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Nucleic acid levels in copepods: dynamic response to phytoplankton blooms in the northern Baltic proper

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ABSTRACT: We examined changes in nucleic acids and concomitant population development of the copepods *Acartia bifilosa* and *Eurytemora affinis* in relation to the progress of the phytoplankton spring bloom in the northern Baltic proper. Individual RNA and DNA concentrations and their ratios in female copepods as well as copepod abundance and population structure were analyzed in 2 coastal areas that differed in the degree of eutrophication and phytoplankton development. During the study period (February to June 2002), bloom conditions were evident, with chlorophyll (chl) *a* values being 42% higher in the eutrophic area than in the reference area. In both areas, diatoms dominated; in the reference area, they were replaced by dinoflagellates toward the end of the bloom. Copepod RNA-DNA concentrations increased rapidly at the onset of the bloom and gradually decreased thereafter. Moreover, in the eutrophic area, both copepods had higher RNA content and RNA:DNA ratios throughout the study period, suggesting higher productivity in this area. In both species, we found positive correlations between RNA-based indices and chl *a*. Thus, as suggested by RNA dynamics, growth rates of *A. bifilosa* and *E. affinis* appear to respond rapidly to both temporal variation in spring phytoplankton stock and spatial variation due to the magnitude of the bloom. In addition, we found that species-specific RNA dynamics and RNA–chl *a* relationships differed between species, indicating possible differences in feeding preferences and growth potential.

KEY WORDS: Biochemical indices · Growth conditions · Population development · RNA and DNA content · Spring phytoplankton bloom

INTRODUCTION

The spring phytoplankton bloom is one of the most widespread phenomena in temperate areas (Voipio 1981). In coastal areas of the northern Baltic proper, phytoplankton blooms are usually observed between March and mid-May, depending on the ice conditions (Hajdu et al. 1997). During this period, phytoplankton biomass is dominated by diatoms and dinoflagellates in the initial phase of the bloom. A post-bloom phase occurs when the nutrient pool is exhausted and is characterized by dinoflagellates, nanoflagellates, and autotrophic ciliates (Hajdu et al. 1997). As primary production rises, zooplankton biomass starts to increase, largely due to the intensive reproduction of copepods (Adrian et al. 1999, Johansson et al. 2004). Fish recruitment is crucially dependent on the success of the first feeding larvae, which in turn depends on the availability of zooplankton food (Arrhenius & Hansson 1999). Many fish, such as herring and cod, spawn their eggs to coincide with the spring bloom so that they are synchronized to hatch into juvenile fish as the copepods, the main prey for many fish species, reach their peak (Cushing 1990). In the Baltic, young-of-the-year herring abundance in spring is positively correlated with zooplankton abundance, thereby allowing predictions

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of fish stock recruitment (Parmanne & Sjoeblom 1988). Therefore, the magnitude and timing of copepod reproduction during spring may affect the Baltic ecosystem and its commercial fisheries. However, while a wealth of information can be found on copepod population dynamics and the physiological status associated with phytoplankton blooms in the North Atlantic, there are only a few studies on this aspect in the Baltic Sea.

To assay the response of copepods to the onset of a phytoplankton bloom, egg production rate measurements (e.g. Dam et al. 1994, Ward & Shreeve 1995), gonad development staging (Niehoff et al. 2002), feeding rate estimates (Dam et al. 1994, Atkinson et al. 1996), and metabolic indices (Hernandez-Leon et al. 2004) have been employed. Nucleic acid content has also been used to assess copepod nutritional conditions during bloom periods (Durbin et al. 2003), providing *in situ* estimates of copepod growth status. The rationale of using nucleic acid quantities as a growth measure is that total RNA content in a somatic cell is primarily a function of ribosome number correlating with protein synthesis, while the amount of DNA is quasi-constant and may therefore be an index of the cell number (Buckley et al. 1999). Nucleic acid concentrations and their ratios have been used as proxies for growth status in a variety of marine organisms, including copepods (Nakata 1994, Durbin et al. 2003). In addition, experimental manipulations of food rations have revealed strong links between growth and the RNA-DNA concentrations and their ratios in several copepod species (Saiz et al. 1998, Wagner et al. 2001, Gorokhova 2003). Laboratory studies on copepods have shown that RNA levels respond to changes in physiological activity within a few days (Saiz et al. 1998), while egg production is a result of feeding history over several days (Hirche et al. 1997) or even weeks (Båmstedt et al. l999). Thus, changes in RNA levels may be useful when detecting a rapid response of biological systems to environmental variability, e.g. the reaction of planktonic copepods to the timing and magnitude of a phytoplankton bloom.

In the northern Baltic proper, the copepods *Acartia* spp. (*A. bifilosa* Giesbrecht, *A. longiremis* Lilljeborg, and *A. tonsa* Dana) and *Eurytemora affinis* Poppe constitute 50 to 90% of the mesozooplankton biomass (Viitasalo 1992, Johansson et al. 2004). Of the 3 *Acartia* species, *A. bifilosa* dominates in the upper mixing layer, constituting about 80% of the *Acartia* biomass (Adrian et al. 1999). Due to their dominance, *A. bifilosa* and *E. affinis* are considered key species for the pelagic food web; they are also main prey for herring and sprat, the dominant fish in this region (Arrhenius & Hansson 1999).

The aim of this study was to assess the importance of a spring bloom to the life cycles of the copepods *Acartia bifilosa* and *Eurytemora affinis*. We examined individual RNA-DNA content and population development of these copepods in relation to the timing and progress of the phytoplankton bloom in 2 coastal areas of the northern Baltic proper. These areas differ in their nutrient loading and phytoplankton development. In spring 2002, the abundance, size, and nucleic acid content of the copepods were measured along with environmental variables, i.e. chlorophyll (chl) *a*, phytoplankton community structure, and water temperature and transparency. A correlative approach and regression analysis were used to determine the effect of environmental variables on nucleic acid-based indices of copepod growth.

MATERIALS AND METHODS

Study area and sampling. The study was carried out during spring 2002 (20 February to 4 June) in the Stockholm Archipelago at 2 study sites (Fig. 1). Samples were taken weekly during April and every 2 wk during the rest of the study. Stn H4, hereafter referred to as the eutrophic site, was located in the middle of Himmerfjärden Bay, which is influenced by discharges from a municipal sewage treatment plant. Stn B1, hereafter referred to as the reference site, was situated in an open coastal area near the Askö Biological Station, Stockholm Marine Research Center, which is separated from the bay by a large archipelago and a 20 m deep sill; this area has not been measurably affected by the nutrient discharge from the treatment plant (Höglander 2005).

On each sampling occasion, water temperature (*T*, °C; 1 to 10 m), chl *a* (mg m^{-3}; 0 to 20 m), and water transparency (Secchi depth, m) were measured according to the standard protocols of the Baltic Monitoring Programme (HELCOM 1988). Phytoplankton samples were collected with a hose (inner diameter, 19 mm) from 0 to 20 m and 0 to 14 m at the reference and the eutrophic stations, respectively, and preserved with acid Lugol's solution (HELCOM 1988). Zooplankton were collected by vertical tows from 5 m above the bottom to the surface using a 90 µm WP-2 net (diameter 57 cm) equipped with a flow meter. From each tow, randomly selected copepods were preserved in bulk using RNA*later* and stored for 1 to 5 wk at 4–5°C until the nucleic acid analysis (Gorokhova 2005). The rest of the single-tow sample was preserved in 4% borax-buffered formaldehyde for microscopic analysis.

Phytoplankton abundance and biomass. Samples were settled in Utermöhl chambers and examined using a NIKON inverted microscope with phase con-

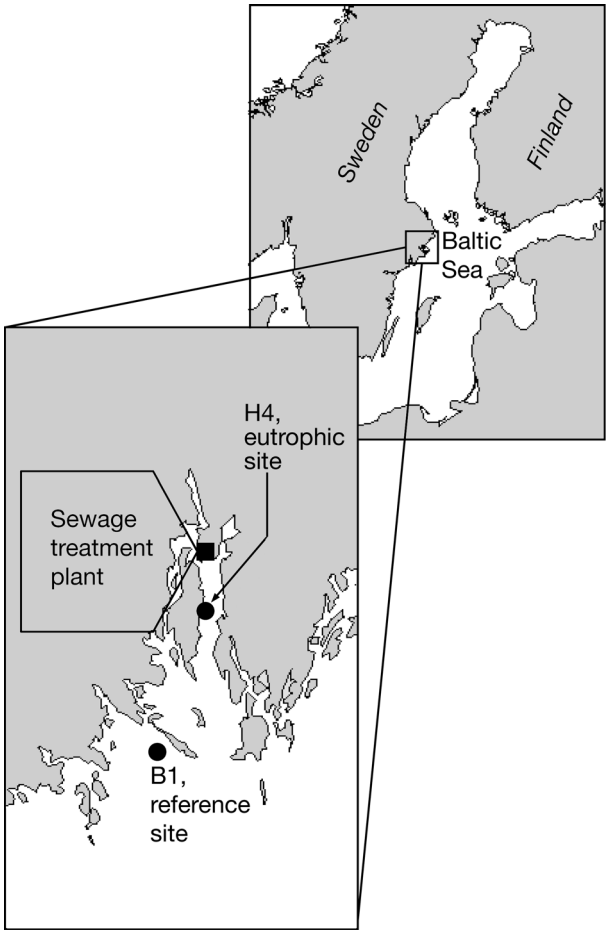

Fig. 1. Study sites: eutrophic site (Stn H4; Himmerfjärden Bay, ~30 m bottom depth; 58° 59′ N, 17° 44′ E) and reference site (Stn B1; Askö area, ~40 m bottom depth; 58° 48′ N, 17° 38′ E). At both sites, salinity was 6 to 7‰ and did not vary appreciably during the year

trast. Phytoplankton (>2 µm; ≥500 cells) was counted in diagonals or on the half/whole chamber bottom at 150× and 400× magnification. Cell volume and carbon biomass were calculated from size measurements (≥25 cells species^{-1}) according to the Baltic Monitoring Programme recommendations. Carbon conversion factors of 0.13 and 0.11 were used for thecate dinoflagellates and for all other groups, respectively (HELCOM 1988).

Copepod abundance. Replicate mesozooplankton subsamples (Kott 1953) were counted (≥500 specimens) with an inverted microscope (Leitz fluovert FS, Leica) at 80× magnification. Copepods were classified according to species, developmental stage, and sex.

Nucleic acid analysis. Adult females of *Acartia bifilosa* and *Eurytemora affinis* were used for the analysis. Prior to extraction, the individual prosome length (PL, mm) was measured in RNA*later*-preserved animals using an inverted microscope equipped with an ocular micrometer. Nucleic acids in individual copepods were quantified using a microplate fluorometric high-range RiboGreen assay after extraction with N-laurylsarcosine followed by RNase digestion (Gorokhova & Kyle 2002). Fluorescence measurements were done in triplicate for each sample using a microplate reader (Fluoroscan II, Labsystems; 485 and 530 nm for excitation and emission, respectively) and black solid flat-bottom COMBO microplates (Labsystems). Measured RNA and DNA concentrations were expressed as % of carbon (C) content using copepod individual weights calculated from the PL measurements and length-weight regressions: $W = e^{3.793PL-2.285}$ for *A. bifilosa* and $W = e^{3.904PL-2.181}$ for *E. affinis*, where W is body C content (µg). These regressions were obtained for specimens within the same size range from coastal areas in Storfjärden (Gulf of Finland; Viitasalo et al. 1995).

Statistics. Data are presented as means ± SD. To approximate normal distributions, environmental (i.e. *T*, chl *a*, and Secchi depth) and biological (i.e. PL and nucleic acid quantities) variables were $[\log_{10}(x+1)]$ transformed, while proportions and ratios (i.e. percentages of different phytoplankton groups and RNA:DNA ratio) were arcsine square root transformed. To test for differences between species or stations, an unpaired *t*-test was performed followed by an *F*-test to compare variances. To test for differences in nucleic acid concentrations and their ratios between the time periods, analysis of covariance (ANCOVA) was applied using temperature as the covariate. Temperature correction was necessary, because nucleic acid concentrations are temperature-dependent (Wagner et al. 2001). Another possible theoretical covariate is body size (Buckley et al. 1999); however, within the size range of the females used for the analysis, no correlations between PL and RNA-DNA concentrations were observed (Gorokhova 2003; this study); consequently, PL was not used as a covariate when comparing RNA-DNA concentrations in copepods collected during different periods. Differences in PL between the time periods were tested using 1-way ANOVA. Before ANCOVA and ANOVA, data were tested for homogeneity of variances using Cochran's test.

The Pearson correlation coefficient and stepwise regression analysis (forward model, entry rule $F = 1.00$) were used to test the effects of environmental (*T*, chl *a*, Secchi depth, and proportions of different phytoplankton groups) and endogenous (PL) variables on nucleic acid based indices using Statistica 6.0 for Windows (StatSoft). In all cases, significance was accepted when $p < 0.05$.

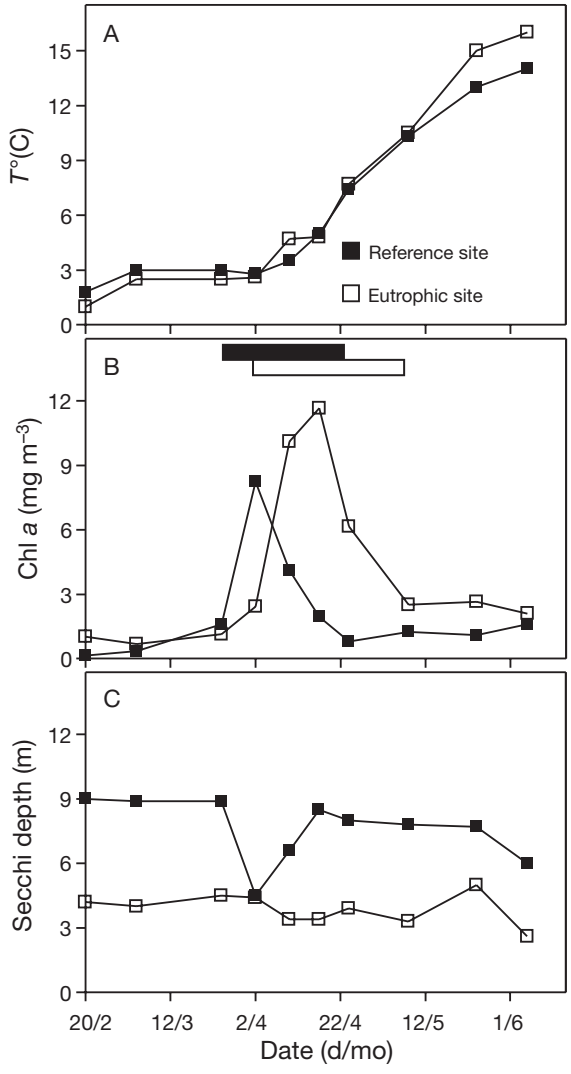

Fig. 2. Variations in (A) temperature (°C), (B) chl *a* concentration (mg m^{-3}), and (C) water transparency (Secchi depth, m) at the reference site and the eutrophic site during the study period. Bloom periods (panel B) are indicated as filled (reference site) and open (eutrophic site) horizontal bars

RESULTS

Environmental conditions. Surface layer temperature increased from 2°C in February to 16°C in June (Fig. 2A). A phytoplankton bloom occurred at both study sites in April starting about 1 wk later and lasting longer in the eutrophic area compared to the reference area (Fig. 2B). The bloom reached 8.2 and 11.7 mg chl *a* m^{-3} at the reference and the eutrophic sites, respectively; thus, the maximal chl *a* value was 42% higher in the eutrophic than in the reference area. In May, the bloom was over, and thereafter chl *a* values remained relatively stable at 2.4 ± 0.20 and 1.3 ± 0.18 mg m^{-3} at the eutrophic and reference sites, respectively. Before, during, and after the bloom, chl *a* concentrations at the eutrophic site were consistently higher than at the reference site (Fig. 2B). At the reference area, water transparency mirrored the chl *a* dynamics, decreasing in concert with the increasing chl *a* and returning to nearly initial values shortly after the bloom. In the eutrophic area, the average Secchi depth was about half that at the reference area and showed little change during the study (Fig. 2C), indicating high quantities of non-photosynthetic particulate matter in the water. The correlation between chl *a* and Secchi depth was significant at the reference site ($r = -0.82$, $p < 0.01$) but not at the eutrophic site ($r = -0.32$, $p > 0.37$), yet resulting in a significant overall correlation (Table 1).

Phytoplankton stocks and composition. The bloom was dominated by the diatoms *Thalassiosira levanderi* Van Goor, *T. baltica* (Grunow) Ostenfeld, and *Chaetoceros wighamii* Brightwell, constituting >70% of the phytoplankton biomass at the peak of the bloom. The average values for the entire study period were 35 and 45% for the reference and the eutrophic sites, respectively (Fig. 3). In the reference area, *Skeletonema costatum* (Greville) P. T. Cleve was also abundant (~14%), but toward the end of the bloom, diatoms were replaced by the dinoflagellates *Peridiniella catenata* (Levander) Balech, *Scrippsiella hangoei* (Schiller) Larsen/*Woloszynskia halophila* (Biecheler), and *Heterocapsa rotundata* (Lohmann) Hansen, and nanoflagellates (<15 µm). In the eutrophic area, diatoms dominated throughout the bloom, while the proportion of dinoflagellates was low (6%; Fig. 3B). By biomass, the autotrophic ciliate *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbrock was subdominant at both sites, contributing most significantly at the eutrophic site (42%; Fig. 3B); however, the abundance of this large species (20 to 40 µm) was relatively low (17 to 88 $\times 10^3$ cells l^{-1}). Calculating the area under the biomass curve (AUC; the integral over time of the biomass stock) for the entire phytoplankton community provided an overall metric of its performance during the study. The AUC value of total phytoplankton biomass derived for the eutrophic site was 29% higher than for the reference site, indicating differences in phytoplankton development between the areas.

The temporal variations in the measured variables, i.e. abundance, PL, and nucleic acid levels in copepods, may be integrated over time to arrive at an estimate of average values for 3 bloom-related periods determined from chl *a* and phytoplankton dynamics (Figs. 2B & 3). These periods are defined as follows: prior the bloom (pre-bloom; reference station: 20 February to 25 March; eutrophied station: 20 February to 2 April), during the peak of the bloom (bloom; reference station: 2 to 16 April; eutrophied station: 8 April to

Table 1. *Acartia bifilosa* and *Eurytemora affinis*. Pearson's product moment correlation (r) between environmental variables (temperature, *T*; chl *a* concentration; and Secchi depth), proportions of different phytoplankton groups (diatoms, Diat%; dinoflagellates, Dino%; and *Mesodinium rubrum*, Meso%), female body size (prosome length, PL, mm), RNA and DNA individual content (µg ind.$^{-1}$), RNA and DNA concentrations (%C), and RNA:DNA ratios observed during the study period. Significant correlations in **bold** ($p < 0.05$); n = 18

	T	Secchi	Chl *a*	Diat%	Dino%	Meso%	PL	RNA	RNA%	DNA	DNA%
Secchi	−0.11	1.00									
Chl *a*	0.02	**−0.58**	1.00								
Diat%	−0.36	−0.27	**0.76**	1.00							
Dino%	0.32	0.40	−0.12	−0.30	1.00						
Meso%	0.29	−0.12	**−0.48**	**−0.62**	−0.45	1.00					
A. bifilosa											
PL	−0.42	0.16	0.37	**0.58**	0.24	**−0.66**	1.00				
RNA	0.01	−0.41	**0.77**	**0.56**	0.10	−0.46	0.45	1.00			
RNA%	0.25	−0.46	**0.73**	0.35	0.14	−0.27	0.18	**0.93**	1.00		
DNA	0.00	−0.10	**0.85**	**0.78**	−0.05	**−0.50**	**0.53**	**0.90**	**0.77**	1.00	
DNA%	0.18	−0.10	**0.58**	**0.66**	−0.16	−0.30	0.14	**0.74**	**0.73**	**0.87**	1.00
RNA/DNA	0.12	−0.37	**0.54**	0.14	0.41	−0.40	0.27	**0.69**	**0.71**	0.45	0.28
E. affinis											
PL	0.05	−0.25	**0.67**	**0.57**	0.06	−0.38	1.00				
RNA	−0.14	**−0.56**	**0.49**	**0.68**	0.09	**−0.63**	**0.60**	1.00			
RNA%	−0.10	**−0.70**	**0.48**	**0.62**	0.14	**−0.61**	0.46	**0.98**	1.00		
DNA	−0.39	**−0.49**	0.37	0.43	0.25	**−0.62**	0.29	**0.60**	**0.60**	1.00	
DNA%	**−0.53**	−0.36	**0.47**	0.37	0.13	**−0.56**	−0.10	**0.53**	**0.57**	**0.91**	1.00
RNA/DNA	0.20	**−0.76**	**0.61**	**0.50**	−0.15	−0.17	**0.56**	**0.70**	**0.68**	−0.08	−0.15

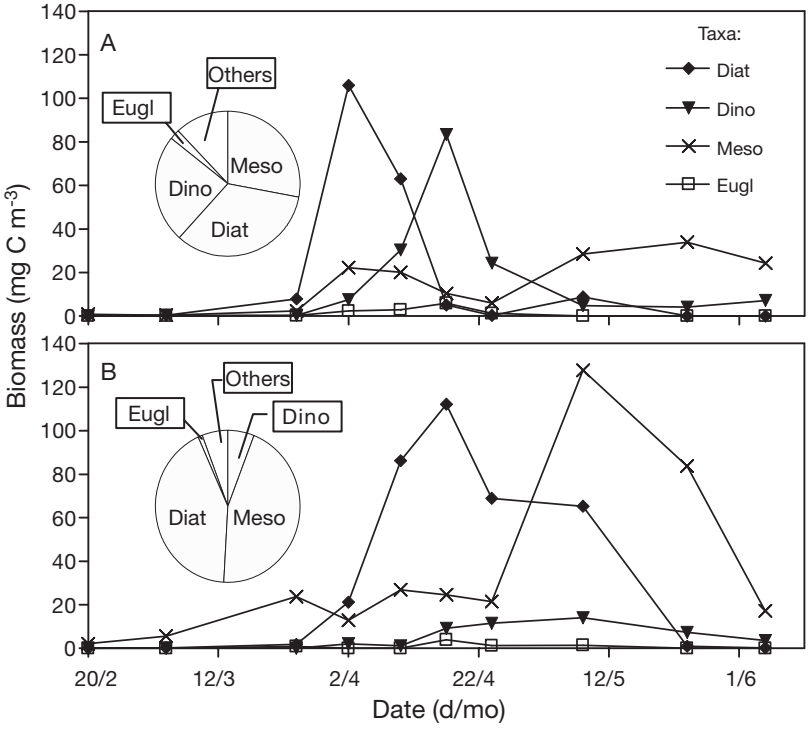

Fig. 3. Variations in carbon biomass of different phytoplankton groups during the study period at (A) the reference site and (B) the eutrophic site. The pie diagrams on the left side of each panel show average proportions of the dominant groups, i.e. diatoms (Diat), dinoflagellates (Dino), euglenophyceans (Eugl), and the ciliate *Mesodinium rubrum* (Meso) in the total biomass of the phyto plankton community

7 May), and after the bloom (post-bloom; reference station: 23 April to 4 June; eutrophied station: 23 May to 4 June).

Zooplankton composition and abundance. At both stations, copepods dominated throughout the study period, averaging 57% of the total mesozooplankton abundance. Other important groups were rotifers (12 to 90%; mainly *Synchaeta* spp. and *Keratella cochlearis*), meroplankton larvae (3 to 19%; bivalves and polychaetes), and appendicularians (2 to 16%; mainly *Fritillaria borealis*). At the reference site, *Acartia bifilosa* and *Eurytemora affinis* comprised on average 70 and 20% of the total copepod numbers, respectively, while at the eutrophic site, the proportion of *E. affinis* was higher, and the 2 species contributed 49 and 38%, respectively.

For both species, the increase in abundance was associated with the onset of the bloom and rising temperature, with pronounced differences in total abundance between the areas. The abundance of *Acartia bifilosa* increased from 100–1000 ind. m^{-3} in

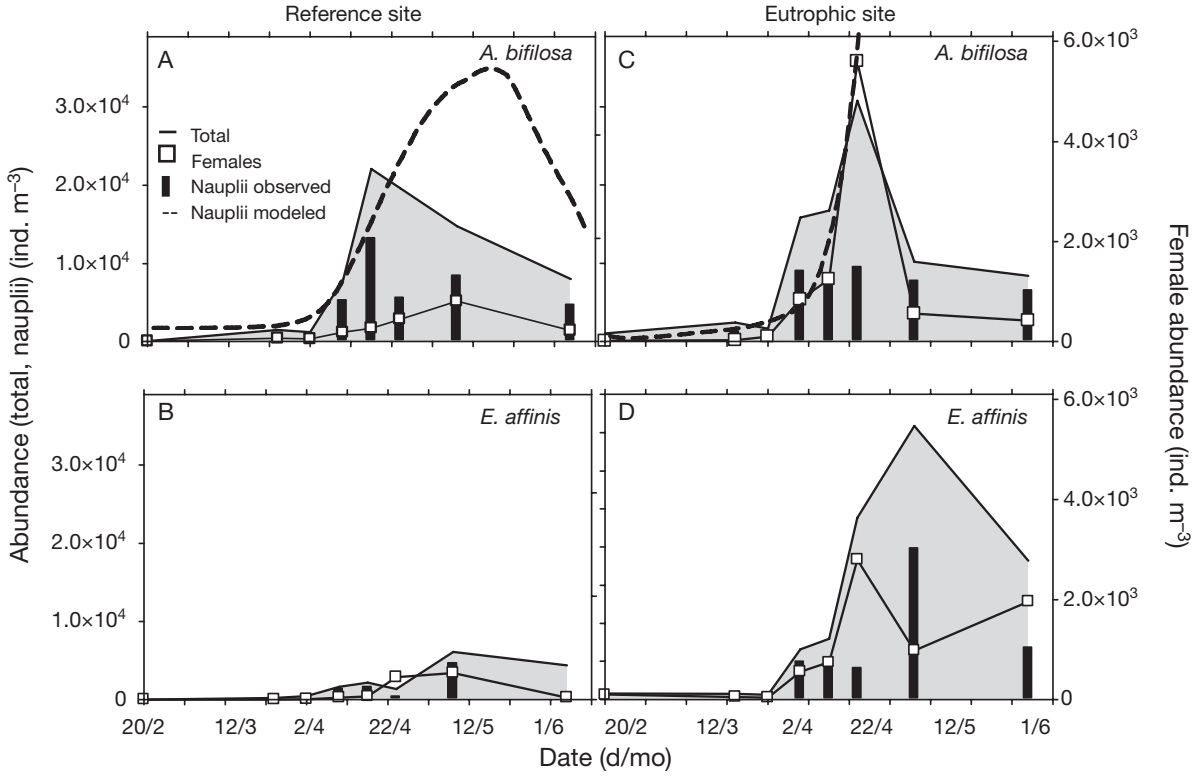

Fig. 4. (A,C) *Acartia bifilosa* and (B,D) *Eurytemora affinis*: variations in total abundance, nauplii, and adult females during the study period at the reference site (A,B) and the eutrophic site (C,D). To illustrate sufficiency of nauplii production based on the bloom-associated reproduction, abundance of *A. bifilosa* nauplii (A,C) was predicted from the model based on observed RNA concentrations and female abundance

February to 20–30 × 10^3 ind. m^{-3} in April, while that of *Eurytemora affinis* increased from 50–800 ind. m^{-3} in February to 6–36 × 10^3 ind. m^{-3} in May (Fig. 4). The increases were attributed to the pulses of nauplii that appeared 2 to 3 wk after the bloom peak. The AUC value of total copepod abundance was 49% higher at the eutrophic site than at the reference site.

Copepod body size. Neither species showed a significant difference in PL grand means between the sites (unpaired *t*-test, $p > 0.05$ in all cases). Mean PL was largest in April, at 0.72 ± 0.01 and 0.68 ± 0.01 mm in *Acartia bifilosa* and *Eurytemora affinis*, respectively. Females sampled during the bloom tended to be larger compared to those during pre-bloom; the difference was significant for the reference site (ANOVA: *A. bifilosa*, $F = 5.20$, $p < 0.01$; *E. affinis*, $F = 4.24$, $p < 0.04$; Fig. 5A,E). At the eutrophic site, *A. bifilosa* females were significantly smaller during the post-bloom period compared to those collected earlier in the season (ANOVA: $F = 4.78$, $p < 0.02$; Fig. 5A).

Nucleic acid levels. In *Acartia bifilosa*, seasonal average RNA and DNA concentrations were 4.3 and 1.6% C, respectively, with a mean RNA:DNA ratio of 2.8. In *Eurytemora affinis*, RNA and DNA concentrations averaged 3.2 and 1.3% C, respectively, with a mean RNA:DNA ratio of 2.4. Based on pooled data, RNA concentration in *A. bifilosa* was the only variable differing significantly between the stations (unpaired *t*-test, $p < 0.04$, $t = 2.255$, df = 16). However, both species exhibited marked temporal variation in nucleic acid concentrations (Figs. 5B,C,F,G & 6B,C,F,G). The lowest RNA-DNA concentrations were recorded in February-March and the highest in mid-April, increasing in concert with chl *a* and the build-up of the diatom bloom. When data were grouped according to the timing of the bloom, there were significant differences in nucleic acid concentrations and their ratios between females collected at the 2 stations (unpaired *t*-test, $p < 0.05$ in all cases indicated as significantly different in Fig. 5) as well as during different periods (ANCOVA; $p < 0.05$ in all cases indicated as significantly different in Fig. 5). In both species, RNA concentrations were significantly higher during the bloom, and in the case of *A. bifilosa*, after the bloom, than in the pre-bloom period (ANCOVA, $p < 0.04$ in all cases). Moreover, in all time periods, RNA concentrations in *A. bifilosa*

were significantly higher in the eutrophic area than in the reference area (unpaired *t*-test, $p < 0.003$ in all cases), while for *E. affinis* this was true for the post-bloom period only (unpaired *t*-test, $p < 0.02$, $t = 2.578$, df = 37).

Relationship between RNA levels in copepods and chl *a*. Individual RNA concentrations and associated chl *a* values were fitted to the Ivlev model (Fig. 7) that has been successfully used to describe growth and feeding kinetics in zooplankton, including copepods (Dam et al.1994). There were significant differences in the response between the 2 species (Table 2): when data for both sites were pooled, the maximum RNA concentration (RNA_{max}) was significantly higher in *Acartia bifilosa* than in *Eurytemora affinis* ($p < 0.007$, $F_{2,30} = 5.86$). However, the rate of RNA increase with increasing chl *a* concentration (as defined by the rate constant *b*) was not ($p > 0.5$, $F_{1,30} = 0.377$). The r^2 values were higher for *A. bifilosa* than for *E. affinis* (Table 2), indicating tighter coupling between chl *a* and RNA concentrations. In both species, RNA approached maximal values (i.e. growth conditions without food limitation) at the eutrophic site at chl *a* exceeding 3.0 mg m^{-3}.

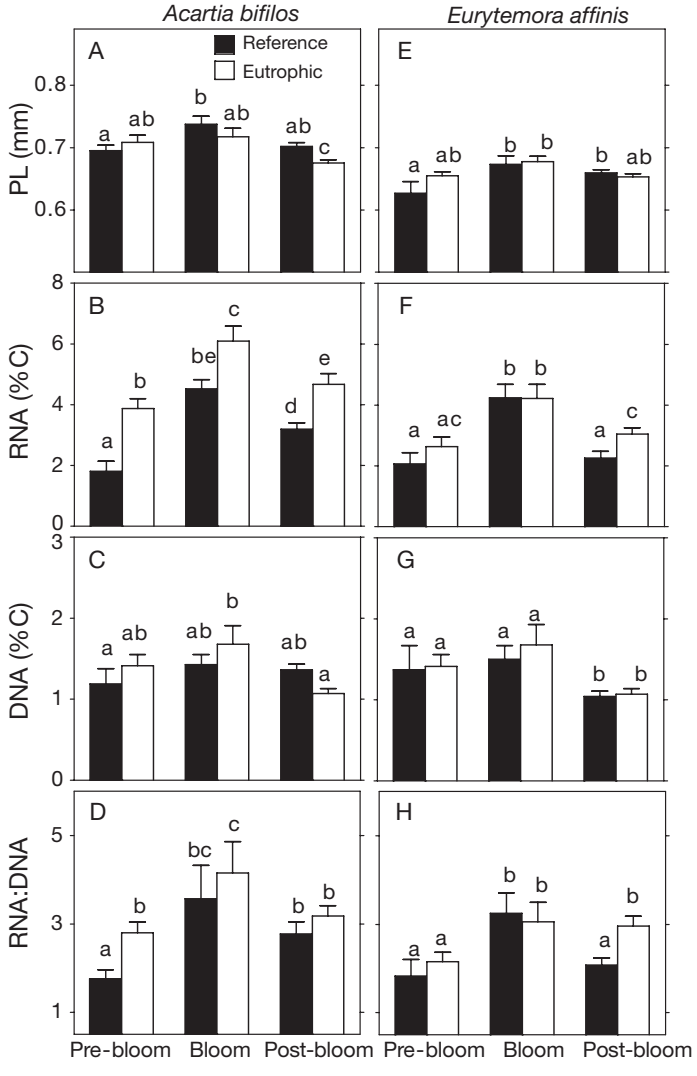

Fig. 5. (A–D) *Acartia bifilosa* and (E–H) *Eurytemora affinis*. Variations (mean ± SD) in female body size measured as prosome length (PL; A,G), RNA concentration (RNA, %C; B,F), DNA concentration (DNA, %C; C,G), and RNA:DNA ratios (D,H) at the reference and the eutrophic sites in relation to the timing of the spring bloom. Non-matching letters above the bars denote statistically significant differences among the groups ($p < 0.05$)

Table 2. *Acartia bifilosa* and *Eurytemora affinis*. The estimated parameters of the Ivlev's model [RNA] = RNA_{max} ($1 - e^{-b\ chl\ a}$), where [RNA]: observed individual RNA concentrations (%C, mean value for each sampling occasion), RNA_{max}: estimated maximal individual RNA concentrations (mean ± SE), *b*: shaping constant (mean ± SE), chl *a*: chl *a* concentration (mg m^{-3}). The parameters of the regression were compared between species using the extra sum-of-squares *F*-test, and significantly different values within each category are depicted in **bold** ($p < 0.01$)

Station and species	RNA_{max}	*b*	r^2
B1			
Acartia	9.86 ± 2.24	0.80 ± 0.45	0.56
Eurytemora	9.52 ± 1.48	0.61 ± 0.21	0.38
H4			
Acartia	**11.52 ± 1.08**	0.80 ± 0.24	0.64
Eurytemora	**8.59 ± 0.66**	0.62 ± 0.14	0.62
Pooled for B1 and H4			
Acartia	**12.48 ± 1.05**	0.69 ± 0.13	0.69
Eurytemora	**8.80 ± 0.66**	0.66 ± 0.13	0.59

Correlation and multiple regression analyses. In both species, chl *a* values were most closely linked to RNA-DNA levels (Table 1). Moreover, the proportion of diatoms in the phytoplankton was positively correlated with chl *a*, while the opposite was true for *Mesodinium rubrum*. Significant positive correlations were observed between the proportion of diatoms and RNA levels in both species, while significant negative correlations were observed between the proportion of *M. rubrum* and nucleic acids in *Eurytemora affinis.* In addition, RNA content in *E. affinis* was related to PL, and RNA-DNA levels were correlated positively with chl *a* and negatively with Secchi depth, while in *Acartia bifilosa*, RNA-DNA levels were correlated with chl *a* only. As shown in the correlation matrix, none of the parameters measured were correlated with temperature, with the exception of *E. affinis* DNA% (Table 1).

In *Acartia bifilosa*, of the 6 environmental variables (*T*, chl *a*, Secchi, Diat%, Dino%, and Meso%) and 1 endogenous variable (PL) examined, nucleic acid levels were correlated the most highly with chl *a* (Table 1), and this was the first variable to enter the

regressions for RNA-based indices. The next variable to enter the model for individual DNA content was PL, while the combined effects of chl *a*, Secchi depth, and Dino% best explained RNA concentrations (Table 3). In *Eurytemora affinis*, Secchi depth was the first variable to enter the models for RNA-based indices, while temperature was important for DNA content and concentration. In both species, equations for individual RNA content and concentration showed a better fit, greater significance (Table 3), and lower standard errors of determination coefficients (not shown) than equations for DNA and RNA:DNA ratio, suggesting greater causal linkage between the dependent and independent variables.

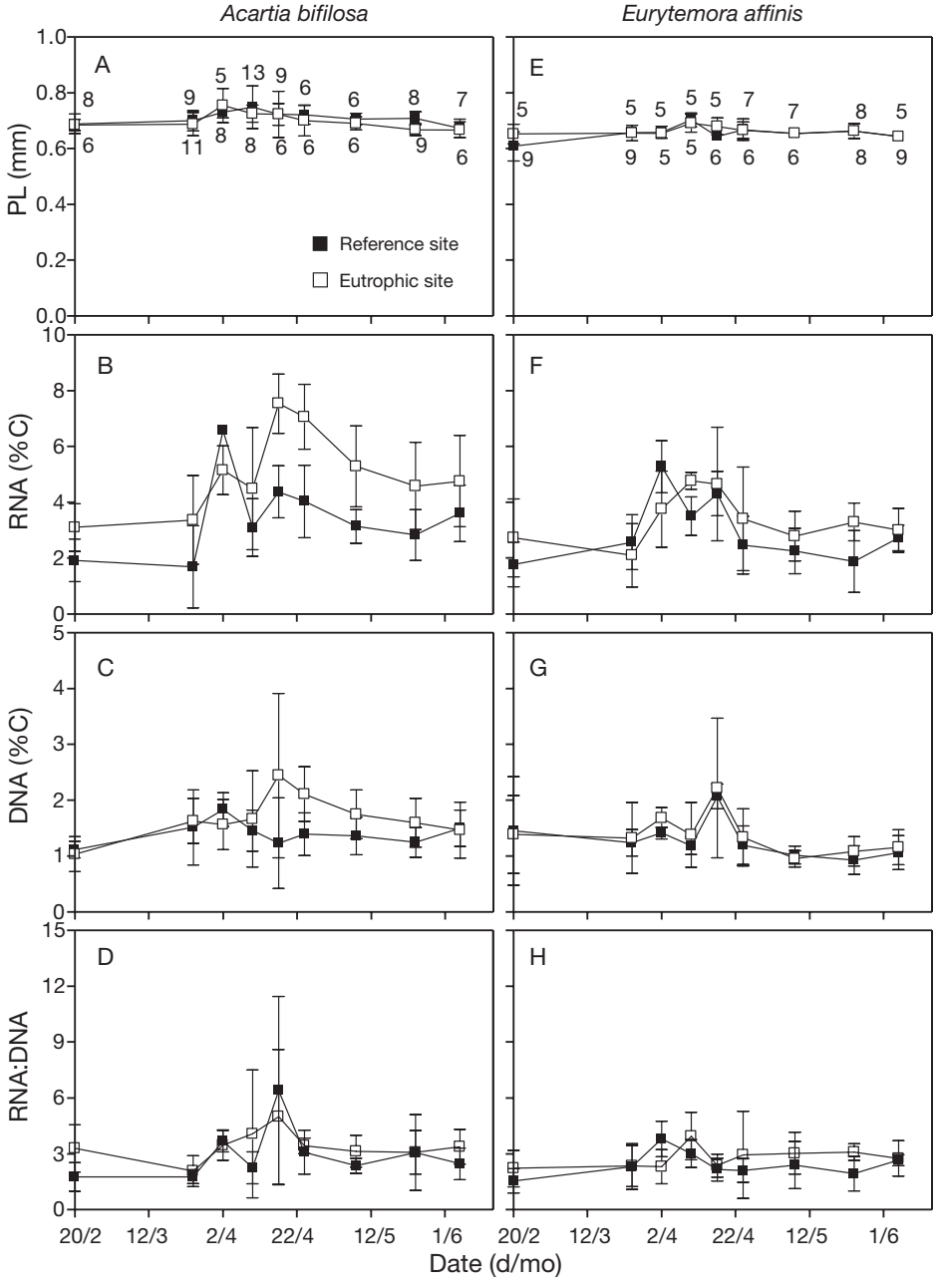

Fig. 6. (A–D) *Acartia bifilosa* and (E–H) *Eurytemora affinis*. Temporal variability (mean ± SD) of female prosome length (PL; A,E), RNA concentration (B,F), DNA concentration (C,G), and RNA:DNA ratios (D,H) at the reference and the eutrophic sites. Numbers of individuals analyzed for each variable (i.e. replicates) are shown as numbers above (reference station) and below (eutrophic station) data points on panels A and E

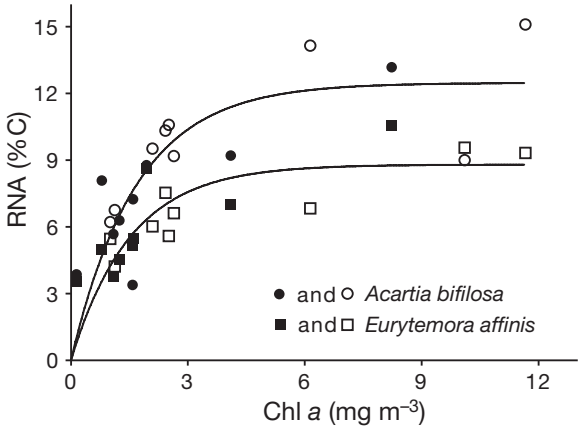

Fig. 7. *Acartia bifilosa* and *Eurytemora affinis*. Ivlev functional response curves (upper: *A. bifilosa*; lower: *E. affinis*) relating individual RNA concentration (%C) to ambient chl *a* (mg m^{-3}) concentration. Data from the reference and the eutrophic sites are shown as filled and open symbols, respectively. Curve fitting was done on non-transformed data

DISCUSSION

In the classic pelagic food web concept, the spring phytoplankton bloom is considered to support secondary production of herbivorous zooplankton and, subsequently, growth of fish and other organisms at higher trophic levels (Voipio 1981). Our findings confirm that in the northern Baltic proper, copepod growth is highly dependent on spring phytoplankton development. The nucleic acid levels appear to respond rapidly to both temporal and spatial variations in food availability despite low temperatures (2 to 5°C; Fig. 2A). These observations are consistent with previous reports on the rapid increase of RNA:DNA ratios in mussels (Dahlhoff & Menge 1996) and krill (Cullen et al. 2003) in response to local and seasonal chl *a* fluctuations. In *Calanus finmarchicus*, RNA:DNA ratios correlated positively with egg production and surface chl *a* concentrations in the Gulf of Maine (Durbin et al. 2003). By contrast, Geiger et al. (2001) found no significant changes in RNA:DNA ratios in copepods in relation to phytoplankton blooms in the Weddell Sea and suggested that the RNA:DNA ratio in adult copepods is not an adequate measure of their growth response to changes in food supply. However, as pointed out by the authors themselves, complications in storage and shipboard treatment compromised the reliability of their data. Moreover, in arthropods, use of the RNA:DNA ratio as a growth biomarker might be restricted by a considerable correlation between DNA concentration and growth rate (shrimps: Moss 1994, lobsters: Parslow-Williams et al. 2001, copepods: Gorokhova 2003). Our data support these earlier findings with significant correlations between DNA-based indices in the copepods and environmental variables. We also found lower significance and weaker regressions between RNA:DNA ratios and environmental determinants compared to those with RNA as the dependent variable (Tables 1 & 3). Thus, both methodological and biological issues may contribute to the strength of the correlation between nucleic acids in copepods and food supply.

The results of the multiple regression analysis suggest that chl *a* (i.e. photosynthetic biomass) and water transparency (i.e. both photosynthetic and non-photosynthetic material) are the key variables controlling growth conditions of *Acartia bifilosa* and *Eurytemora affinis* in the northern Baltic proper, whereas the effects of temperature and proportion of dinoflagellates in the phytoplankton community are less impor-

Table 3. *Acartia bifilosa* and *Eurytemora affinis*. Multiple linear regressions for nucleic acid-based indices using data for individuals collected at Stns B1 and H4 combined. Equations contain only variables that significantly improved the model R^2 value (Adj.: adjusted). Indices used as dependent variables were individual RNA and DNA content (RNA and DNA, µg ind.$^{-1}$), RNA and DNA concentrations ([RNA] and [DNA], µg mg C^{-1}), and RNA:DNA ratios. Chl *a* concentration (mg m^{-3}), water transparency (Secchi, m), water temperature (*T*, °C), relative abundance of dinoflagellates (Dino%), and copepod body size (prosome length, PL, mm) are independent variables. Input values are daily means of measured parameters; all values were either log or arcsine square root transformed as specified in 'Materials and methods'; n = 18

Species	*y*	x_1	x_2	x_3	Equation	Adj. R^2	β for x_1	β for x_2	β for x_3	p <
A. bifilosa	RNA	Chl *a*			$y = 0.022 + 0.055\ x_1$	0.57	0.770			0.0002
	DNA	Chl *a*	PL		$y = 0.009\ x_1 + 0.246\ x_2$	0.74	0.610	0.337		0.0005
	[RNA]	Chl *a*	Secchi	Dino%	$y = 0.220\ x_1 - 0.684\ x_2 + 0.269\ x_3 + 1.294$	0.76	0.635	–0.440	0.411	0.0000
	[DNA]	None								
	RNA:DNA	Chl *a*			$y = 0.265\ x_1$	0.51	0.608			0.005
E. affinis	RNA	Secchi	*T*		$y = -0.051\ x_1 - 0.015\ x_2$	0.88	–1.058	–0.307		0.0000
	DNA	*T*	Chl *a*		$y = -0.010\ x_1 + 0.009\ x_2 + 0.015$	0.48	–0.638	0.624		0.011
	[RNA]	Secchi	Dino%	*T*	$y = -0.448\ x_1 + 0.168\ x_2 - 0.097\ x_3 + 1.887$	0.83	–1.082	0.355	–0.232	0.0000
	[DNA]	Chl *a*	*T*	PL	$y = 0.229\ x_3 - 8.004\ x_2 - 0.199\ x_1 + 2.292$	0.58	0.862	–0.742	–0.553	0.012
	RNA:DNA	Secchi			$y = -0.221\ x_1 + 0.389$	0.57	0.877			0.007

tant (Table 3). The RNA–chl *a* relationships (Table 2, Fig. 7) indicate that although other prey such as detritus or ciliates could be eaten by these copepods, phytoplankton was the main food source during the study period. In both species, RNA concentration increased with chl *a* and became saturated at about 3.0 mg chl *a* m^{-3}. This implies that if phytoplankton is the main food source of the copepods, food limitation might be quite common during most of the growing season in the northern Baltic proper, where chl *a* concentration is 2.5 ± 1.25 (June to September 1994 to 1998; HELCOM 2002). Note that post-bloom chl *a* values observed in our study were even lower (1.3 ± 0.18 and 2.4 ± 0.20 mg m^{-3} at the reference and the eutrophic sites, respectively; Fig. 2). However, this limitation might be modulated by ciliate prey available during summer (Johansson et al. 2004).

Our data suggest that *Acartia bifilosa* reacts more rapidly to the increasing algae availability than *Eurytemora affinis*, as indicated by consistently higher *b* values, and has significantly higher RNA_{max} (Table 2, Fig. 7). This may indicate that, similar to *A. tonsa* (Escaravage & Soetaert 1995), *A. bifilosa* has higher growth efficiency than *E. affinis*, particularly at low food concentrations. Based on the inherent differences in specific growth rates between broadcast-spawning and egg-carrying copepods (Kiørboe & Sabatini 1995), higher growth in *A. bifilosa* is not unexpected. However, the absolute RNA concentration is not an adequate indicator of growth rate per se for interspecific comparisons, because ribosomal (i.e. rRNA) requirements for protein production and maintenance costs are species-specific (Karpinets et al. 2006). Moreover, if *A. bifilosa* has a higher demand for RNA allocation for a given growth rate, it may require a higher food density than *E. affinis* in order to maintain similar growth. Further, the comparison of RNA–chl *a* relationships suggests interspecific differences in reliance on phytoplankton as a food source and the importance of non-algal seston in the diet. In particular, *A. bifilosa* appears to utilize phytoplankton more efficiently than *E. affinis* as indicated by the higher r^2 values of Ivlev fit (Table 2). These results corroborate differences in feeding between the 2 species reported from other North American (e.g. Patuxent River; Heinle & Flemer 1975) and European (Gironde; Irigoien 1995) estuaries, where these copepods are usually seasonally and spatially separated: *E. affinis* occupies upper reaches of the estuaries with maximal abundance in spring, and, as one moves seaward, it is replaced by *A. bifilosa*, which is more abundant in summer. In these habitats, the diet of *A. bifilosa* is dominated by autotrophic forms, whereas heterotrophic organisms and detritus are more common in the diet of *E. affinis* (Gasparini & Castel 1997). In the northern Baltic proper, these copepods co-occur in time and space and share the feeding environment (Viitasalo et al. 1994, Johansson et al. 2004). Previously, *E. affinis* has been found to dominate the eutrophic area, while *Acartia* spp. dominated in the reference area (Adrian et al. 1999). Our findings partially corroborate these observations with the reference area having ~5-fold more abundant *A. bifilosa* than *E. affinis*, while in the eutrophic area, both species contributed nearly equally (based on AUC values; Fig. 4). During our study, the nutritional environment of the Himmerfjärden Bay was characterized by ~30% higher average phytoplankton biomass with greater proportion of diatoms compared to the reference area. Moreover, high turbidity observed in the eutrophic area together with uncoupled dynamics of chl *a* and Secchi depth (Fig. 2B,C) indicate the presence of large amounts of detritus and associated bacteria. Interestingly, *A. bifilosa* and *E. affinis* responded in different ways to these between-area differences. In *A. bifilosa*, RNA concentrations were significantly higher in the eutrophic than in the reference area in any time period, while in *E. affinis*, this was true for the post-bloom period only (Fig. 5). Enhanced growth conditions in the eutrophic area might be related not only to the higher chl *a* stocks during and after the bloom but also to the differences in phytoplankton species composition (Fig. 3), stoichiometric composition of algae due to the nutrient load (Hajdu et al. 1997), higher availability of microzooplankton (e.g. ciliates and flagellates, which are other important prey for the copepods in spring; Johansson et al. 2004) and of detritus (which is a plausible food source for both *A. bifilosa* [Irigoien 1995] and for *E. affinis* [Gasparini & Castel 1997]). Thus, the differences in nutritional environment between the areas together with the RNA–chl *a* relationships and multiple regressions can provide a causal explanation for the copepod distribution pattern. Taken together, our data suggest that while in the reference area at lower seston and chl *a* concentrations *A. bifilosa* outnumbers *E. affinis*, the feeding environment of the Himmerfjärden Bay can sustain higher abundances and near maximal RNA levels in both species.

High RNA levels during the bloom indicate elevated protein synthetic activity for egg production. Indeed, strong positive relationships have been observed between RNA levels and egg production rates in several copepod species (Nakata et al. 1994, Saiz et al. 1998), including *Acartia bifilosa* (Gorokhova 2003). High egg production together with increasing abundance of females should lead to high abundance of nauplii. However, the time lag between the peak of egg production and the mass appearance of nauplii could be as long as several weeks, because of the long egg development time at low temperatures (up to 16 d in *A. bifilosa* [Katajisto 2003] and up to 14 d in *Eury-*

temora affinis [Andersen & Nielsen 1997]). In accordance with these development times, the abundance of juvenile copepods increased as the spring bloom progressed, with the time lag of ~2 to 3 wk between the chl *a* peak and the copepod abundance peak (Figs. 2 & 4). This increase may result from hatching of both subitaneous, i.e. ready-to hatch, eggs produced in the spring and benthic resting eggs. Many estuarine copepods depend on the hatching of resting eggs for the recruitment of nauplii, and egg banks are important for both copepod species in the northern Baltic proper (Viitasalo 1992). In this area, *A. bifilosa* occurs throughout the year (Viitasalo 1992, Viitasalo et al. 1994) producing subitaneous eggs that hatch continuously. Unfavorable conditions, however, may induce quiescence of eggs that survive in sediment and hatch when growth conditions improve (Katajisto 2003). In spring, predation is low, food is plentiful, and therefore nauplii survival is high, resulting in the rapid population build-up. Thus, in this species, hatching of quiescent eggs is likely to be particularly important in early spring; however, quantifying this importance is a challenging task. To test whether egg production during the bloom is sufficient for observed nauplii production in *A. bifilosa*, abundance of nauplii was predicted from (1) observed RNA concentrations and female abundance using the regression between egg production rate and RNA established earlier (Gorokhova 2003), (2) observed temperature, and (3) published temperature-dependent development rates for eggs (Katajisto 2003) and nauplii (Klein Breteler & Schogt 1994). The model was $A_n(t) = A_n(t - \mathrm{d}t) + [ER - (A_n \times M_n) - (A_n \times DT_n^{-1})] \times \mathrm{d}t$, where $A_n(t)$ is the abundance of nauplii at time t, $\mathrm{d}t = 1$ d, ER is egg recruitment (egg m^{-3}), M_n is nauplii mortality rate (= 0.1), considered a constant fraction of the population (Hirst & Kiørboe 2002), and DT_n is the published temperature-dependant development time (d) of nauplii (Klein Breteler & Schogt 1994). $ER = EPR \times A_F \times (1 - M_e) \times H \times DT_e^{-1}$, where EPR is individual egg production rate (egg $female^{-1}$ m^{-3}), A_F is observed female abundance (ind. m^{-3}), M_e is egg mortality rate (= 0.2), considered a constant fraction of the population (Hirst & Kiørboe 2002), H is egg hatching success of 0.56 (Katajisto 2003; lowest values for egg hatching success observed in this study were adopted to provide a conservative estimate), and DT_e is the published temperature-dependant development time (d) of eggs (Katajisto 2003). $EPR = (0.0043 \times RNA - 0.125) \times W_F/W_e$ (Gorokhova 2003), where RNA is observed individual RNA concentration (µg mg^{-1} C), W_F is female body weight (µg C $female^{-1}$) calculated using average PL values as described in 'Materials and methods', and W_e is egg weight (0.04 µg C egg^{-1}; Gorokhova 2003). To calculate A_n on a daily basis, data on temperature, female abundance, body size, and RNA concentrations were linearly interpolated between the sampling dates. The modeled nauplii abundances were substantially higher than those observed, particularly for the eutrophic area (Fig. 4A,C), suggesting that under the conditions of this study and our assumptions, additional sources (i.e. benthic eggs) would be unnecessary to account for the observed nauplii abundance. Although rough, these calculations indicate that egg production by the overwintering females is sufficient to account for the nauplii pulse observed in spring.

As a related issue, evidence is accumulating that diatoms, a major component of the spring algal bloom in temperate waters, are harmful for copepod reproduction (Ianora et al. 2004 and references therein). In particular, the presence of toxic or inhibitory components produced by diatoms may have negative impacts on reproductive success via abnormal embryonic or post-hatching development, although alternative food sources may compensate for these effects in natural communities (Ianora et al. 2004). In our study, diatom abundance and chl *a* changed in concert due to the diatom dominance during the bloom. As a result, RNA was correlated positively with both chl *a* concentration and, albeit to a lesser degree, to the diatom stocks (Table 2). However, as we did not measure the ingestion of different food items, selective feeding and its consequences for growth cannot be ruled out. Moreover, it is not clear if and how female RNA concentration is related to egg hatching success. If the RNA concentration simply reflects the synthesis rate of total mixed proteins, and the egg protein content is relatively stable, then female RNA allocation per unit of growth would not be indicative of hatching success. However, Standiford (1988) suggested that in the copepod *Acanthocyclops* during oogenesis, when demand for rRNA is high, oocytes supply all the rRNA needed by the embryo. If an inadequate supply of maternal rRNA compromises egg hatching and nauplii survival, then female RNA production may reflect offspring viability. In this case, RNA content of eggs and nauplii would be most useful for an *in situ* assessment of diatom effects on copepod recruitment. To understand these connections, further studies are needed that will include egg production and hatching experiments with concurrent nucleic acid measurements in females and eggs.

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