0.35)	5.3 (0.27)	4.7
GFF	5.3 (0.22)	6.3 (0.07)	5.6 (0.67)	4.7 (0.07)	6.4 (0.43)	2.4 (0.36)	5.3 (0.46)	7.1 (0.40)	5.9 (0.47)	4.7
CB	–	–	–	–	–	–1.7 (0.05)	4.5 (0.08)	–	–	6.2

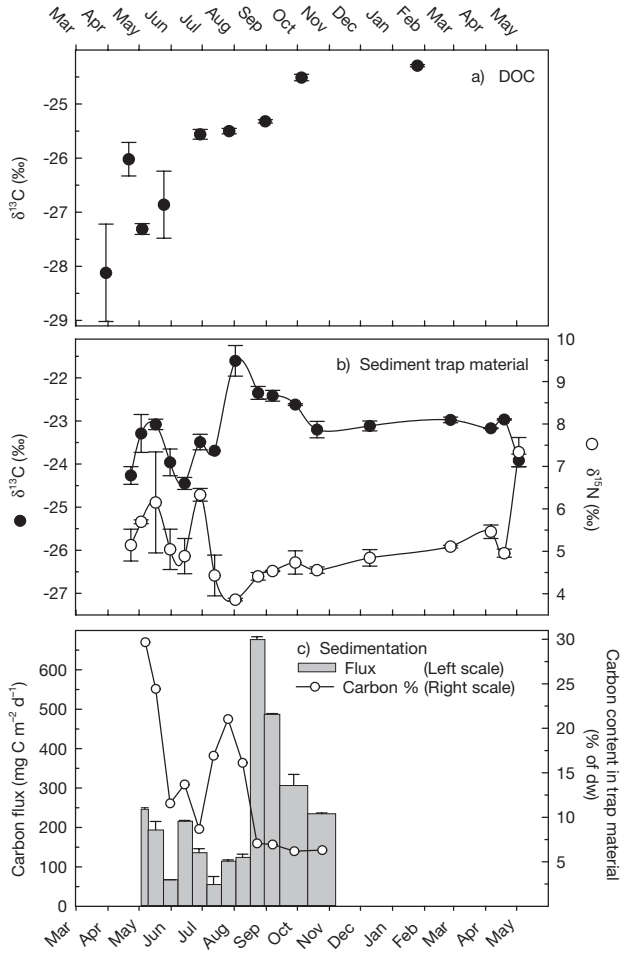


Fig. 4. Averages (SE) from 1994 to 1995 of (a) $\delta^{13}C$ in COC, and (b) $\delta^{13}C$ and $\delta^{15}N$ in sediment-trap material. (c) Carbon flux and salt-corrected carbon content as percent of dry weight in sediment-trap material. Scale marks on abscissas indicate beginning of relevant months

was most enriched in early May, but in samples PL_3 to PL_5 the peak occurred at the end of May (Fig. 3). For all samples (GFF to PL_5) $\delta^{13}C$ reached a synchronous local minimum in late June. The autumn maximum occurred in August to October and was initiated in the smallest size-fractions (GFF and PL_1). It was broader than the spring maximum (Fig. 2), and non-significant differences between sampling occasions were more common (Fig. 3, Table 4).

In DOC, a gradual enrichment of $\delta^{13}C$ was found, from –28.1‰ in the early spring to –24.3‰ in the winter (Table 5), accompanied by a decreasing trend of within-sample variability. There was no clear seasonal $\delta^{13}C$ cycle in DOC, corresponding to the plankton samples (Fig. 4a). The ANOVA post hoc tests (Table 4) showed few significant differences between consecutive samplings. DOC-$\delta^{13}C$ was more variable during the spring bloom than in the other periods.

Nitrogen

In the size-classes GFF to PL_6 the seasonal range of $\delta^{15}N$ was between 2.8 and 7.5‰ (Table 5). The cycle was less distinct than that seen in $\delta^{13}C$, but was generally trimodal, with 3 local maxima (Fig. 2). With some time lags, the maxima seen in $\delta^{15}N$ coincided with the minima of $\delta^{13}C$, and vice versa (Fig. 2). The first local maximum occurred at the beginning of April and the first local minimum in early to mid-May (Fig. 3). The larger size-classes (PL_4 and PL_5) appeared to reach their local extremes earlier than the smaller size-classes. For all size-classes, a synchronous local maximum coincided with the local $\delta^{13}C$ minimum in late June. The late-summer local $\delta^{15}N$ minimum was the most pronounced in all size-classes. It varied in time between size-classes, but was statistically distinct (Table 4) in all size-classes, and coincided with the development of a large cyanobacterial bloom. The nitrogen isotopic composition of pure cyanobacteria (CB, Table 5) from the earlier part of the bloom was considerably depleted (–1.7‰), indicating uptake of depleted gaseous nitrogen through nitrogen-fixation. The cycle was ended by a broad local autumn maximum and a return to more depleted $\delta^{15}N$ in winter.

Sediment traps

The isotopic ratios found in sediment-trap material were less variable on a seasonal basis than those found in plankton. Averages of $\delta^{13}C$ between –24.5 and –21.6‰ were found, and of $\delta^{15}N$ between 3.9 and 7.3‰ (Table 6).The sediment traps covered a longer period than the other samples but were only replicated by 2 collection tubes and the trends (Fig. 4b) were therefore uncertain from a statistical point of view (Table 6). The only clear trend was that $\delta^{13}C$ of seston became more enriched from spring to autumn and then became more depleted again. In nitrogen the opposite occurred, with seston $\delta^{15}N$ becoming more depleted in summer than in spring, and then returning to a more enriched state. Since only 2 replicates exist per period, the power of the post hoc tests was low. In the second spring bloom (1995), $\delta^{13}C$ returned to the levels seen in the 1994 samples, whereas $\delta^{15}N$ appeared to become more enriched during the second spring bloom. High carbon content in spring seston coincided with more enriched carbon and nitrogen isotopes, whereas the same situation in the summer coincided with enriched $\delta^{13}C$ and depleted $\delta^{15}N$. Low carbon content and high fluxes in the autumn sediment trap (Fig. 4c) indicate resuspension (Blomqvist & Larsson 1994), which had no clear impact on either isotopic ratio. The variabil-

Table 6. Averages and standard deviation (n = 2) of $\delta^{13}C$ and $\delta^{15}N$ in sediment-trap material and ANOVA post hoc tests for all periods. Date and dot indicate middle of sampling period; vertical bars indicate the distance to nearest significantly different sampling occasion ($p < 0.05$). For $\delta^{13}C$ of 12 July 1994 one replicate was lost, which prevented testing of this date. For $\delta^{15}N$ a Box-Cox transformation ($\delta^{15}N^{-1.57}$) was applied in the test to eliminate non-homogeneous variances

Date	$\delta^{13}C$			$\delta^{15}N$		
(d.mo.yr)	Avg	(SD)	Nearest significant neighbour	Avg	(SD)	Nearest significant neighbour
22.04.1994	–24.3	(0.29)		5.1	(0.53)	
03.05.1994	–23.3	(0.62)		5.7	(0.06)	
17.05.1994	–23.1	(0.17)		6.2	(1.68)	
31.05.1994	–24.0	(0.44)		5.0	(0.67)	
14.06.1994	–24.5	(0.20)		4.9	(0.59)	
28.06.1994	–23.5	(0.25)		6.3	(0.21)	
12.07.1994	–23.7			4.4	(0.68)	
02.08.1994	–21.6	(0.50)		3.9	(0.04)	
23.08.1994	–22.4	(0.21)		4.4	(0.13)	
07.09.1994	–22.4	(0.18)		4.5	(0.02)	
29.09.1994	–22.6	(0.03)		4.7	(0.39)	
19.10.1994	–23.2	(0.27)		4.6	(0.11)	
10.12.1994	–23.1	(0.16)		4.8	(0.28)	
26.02.1995	–23.0	(0.09)		5.1	(0.04)	
05.04.1995	–23.2	(0.01)		5.5	(0.23)	
19.04.1995	–23.0	(0.02)		5.0	(0.13)	
03.05.1995	–23.9	(0.21)		7.3	(0.49)	

ity in $\delta^{13}C$ and particularly $\delta^{15}N$ was greater during the spring bloom than during the summer and winter periods.

Size-dependence of $\delta^{13}C$ and $\delta^{15}N$

The dependence of isotopic composition on logarithmic particle size (excluding filamentous cyanobacteria) was analysed separately for each sampling occasion (Fig. 5). The unknown size-class of the DOC fraction is for graphical reasons indicated in Fig. 5 as ~0.1 µm, which is likely to be an overestimate. The midpoint between mesh sizes (Table 1) was used as an estimate of average particle size in the size-classes GFF to PL_5. For the other organisms (PL_6, *Mysis mixta*, *Neomysis integer* and *Aurelia aurita*), their average length as measured in the samples was used.

Carbon

The maximum range (DOC to PL_1) between averages of $\delta^{13}C$ in different size-classes on a single sampling occasion (4 May) was 9.9‰ (Table 5). The carbon isotopic composition of DOC was generally slightly more depleted than that of the smallest particles (GFF). Coinciding with the local maxima of $\delta^{13}C$ in spring and autumn a strong enrichment of $\delta^{13}C$ was found in the smallest size-classes, GFF to PL_1 or PL_2 (Fig. 5). At the spring maximum of $\delta^{13}C$ (4 May) the difference ($\Delta^{13}C$) between GFF and PL_1 was 7.5‰, and at the autumn peak (31 August) $\Delta^{13}C$ of GFF to PL_2 was 7.2‰. There was generally a declining or no clear trend in $\delta^{13}C$ above PL_1. The 2 species of mysids and the jellyfish *Aurelia aurita* were not statistically different in their carbon isotopic composition.

Nitrogen

Since the inorganic molar N/P-ratio of the upper 20 m was less than the Redfield ratio of 16:1 (Fig. 2), nitrogen is considered to have been the limiting nutrient during the entire phytoplankton production cycle. The maximal encountered range of $\delta^{15}N$ averages within 1 sampling date (31 August, PL_2 to MM) was 9.6‰, and the size-dependence was clearer than in $\delta^{13}C$. Generally the GFF samples were more enriched than PL_1 samples, but from PL_2 or PL_3 on, there was generally a continuous enrichment of $\delta^{15}N$ with increasing organism size. This trend is broken by the late-summer diatom bloom reflected in the PL_3 samples of 27 July to 5 October. The 2 mysid species were the most enriched organisms during the summer period. In winter samples, PL_5 to mysids were equally enriched. The mysid species were well separated on all 3 sampling occasions (ANOVA post hoc, $p < 0.01$) but with a significant interaction between time and species ($p < 0.001$). On the last sampling occasion *Neomysis integer* was more enriched than *Mysis mixta*, but on the 2 previous occasions their

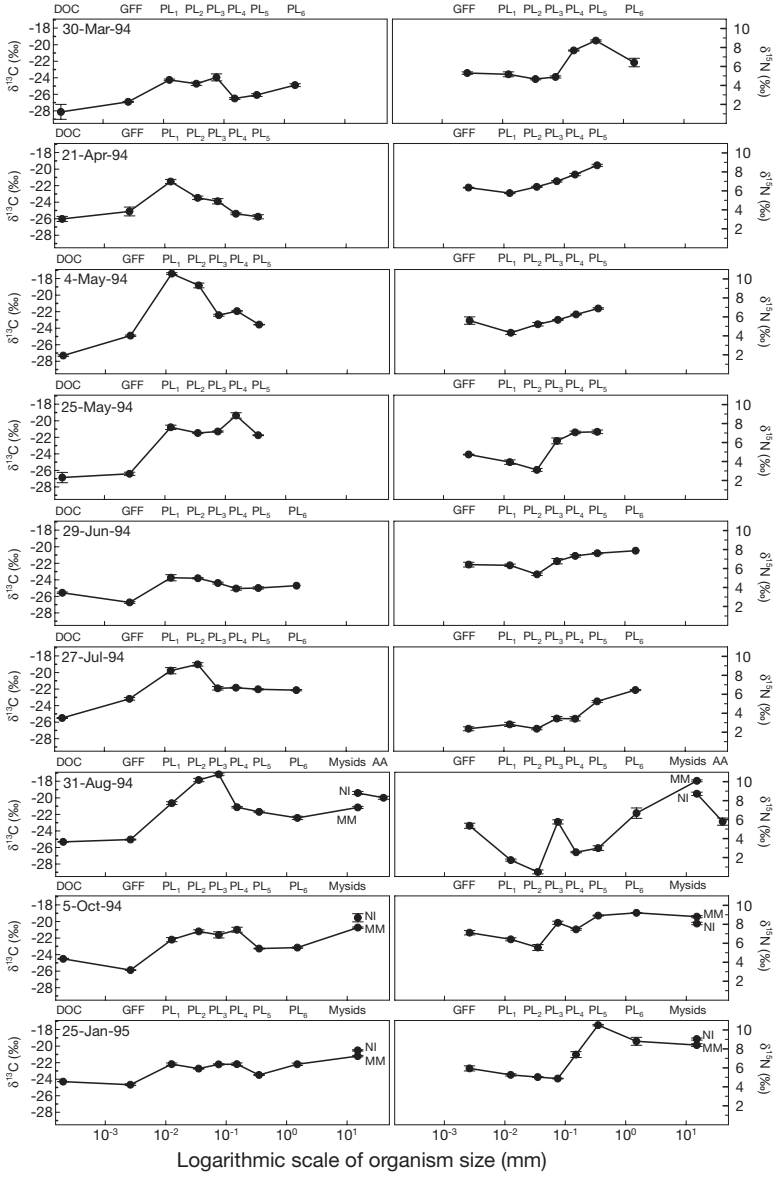


Fig. 5. Logarithmic size-dependence of isotopic ratios in all samples for each sampling date (averages ±SE). Left graphs: $\delta^{13}C$; right graphs: $\delta^{15}N$. Bottom abscissa of figure shows logarithmic particle size; upper abscissa of each graph shows position of sample types. Note there is no dissolved fraction for $\delta^{15}N$

interrelation was the opposite, precluding any conclusive inferences concerning their trophic interrelation. The $\Delta^{15}N$ between them was comparatively small (<1.3‰), and both appeared to be planktonic top-predators in agreement with previous findings (Rudstam et al. 1989). *Aurelia aurita* occupied a markedly lower trophic (~3.6‰ more depleted) position than mysids.

Food chain

The isotopic information suggests 2 trophic structures in the plankton. The smallest size-fractions (DOC or GFF to PL_1) are generally characterised by strong enrichment of $\delta^{13}C$ and depletion of $\delta^{15}N$ with increasing size. In the larger organisms (PL_2 or PL_3 to mysids), $\delta^{13}C$ is variable and $\delta^{15}N$ enriched with increasing size. It is reasonable (Tables 1 & 3) to assume that these 2 structures correspond to the micro-heterotrophic food-web and the classical phytoplankton-based micro- and mesozooplankton food-web, respectively. The taxonomic identification and size resolution

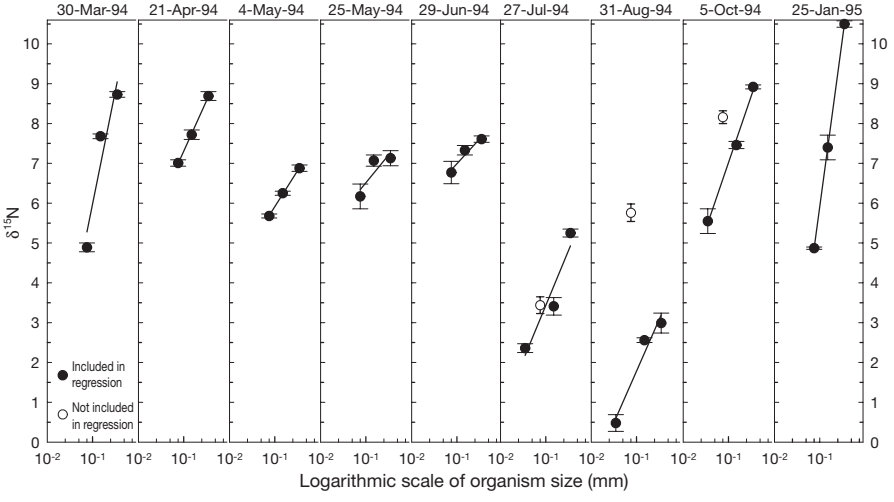


Fig. 6. Regressions of $\delta^{15}N$ on logarithm of organism size ($\delta^{15}N$ = slope × log(mm) + intercept) for each sampling date. For all dates except 27 July, 31 August and 5 October, regressions were calculated using Samples PL_3 to PL_5. In the latter 3 cases, PL_2 sample was used (see 'Results'); position of PL_3 sample in those graphs is indicated by open symbol. Averages (±SE) are indicated, but regressions were calculated using all replicates (Table 1). Significance levels and parameters of regressions are given in Table 7

Table 7. Statistics and parameter (slope, R^2) estimates of $\delta^{15}N$ regressions on logarithm of organism size (Fig. 6). Deviations from linearity in slope were found on 2 occasions ('Methods')

Date (d.mo.yr)	Samples included in regression	Slope	SE of slope	R^2	Significance of slope H_0: $\beta = 0$ $F_{(1,13)}$	p	Deviations from linearity H_0: line is straight $F_{(1,12)}$	p
30.03.1994	PL_3–PL_5	5.63	(0.54)	0.89	110.4	***	110.6	***
21.04.1994	PL_3–PL_5	2.51	(0.22)	0.91	136.5	***	0.1	ns
04.05.1994	PL_3–PL_5	1.79	(0.12)	0.94	222.4	***	0.17	ns
25.05.1994	PL_3–PL_5	1.38	(0.51)	0.36	7.3	*	2.82	ns
29.06.1994	PL_3–PL_5	1.23	(0.38)	0.45	10.6	**	0.63	ns
27.07.1994	PL_2, PL_4–PL_5	2.76	(0.32)	0.85	75.2	***	17.59	**
31.08.1994	PL_2, PL_4–PL_5	2.60	(0.30)	0.85	74.8	***	4.25	ns
05.10.1994	PL_2, PL_4–PL_5	3.33	(0.26)	0.93	166.3	***	0.85	ns
25.01.1995	PL_3–PL_5	8.40	(0.38)	0.97	496.0	***	0.001	ns

were not sufficient to analyse food-chain dynamics in the smallest size-classes (Samples DOC to PL_1), but the isotopic patterns agree with little transport of material from the microheterotrophic food-web to the mesozooplankton community (Ducklow et al. 1986).

In the interval 20 to 500 µm, $\delta^{15}N$ increased linearly with the logarithm of organism size, indicating size-dependent consumption (Fig. 6). Only samples obtained on every sampling occasion were included in the regressions to facilitate comparison of regression slopes (Table 7). PL_3 was generally the sample most clearly dominated by autotrophs (Table 3), whereas unidentified flagellates with possible heterotrophic tendencies were often present in the PL_2 samples. Size regressions were therefore generally performed on samples PL_3 to PL_5 (Table 7). An exception was made for the late summer and autumn bloom of small diatoms, were the $\delta^{15}N$ position of the PL_3 samples distinctly diverged from the general pattern (Fig. 5). The PL_3 sample of 27 July consisted of an almost pure sample of rotifers (*Keratella* spp.) and copepod nauplii. On these occasions the PL_2 samples were more representative of autotrophic phytoplankton and replaced the PL_3 samples in the regressions (Table 7, Fig. 6). All regressions were significant ($p < 0.05$ or better), slopes were between ~1.2 and ~8.4, and (R^2) between 97 and 36%. Slopes and R^2 showed a similar seasonal pattern, with steep slopes in spring and autumn, coinciding with high regression-explained variability (Fig. 7). On 2 occasions significant regressions with high R^2 deviated from linearity (Table 7), indicating that the linear logarithmic dependence between size and $\delta^{15}N$ was less accurate on these sampling occasions.

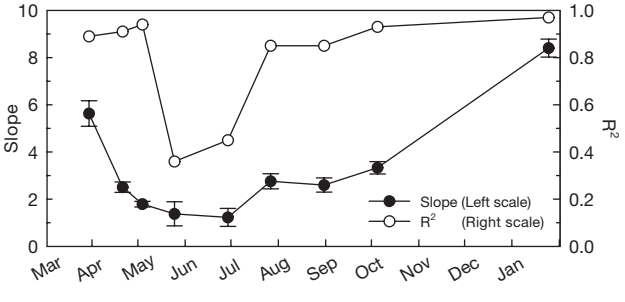


Fig. 7. Seasonal changes in slopes (±SE) and regression-explained variability (R^2) in regressions of $\delta^{15}N$ on logarithm of organism size (Fig. 6, Table 7). Scale marks on abscissa indicate beginning of relevant months

DISCUSSION

Seasonal isotopic cycles

A statistically valid description of seasonal changes in plankton isotopic composition was achieved by the ANOVA design used in this study. It aimed at reliably describing the seasonal cycle per se and the propagation of isotopic signals in different size-fractions and was therefore less suited for correlation studies between the isotopic cycles in the size-classes, or with the environmental factors potentially affecting them. Correlation requires high frequency in sampling and evenly spaced representation along an entire gradient. Cyclic phenomena with lags generally lead to elliptic or irregular patterns in phase-plots (1 variable plotted against the other). Correlation studies of such data may, therefore, by small lags, change drastically from complete correlation when the cycles are in phase or antiphase to very weak correlation in intermediate situations. The apparent negative dependence of the $\delta^{13}C$ and $\delta^{15}N$ cycles in the plankton was therefore not analysed by correlation. A similar situation occurs when comparing the isotopic cycles with other oceanographic variables (Fig. 2). Several significant and high correlations were found, but were highly dependent on which time periods were included. Correlation is also likely to be flawed, since the causes for isotopic

fluctuation will differ between seasons. The ample background data does however suggest explanatory patterns, and the seasonal cycles will therefore be discussed in relation to some known causes of isotopic fluctuations.

The trend in DOC differed from all plankton samples, and $\delta^{13}C$ became increasingly heavy during the study (Fig. 4a), accompanied by decreasing within-sample variance. A trend resembling the first phytoplankton maximum was found in the first 4 samples (Fig. 2), suggesting that the phytoplankton production affected $\delta^{13}C$ in DOC. The DOC concentration is comparatively high in the Baltic Sea (~3 to 5 mg C l^{-1}) and relatively stable year-round. Zweifel et al. (1995) found an annual variation of ~20% in the Bothnian Bay. Since exudates are used almost instantaneously by bacteria (Larsson & Hagström 1979), it is unlikely that exudates per se would quantitatively affect bulk DOC in the way suggested by $\delta^{13}C$. The zooplankton population is low in this period and excretion is equally unlikely to quantitatively influence DOC. A plausible explanation is therefore leaching of organic matter, with light isotopic composition (e.g. lipids), from dead or decaying phytoplankton cells during high production and low grazing. Leaching from the organic matter on the filter is unlikely, since low vacuum was used and no dependence was found between the average amount of carbon per filter, and the variance of $\delta^{13}C$ in DOC (Spearman rank correlation p = 0.90).

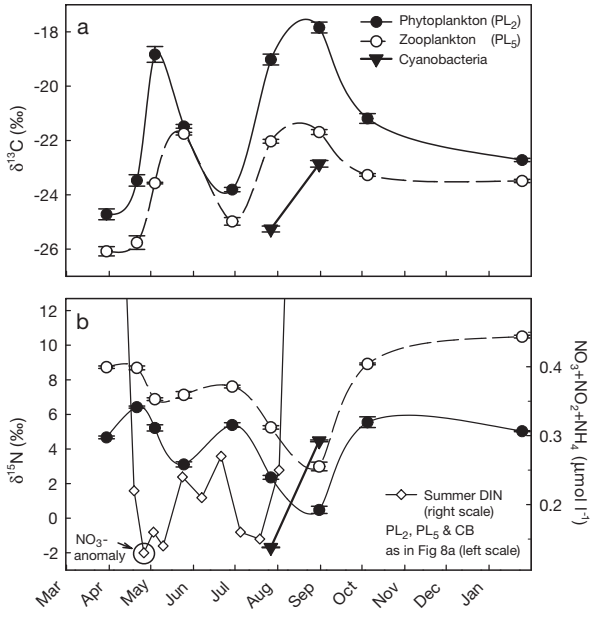


Fig. 8. Isotopic composition of phytoplankton (PL_2) zooplankton (PL_5) and cyanobacteria (CB). (a) $\delta^{13}C$; (b) $\delta^{15}N$ and summer concentrations of dissolved inorganic nitrogen (DIN) in the top 20 m (NO_3-anomaly is indicated, cf. Fig. 2). Scale marks on abscissa indicate beginning of relevant months

Statistically validated bi- and trimodal patterns were found in $\delta^{13}C$ and $\delta^{15}N$, respectively and occurred in all size-classes from GFF to PL_5 (Figs. 2 & 3, Table 4). The spring bloom caused a time lag between phytoplankton and zooplankton isotopic signals (Fig. 8). In the middle of the summer, tight coupling between primary and secondary production appear to have brought the isotopic cycles of phyto- and zooplankton in phase. In $\delta^{13}C$ the local maxima of the smaller size-classes precedes those of the larger size-classes (Fig. 3), in agreement with the assumption that $\delta_{consumers}$ is dependent on δ_{food}. In $\delta^{15}N$ this relation was less clear and the local extremes were also less distinct (Fig. 3), possibly caused by slower turnover rate in nitrogen-rich tissue, creating an isotopic 'memory'. Hesslein et al. (1993) however found similar replacement rates of sulphur, carbon and nitrogen isotopes in broad whitefish, whereas Fry & Arnold (1982) found rapid replacement of carbon isotopes in brown shrimp. Generally $\delta^{13}C$ was more depleted and had a smaller amplitude in the GFF samples than in other plankton samples. These samples are likely to be dominated by flagellates, cyanobacteria and bacteria, of which the latter appear to be highly variable in $\delta^{13}C$ (Coffin et al. 1989).

Carbon

Dissolved carbon dioxide in ocean surface water is negatively correlated to $\delta^{13}C$ in marine POM. This may be proximately caused by the influence of temperature on dissolved CO_2 (Rau et al. 1989, 1992, Kennedy & Robertson 1995, Dehairs et al. 1997, Korb et al. 1998). Factors influencing $\delta^{13}C$ in marine diatoms were reviewed by Fry (1996), who found the external CO_2 concentration to have the strongest demonstrated effect of several investigated factors (temperature, salinity, pH, growth rate and CO_2 supply), whereas Thompson & Calvert (1994), in controlled cultures, found irradiance and daylength to be more important. In stagnant, eutrophicated lakes, where carbonate buffering is less efficient than in marine water, CO_2 supply often limits phytoplankton growth. The assimilation reduces CO_2 concentration, which is reflected in heavier phytoplankton during blooms (Yoshioka et al. 1994, Zohary et al. 1994, Gu & Schelske 1996, Gu et al. 1996). During bloom periods with nutrient excess, CO_2 availability can limit phytoplankton production also in marine systems (Riebesell et al. 1993) and high growth rate appears to cause enrichment of ^{13}C also in marine phytoplankton (Fry & Wainright 1991, Rau et al. 1996).

The bimodal pattern seen in plankton $\delta^{13}C$ agrees well with carbon enrichment in the remaining CO_2 during bloom periods (Fig. 2). Both $\delta^{13}C$ maxima coin-

cided with the peaks of primary production, chlorophyll *a* and high pH. Exhaustion of CO_2(aq) by the vigorous spring bloom (maximally ~1 g C m^{-2} d^{-1}) causes pH to increase from ~7.8 to ~8.5 (Fig. 2). Assimilation causes remaining CO_2 to become enriched in ^{13}C, leading to progressively enriched phytoplankton. At high pH, a greater proportion of dissolved inorganic carbon occurs as bicarbonate which is generally, depending on temperature, ~8 to 9‰ more enriched than CO_2 (Tan 1989, Hayes 1993, Thompson & Calvert 1994). Use of bicarbonate by phytoplankton would thus further enrich their carbon isotopic composition. To what extent this occurred in the present study is not known. A consequence of assuming incipient CO_2 limitation as the cause of the 2 local maxima seen in $\delta^{13}C$ (Fig. 5), is that the enrichment should be more pronounced in the larger size-class of phytoplankton (PL_3), since their less favourable surface to volume ratio should cause steeper CO_2 gradients. The results however suggest the opposite, with generally more pronounced enrichment in the smaller phytoplankton size-classes (Figs. 2 & 5). Leboulanger et al. (1995) found considerably enriched $\delta^{13}C$ (–12.7‰) in a culture of the strictly heterotrophic dinoflagellate *Crypthecodinium cohnii*, and depleted carbon may have been lost in heterotrophic respiration by flagellates in the small size-classes (PL_1 and PL_2). The quantitative extent to which heterotrophy occurred in these size-fractions in the present investigation was not assessed.

The local $\delta^{13}C$ minimum seen in summer coincided with the period when zooplankton production is normally high (Johansson 1992) and exchange with deeper water was limited by a distinct thermocline. The nitrogen supply shifts from NO_3 which has been exhausted, to NH_4 (Fig. 2) supplied by excretion from zooplankton who also (by respiration) supply isotopically depleted CO_2(aq) (DeNiro & Epstein 1978, McConnaughey & McRoy 1979, Checkley & Enzenroth 1985). Primary production using respired carbon also becomes isotopically depleted, and the summer local minimum of $\delta^{13}C$ coincided with a local pH minimum of ~8 (Fig. 2). The species composition also shifted from mainly diatoms to small dinoflagellates (Table 3). Rapidly growing diatoms have been suggested to fractionate carbon less effectively than other phytoplankton (Fry & Wainright 1991, Wainright & Fry 1994), whereas Montoya & McCarthy (1995) suggested that the motility of flagellates, making them less sensitive to nutrient limitation, may also affect their isotopic fractionation. The latter study considered nitrogen fractionation in nitrate uptake but the principle should also be valid for carbon. The shift in species composition may therefore also contribute to the summer local minimum of $\delta^{13}C$.

The summer local minimum was succeeded by a progressive enrichment of $\delta^{13}C$ leading to the broad autumn local maximum. The onset of this enrichment coincided with the extensive summer bloom of nitrogen-fixing cyanobacteria (*Aphanizomenon* sp. and *Nodularia spumigena*), characteristic of the Baltic (Karhu et al. 1994). The information on $\delta^{13}C$ in cyanobacteria is scarce and contradictory. Two investigations of marine species found carbon to be more depleted than in other phytoplankton (Falkowski 1991, Wainright & Fry 1994). Falkowski found extremely depleted carbon isotopes ($\delta^{13}C$ = –28.8‰), whereas Leboulanger et al. (1995) found a culture of *Synechocystis* sp. to be generally more enriched ($\delta^{13}C$ = –14.4‰) than phytoplankton from other phylogenetic groups. Three limnic field studies also found cyanobacteria to be enriched in ^{13}C (Estep & Vigg 1985, Boon et al. 1994, Gu & Schelske 1996). In the present investigation, $\delta^{13}C$ in cyanobacteria (heavily dominated by *Aphanizomenon* sp.) was always more depleted than in other contemporary phytoplankton samples (Table 5, Fig. 8a). Since all samples above PL_1 were cleansed from cyanobacteria with gas vacuoles, the size-fractionated phytoplankton samples represent the isotopic composition in other groups, mainly dinoflagellates and diatoms (Table 3). Disintegration of the cyanobacterial bloom started in early August, and the cyanobacterial samples of 31 August showed signs of decay and were more enriched than the July samples.

A vigorous late-summer diatom bloom succeeded the cyanobacterial bloom. From the onset of the cyanobacterial bloom at the end of June, pH increased continuously to a maximum of ~8.5, which coincided with the temperature maximum of ~20°C in the top 20 m. The peaks of daily primary production in these 2 blooms (maximally ~1.2 g C m^{-2} d^{-1}) exceeded that of the spring bloom. It is likely that renewed incipient CO_2 limitation caused enrichment of $\delta^{13}C$ in the phytoplankton. The $\delta^{13}C$ of the zooplankton was lighter than would be expected from the non-cyanobacterial phytoplankton samples from this period, suggesting use of depleted cyanobacterial carbon. Particularly the PL_4 and PL_5 samples were rich in rotifers and cladocerans and had comparatively depleted $\delta^{13}C$. *Bosmina longispina maritima* has been shown to utilise both *Aphanizomenon* sp. and *Nodularia spumigena* under experimental conditions (Sellner et al. 1994).

Nitrogen

The tri-modal $\delta^{15}N$ cycle was more complex than that of $\delta^{13}C$. The first local maximum in early spring (Fig. 2) is in agreement with what is generally found during blooms in marine and limnic environments (Goering

et al. 1990, Wainright & Fry 1994, Yoshioka et al. 1994, Gu et al. 1996, Voss et al. 1996). As the nitrate stock is used up by the bloom, the remaining nitrate becomes enriched by discriminative phytoplankton uptake, causing progressively enriched phytoplankton. Since the zooplankton biomass is low, most of the bloom sediments out of the water column, removing ^{15}N-enriched diatoms (Fig. 4b,c). As the nitrate is consumed and the zooplankton population increases, the nitrogen supply (DIN) becomes dominated by depleted ammonium nitrogen excreted by heterotrophs (Fig. 2).

The second local maximum in late June can be given several interpretations, but a detailed explanation requires more data. Other published seasonal studies of $\delta^{15}N$ in plankton, seston or sediment have generally suggested a bimodal annual cycle (Gu et al. 1994, Wainright & Fry 1994, Yoshioka et al. 1994), but a recent study suggested a trimodal $\delta^{15}N$ seasonal pattern in coastal marine sediment-trap material (Ostrom et al. 1997). The seasonal patterns found in these studies were however not statistically tested. In this study, the second local minimum is statistically separable from the first and second local maxima in all size-classes, and some possible explanations will therefore be briefly discussed. Following the first $\delta^{15}N$ maximum, the shift from nitrate to ammonium dominance was briefly broken by a period of new nitrate dominance (Fig. 2). Cold weather and persistent, northeasterly winds (~9 m s^{-1}) eroded the weak thermocline and temporarily caused upwelling (Fig. 1). It cannot be excluded that the first local minimum was caused by replenishment of depleted nitrate. There was however no evident effect on primary production (Fig. 2), and total DIN was lower than in the week preceding the anomaly (~0.1 vs ~0.2 µmol l^{-1}, respectively, Fig. 8b). It is therefore more likely that the renewed dominance of nitrate was of short duration and that the depleted $\delta^{15}N$ in primary-produced material was caused by a shift to regenerated ammonium. Ammonium excreted by copepods has been found to be ~3‰ depleted in relation to the animal (Checkley & Enzenroth 1985, Checkley & Miller 1989), and Mullin et al. (1984) found marine copepods to be more depleted in $\delta^{15}N$ in areas where ammonium was the primary nitrigen source for phytoplankton than in those where nitrate dominated. Hoch et al. (1996) found nitrogen isotope discrimination to be 3 to 5‰ between biomass and excreted ammonium for protists growing in batch cultures, and 1 to 2‰ for flagellates growing in continuous cultures. The post-spring bloom period in the study area is dominated by high bacterial and ciliate biomass. Larsson (1986) estimated the biomass of bacteria and heterotrophic ciliates for the upper 20 m in the area to be ~0.5 and ~0.25 g C m^{-2}, respectively in mid-May. In the present study, samples from this period were rich in ciliates and rotifers. Ammonium generated from this community and heterotrophic flagellates is the most likely explanation for the local $\delta^{15}N$ minimum. Processes leading to the second local maximum are difficult to explain, but it is significant in size-fractions GFF to PL_5. Little or no fractionation because of nitrogen limitation or competition between phytoplankton and nitrifying bacteria may explain the process (Mariotti et al. 1984). Complex interactions of protist grazing, release of dissolved organic nitrogen (DON) and DON uptake by bacteria were suggested to account for variations around 2‰ in $\delta^{15}N$ by Hoch et al. (1996). Owens (1985) showed that the $\delta^{15}N$ of particulate matter was considerably enriched by microbial activity in the turbidity maximum in the River Tamar estuary. The auxiliary data in this study do not suffice to elaborate on these issues, but it can be noted that the summer DIN concentration increased slightly in connection with the first local $\delta^{15}N$ minimum in phytoplankton (Fig. 8b), possibly causing a transient relaxation of nitrogen limitation.

The onset of the cyanobacterial bloom at the end of June drastically affected $\delta^{15}N$ in all size-fractions. A mid- to late-summer local minimum developed, with time-lags between size-classes (Fig. 3). In the early stage of the bloom the nitrogen in N_2-fixing cyanobacteria (CB) was considerably depleted ($\delta^{15}N$ = –1.7‰, Fig. 8b, Table 5) relative to other phytoplankton. The result is consistent with other studies on N_2-fixing cyanobacteria, in which they have been found to be depleted relative to other phytoplankton species (Estep & Vigg 1985, Angradi 1993, Gu & Alexander 1993).

Cyanobacteria are often considered to be unpalatable or toxic to zooplankton and therefore not an important food source. Horstmann however (1975) observed Baltic rotifers ingesting filamentous cyanobacteria, and Sellner et al. (1994) found cladocerans but not copepods to use filamentous cyanobacteria in the Baltic Sea. In the present study, depleted fixed nitrogen was detected in all size-fractions (Fig. 2). In the PL_4 samples, dominated by rotifers and cladocerans, the cyanobacterial signal was particularly strong (Fig. 2), reflecting direct consumption of cyanobacteria, or secondary consumption of bacterio-, phyto- or zooplankton using depleted cyanobacterial nitrogen. The PL_6 samples of large copepodites appear less affected, but the signal is found also in these samples (Fig. 2, Table 5). Since the other phytoplankton samples from the period were cleansed of filamentous cyanobacteria, their depleted $\delta^{15}N$ signal must have been caused by secondary use of fixed nitrogen. This could have been derived from ammonium excreted from rotifers and cladocerans or by direct leaching from cyanobacterial cells. Capone et al. (1994) found extensive release of the amino acids glutamine and glutamate

from intact colonies of the cyanobacterium *Trichodesmium thiebautii*. The release of glutamate was typically 100 pmol N colony^{-1} h^{-1}. The rapid propagation of the depleted nitrogen signal to all size-fractions found in this study suggests that bacterial use of released amino acids may well constitute a significant pathway for fixed nitrogen to the non-cyanobacterial community. The nitrogen fixed in Baltic cyanobacterial blooms may therefore have a greater direct impact on summer production than previously assumed.

The late-summer samples of cyanobacteria were more enriched than other phytoplankton (Table 5). The floating filaments were on this occasion shapeless, rugged and greyish yellow, indicating bacterial degradation. The enriched carbon and nitrogen isotopes of the late-summer cyanobacteria indicate that for this sampling occasion they should be viewed as bacterial aggregates rather than phytoplankton. As the decaying cyanobacterial aggregates sediment or are degraded in the water column, the annual $\delta^{15}N$ cycle is ended by a broad autumn and winter local maximum. The thermal stratification breaks down and nitrate from deeper water enters the trophogenic layer, inducing an autumn diatom bloom (mainly *Thalassiosira* spp.). This bloom differs from the spring-bloom in that it is not ultimately limited by nutrients (since nitrate is continuously supplied from deeper waters) but rather by day length and irradiance. Montoya & McCarthy (1995) found a strong interaction between photon-flux intensity and nitrogen fractionation in *T. weissflogii*. The primary production causes a temporary depression in the increasing trend of DIN, and it is likely that some nutrient competition combined with short daylight causes nitrogen to become enriched in the plankton. The fact that the isotopic composition remained enriched during winter even though DIN was high supports this explanation.

Trophic enrichment of $\delta^{15}N$

The plankton food-web can be seen as a set of trophic interactions structured by critical size differences between consumers and food items. With an increasing number of taxa and developmental stages, the number of such critical size differences increases. Trophic fractionation of stable isotopes occurs when biomass is assimilated from one organism to another. The isotopic distance between the end members of the food web will therefore theoretically increase with an increasing number of such interactions (i.e. increasing food-web complexity). The zooplankton in this study are generally non-specific suspension-feeders or raptorial feeders (Johansson 1992). It therefore appeared appropriate to analyse the isotopic enrichment in relation to average particle size estimated by the midpoint between mesh sizes.

The regression slopes of $\delta^{15}N$ on logarithmic size (Fig. 6) show a seasonal pattern (Fig. 7). The ^{15}N enrichment per unit of size (slope) and the regression-explained variability (R^2) were most pronounced in spring and autumn. The development of the mesozooplankton community at the station suggests that the complexity of the food-web is also reflected in the enrichment of ^{15}N. The community of ciliates and rotifers (Table 3) that succeed the phytoplankton spring-bloom are characterised by low enrichment of ^{15}N with increasing size (Table 7, Fig. 6) and little linear dependence on size ($R^2 \approx 40\%$). The number of mesozooplankton size-classes is low in this community, and hence the potential number of consecutive trophic interactions is also low. In July and August a complex mesozooplankton community develops (Johansson 1992), whereby most species found at the station occur simultaneously in several developmental stages (i.e. size-classes). The number of potential trophic interactions increases as does the trophic enrichment of $\delta^{15}N$ per size-class and the regression-explained variability (R^2~90%). The development of the massive autumn bloom causes a problem in interpretation, since the enriched nitrogen isotopic composition of the diatoms (Fig. 2, PL_3) is not instantaneously found in the mesozooplankton. When later incorporated in the zooplankton, the diatom material causes all zooplankton size-classes to become enriched by 2.5 to 6‰ from August to October. The high enrichment slopes in early spring (5.6 and 8.4; Table 7) are most likely to have been caused by enrichment with age, since the large copepods and copepodites found in January are over-wintering individuals, growing at low rates or starving. Assuming dependent periodic cycles in $\delta^{15}N$, the slopes of size-dependent regressions would theoretically be most affected by the complexity of the food-web (i.e. trophic interactions per size-class), whereas R^2 would be most sensitive to phase differences between size-classes. The spring and early summer is characterised by large phase differences and few species and developmental stages (Fig. 8b, Table 3), resulting in low slopes and R^2s, whereas the opposite is true for late-summer and autumn.

In this study the slopes were between 1.2 and 8.4 (July ~2.8; Table 7) and between 2.0 and 4.0 in an offshore investigation (Rolff & Elmgren 2000) for the same plankton size-classes in different basins of the Baltic Sea (July 1994). Literature data on isotope enrichment in size-fractionated marine plankton are scarce. Recalculating figure data I found slopes of $\delta^{15}N$ on logarithmic size (in mm) of between 1 and 2 in plankton of the size-classes 0.25 to 8 mm (Fry & Quinones 1994; Fig. 2: NW Atlantic), whereas plankton between 0.02 and

0.5 mm gave a slope of ~3 (Sholto Douglas et al. 1991; Table 2: S. Benguela). Mediterranean plankton (0.003 to 0.15 mm) in Rau et al.'s (1990) (Fig. 1b) study had slopes of 1.6, 1.9, 3.8 and 2.4, depending on season (April, July, September and December). The slope estimates found in the present study cover a greater range than those found in the previous study and in the literature. The time pattern suggested by Rau's data is similar to the pattern found in the present investigation. My estimates of the slopes in the literature data are rough estimates from figures, and in the case of the Benguela data are based only on the 2 end-members of the size distribution. During summer and autumn mysids appear to be the top predators among the organisms studied, but in the winter season they compete with large copepods for the same resource (Fig. 2). Biomass production in August is heavily dominated by copepod nauplii and copepodites (Rudstam et al. 1992). Mysids feeding selectively on larger size-classes of copepodites or adult copepods may thereby become enriched in ^{15}N (Hansson et al. 1997). The $\delta^{15}N$ of *Aurelia aurita* (40 mm) suggests that they mainly feed on smaller zooplankton, possibly reflecting non-selective predation determined mainly by the current environmental size-spectrum.

The size-specific approach has several advantages over the more common assumption of a general enrichment of $\delta^{15}N$ (e.g. 3 to 5‰) per trophic level. Size is more likely to represent the trophic reality of marine mesozooplankton food-webs. It is also independent of the vaguely defined concept of intermediate trophic levels originally defined as integers. The trophic level concept is also difficult to apply when assimilation and diet composition may be variable and difficult to estimate. Assuming that the copepodites, generally found dominating the PL_5 samples, represent 1 to 1.5 trophic-levels distance from the phytoplankton in the PL_2 samples, the estimates of 1 trophic-level $\delta^{15}N$ distance would vary from 1.7 to 5.5‰ during the annual cycle (Table 8). For $\delta^{13}C$ the corresponding absolute difference would be from 0.3 to 4.7‰. As long as the cycles are in phase, the estimates of trophic distance between sample types will be fairly stable (Fig. 8a,b). When a time lag occurs, the isotopic distance between samples can vary drastically, and the estimates of a general distance per trophic level will become erratic. The chemical composition of a consumer can, by definition, not be in steady state with its food resource until the resource reaches steady state. Since the phytoplankton community is a continuous succession of species using resources with variable isotopic composition, a steady state will, by definition, never occur. The size-spectrum of marine mesozooplankton has been well investigated, and provides a simpler model of food-web complexity than the trophic-level concept. Considering the results from this study, and from Rolff & Elmgren (2000), I suggest that a size-dependent approach is preferable in the analysis of trophic isotope enrichment in marine micro- and mesoplankton food-webs.

Table 8. Difference ($\Delta = \delta_{zoopl} - \delta_{phytopl}$) between averages of $\delta^{13}C$ and $\delta^{15}N$ in zooplankton (PL_5) and phytoplankton (PL_2) on all sampling occasions

Date (d.mo.yr)	$\Delta^{13}C$ PL_5-PL_2	$\Delta^{15}N$ PL_5-PL_2
30.03.1994	−1.4	4.1
21.04.1994	−2.3	2.3
04.05.1994	−4.7	1.7
25.05.1994	−0.3	4.0
29.06.1994	−1.2	2.2
27.07.1994	−3.0	2.9
31.08.1994	−3.8	2.5
05.10.1994	−2.1	3.4
25.01.1995	−0.8	5.5

Conclusions

Marked bi-modal ($\delta^{13}C$) and tri-modal ($\delta^{15}N$) seasonal cycles were found in all plankton size-classes. Undetected time lags between such cycles may cause misinterpretation of food-chain interactions in studies of relations between isotopic changes and other environmental variables. The ANOVA design used here is aimed at separating the sampling occasions and sample types. Resource-limitation will therefore cause the temporal variation to be less frequently measured and limit the use of data in correlation studies. If, however, the timing of the seasonal cycle is unknown, inclusion of inappropriate time periods will frequently cause spurious correlations. This risk also occurs when analysing data from large-scale expeditions during which stations may only be visited once and a seasonal gradient such as the one studied here corresponds to geographical distances. A reliable description of the seasonal cycle is therefore a prerequisite for many studies, and it may not be appropriate to compare samples from different trophic groups on the same sampling occasion. The recorded transport of depleted fixed nitrogen from the cyanobacterial bloom to all size-classes of the plankton indicated that nitrogen-fixing cyanobacteria are ecologically more important as instantaneous nitrogen sources in the Baltic than previously assumed. In plankton of the size-classes 0.05 to 1.5 mm, $\delta^{15}N$ was linearly related to the logarithm of size. The trophic enrichment of ^{15}N increased as the complexity of the plankton community increased during summer, possibly reflecting more numerous

trophic interactions per size-class. The results indicate that a size-dependent approach is more applicable to isotopic studies of marine mesozooplankton than the assumption of a fixed fractionation per trophic level.

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