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Relationships between nucleic acid levels and egg production rates in *Acartia bifilosa*: implications for growth assessment of copepods *in situ*

Elena Gorokhova*

Department of Systems Ecology, Stockholm University, 106 91 Stockholm, Sweden

ABSTRACT: To evaluate the applicability of RNA-based indices in copepod growth assessment, concentrations of nucleic acids in *Acartia bifilosa* were calibrated against growth rates estimated via egg production experiments, and the relationships between levels of RNA, DNA, and the RNA:DNA ratio and growth rates were examined. Furthermore, to investigate effects of temperature and food availability on the relationships between weight-specific fecundity and nucleic acid levels, incubations were carried out at 9 and 16°C, each at 3 food concentrations. There were positive relationships between nucleic acid concentrations and their ratios and weight-specific egg production rates. Overall, RNA concentration was the best predictor of specific growth rate. No correlations between either of the measured variables and female body size were observed. When egg production was elevated by manipulating the feeding regime, RNA concentration and the RNA:DNA ratio increased in concert. Neither growth nor RNA indices were significantly affected by temperature, while a significant increase in DNA concentrations was observed at high food levels and low temperatures. The lack of temperature dependence in RNA-growth relationships allows their direct application for *in situ* growth estimates in summer populations of *A. bifilosa* in the northern Baltic Proper.

KEY WORDS: Nucleic acid concentrations · RNA:DNA · Growth rate · Egg production · Weight-specific fecundity · Baltic · *Acartia bifilosa*

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INTRODUCTION

The lack of appropriate methodology for measuring site- and time-specific growth rates remains the greatest obstacle to estimating *in situ* zooplankton production (Poulet et al. 1995). In copepods, which are ecological key species in marine, and often in freshwater, ecosystems, production consists primarily of juvenile growth and adult egg production. Direct measurement of egg production is currently most used to estimate copepod growth (Poulet et al. 1995). This method is highly sensitive to changes in environmental variables (Saiz et al. 1997), and has the advantage of site- and time-specific estimates of production. However, egg production experiments are both time and labor consuming. Moreover, the risk of introducing artifacts due to the handling and use of various anesthetics is high, and bottle-effects are unavoidable (Miller et al. 1984). Consequently, there is still no definitive routine method for measuring copepod growth *in situ* (Poulet et al. 1995), and the search for new analytical tools and techniques for growth assessment is a significant issue for nearly all plankton biologists.

Since <u>Sutcliffe (1965, 1970)</u> suggested that growth could be estimated from the RNA content in small crustaceans, nucleic acid measures have generated increasing interest and have come to be recognized as a promising biochemical tool for evaluating growth and nutritional conditions in a variety of species. Accumulating research suggests a characteristic biochemical signature of rapid organism growth, manifested in elevated RNA concentrations, which is driven by the fundamental association of rapid growth with ribosomal RNA (Elser et al. 2000). Several studies have tested the applicability of nucleic acid analysis for growth rate assessment using RNA concentrations (Dagg & Littlepage 1972, Ota & Landry 1984, Båmstedt & Skjodal 1980) or RNA:DNA values (Wagner et al. 1998, 2001) of mono- or multispecies zooplankton samples. Although most of the early studies focused on using RNA:DNA ratios as an index of nutritional condition (Wagner et al. 1998), a calibration of zooplankton growth rates against nucleic acid content has also been attempted, and linear relationships have been found between growth rate and RNA content (and/or RNA:DNA ratio) for various species of marine copepods (Nakata et al. 1994, Saiz et al. 1998, Wagner et al. 2001) and freshwater cladocerans (Vrede et al. 2002). In some cases, however, the relationship between RNA content and growth rate has been found to lack sufficient predictability (Dagg & Littlepage 1972, Ota & Landry 1984). Of great interest, therefore, are the 'rules' used to estimate growth from nucleic acid measurements, and these need to be defined. For example, it has been shown that temperature influences the relationship between RNA and growth in copepods (Saiz et al. 1998, Wagner et al. 2001) and lobsters (Juinio et al. 1992), while a correlation was found between body size and the RNA:protein ratio in squid (Moltschaniwskyj & Jackson 2000), and between developmental stage and RNA-based indices in copepods (Wagner et al. 1998, 2001) and daphniids (Gorokhova & Kyle 2002). Thus, RNA-growth relationships need to be thoroughly evaluated and calibrated with independent estimates of growth rates, also in relation to biotic and ambient environmental conditions. In the case of copepods, growth rates determined via egg production experiments may serve as such independent measures.

In the northern Baltic Proper, the copepods Acartia bifilosa Giesbrecht and A. longiremis Lilljeborg constitute 22 to 44% of the total zooplankton biomass (Hansson et al. 1990, Viitasalo 1992). Of these 2 species, A. bifilosa dominates, especially in the upper mixed layer, and constitutes ca. 80% of the Acartia biomass in the coastal areas of the Stockholm Archipelago (Adrian et al. 1999). It is also one of the main prey species for fish (Hansson et al. 1990); therefore, A. bifilosa is considered to be a key species for zooplankton production in this region, and availability of rapid-yetsensitive methods to measure its growth would allow us to address many interesting ecological questions.

The primary objective of this study was to evaluate the applicability of RNA-based indices to assess copepod growth by examining: (1) relationships between reproductive growth rates and RNA-DNA concentrations and RNA:DNA ratios in *Acartia bifilosa*, and (2) the effects of temperature and food availability on these relationships.

MATERIALS AND METHODS

Collection sites and methods. Egg production rates of Acartia bifilosa were estimated from shipboard incubations carried out in August 1998 during the second BASYS (Baltic Sea System Study) cruise in the eastern Gotland basin of the northern Baltic Proper (Fig. 1). Data on environmental conditions were obtained from simultaneous measurements during the cruise, according to standard protocol of the Baltic Monitoring Programme (HELCOM 1988). Copepods were obtained either from slow vertical or drift tows made with a 90 µm WP-2 plankton net, or with a 50 l water bottle within the upper 10 to 15 m. The zooplankton were transferred into a plastic insulated container with ambient seawater and sorted within an hour. Copepods were gently removed from this container using a sieve, washed into a petri dish, and sorted under a dissecting microscope with a wide-mouthed pipette.

Egg production under ambient conditions. Groups of 3 to 5 adult females of *Acartia bifilosa*, which is a free-spawning species, were incubated in either 1 or 0.3 l bottles for 24 to 28 h at the ambient water temperature (15.6 to 17.8°C). Surface water was filtered through a 60 μ m net and used as an experimental medium. The 1 l bottles were placed in a plankton wheel, turning at a speed of 0.5 rpm, and the 0.3 l bot-



Fig. 1. Study area: samples were taken during a scientific cruise with RV 'Baltica' in August 1998 in the northern Baltic Proper. Most of the experimental animals were collected at Stns T1 (57°36'N 19°21'E, bottom depth 36 m) and BY15 (57°16'N 20°05'E, 114 m)

tles were incubated in a covered 50 l plastic container, through which surface seawater was pumped continuously. The ship's motion was assumed to be sufficient for keeping phytoplankton in suspension. Although none of the experimental incubation bottles had a screen to prevent females from ingesting their eggs, the low density of females was assumed to minimize the possibility of egg cannibalism (Laabir et al. 1995), and no empty or damaged eggs were observed. At the end of incubation, the contents of each incubation bottle were poured through a 35 µm submerged sieve, and eggs and nauplii were counted under a dissecting microscope. Females were removed, rinsed with 0.2 µm filtered surface seawater, and their prosome length (PL, mm) was measured under a microscope equipped with a micrometer eyepiece. They were then transferred into 1.5 ml Eppendorf tubes (1 per experimental replicate, i.e. a group of 3 to 5 ind. per tube) and stored at -80°C until nucleic acid analysis.

Effects of temperature and food on egg production and nucleic acid levels. To study effects of temperature (T) and food availability (F) on growth rates and nucleic acid concentrations, a 2×3 factorial experimental design was used ($T \times F$ experiment hereafter). In this experiment, 3 females of Acartia bifilosa were placed in each of 18 bottles (0.3 l). The bottles were incubated at 9 or 16°C (9 bottles at each temperature) and 3 alternative feeding regimes (high, medium, and low; 3 replicates for each feeding regime and temperature). To create a gradient in food availability, prescreened (60 µm) ambient water (referred as 'medium food level'; chl $a \sim 2.4 \text{ mg m}^{-3}$, particulate organic carbon [POC] ~640 mg C m⁻³) was either 5-fold diluted ('low food level'; chl $a \sim 0.5 \text{ mg m}^{-3}$,) or 5-fold concentrated ('high food level'; chl $a \sim 12.0 \text{ mg m}^{-3}$) by tension-flow filtration. All experimental animals were adapted to temperature and feeding conditions by incubating them in the respective temperature and food combination for ~32 to 42 h prior to the experiment. A few males were added to containers with females during the adaptation period to ensure insemination. Egg production measurements and preservation of the experimental animals were conducted in the same way as in experiments under ambient conditions.

Growth estimates. Egg production rates (EPR, eggs female⁻¹ d⁻¹) were calculated as:

$$EPR = \frac{E+N}{F} \times \frac{1}{t}$$
(1)

where *E* is the number of eggs at the end of incubation, *N* is the number of hatched nauplii, *F* is the number of incubated females, and *t* is the duration of the experiment (d). Weight-specific egg production rates (SEPR, d^{-1}) of females were calculated as:

$$SEPR = EPR \times \frac{W_e}{W_f}$$
(2)

where $W_{\rm f}$ and $W_{\rm e}$ are female and egg weights as carbon. Egg weight for *Acartia bifilosa* was estimated to be 0.04 µg C egg⁻¹, based on the measured average egg diameter of 83 µm, and assuming a density of 0.14 ng C µm⁻³ (Kiørboe & Sabatini 1994).

The carbon content of a female was estimated from the length-carbon regression:

$$W = e^{3.793 \, \text{PL} - 2.285} \tag{3}$$

where W is body carbon content (µg) and PL is prosome length (mm). This regression was obtained for *Acartia bifilosa* within the same size range, and sampled in late summer at coastal stations in Storfjärden (Gulf of Finland; Viitasalo et al. 1995).

Nucleic acid analysis. Nucleic acids were quantified according Wagner et al. (1998), with some modifications; measured RNA and DNA concentrations were expressed as $\mu g mg C^{-1}$ using copepod individual carbon weights (Eq. 3).

Reagents: RNA (Type III: from Bakers Yeast, Sigma, cat. # R-7125); DNA (calf thymus, Sigma, cat. # D-1501); RNase, DNase-free (Boehringer Mannheim, cat. # 1579681), 50 Kunitz U ml⁻¹ in TE buffer; N-lauroysarco-sine (sarcosyl, Sigma, cat. # L-5125); ethidium bromide, EtBr, nuclease-free (Fisher Scientific); sterile water was used in all solutions.

Buffers: TE buffer; extraction buffer: 1 % w/v sarcosyl in TE buffer; standard buffer: 0.25 % sarcosyl in TE buffer.

Standards: RNA and DNA standard sets were prepared from frozen (-80° C), aliquoted stock. Working solutions were diluted in Standard buffer in concentrations ranging from 0.2 to 8.0 µg ml⁻¹ for RNA and from 0.2 to 6.0 µg ml⁻¹ for DNA. The standard sets were aliquoted and stored at -80° C until analysis.

Controls: Negative controls containing all chemicals but no copepods were included in every set of samples and processed in the same way. Positive controls were prepared as follows: ca. 0.5 mg of fresh organisms (all stages, a mixture of species retained on 500 μ m net) were crushed in 1 ml of water and centrifuged; supernatant was aliquoted, stored at -80° C, and a 10 μ l aliquot was used as a positive control sample with each set of experimental samples.

Extraction procedure: Extraction buffer (200 µl) was added directly to the tubes containing frozen copepods and samples were shaken at room temperature for 2 h. After extraction, TE buffer was added (600 µl), samples were shaken again for 15 min and centrifuged for 1 min at 7500 rpm.

Fluorometric determinations: Fluorescence measurements were conducted using a Fluoroscan II fluorometer (Labsystems, microplate reader, filters: 544 nm for excitation and 590 nm for emission) and black solid flat-bottom COMBO microplates (Labsystems, cat. # 9502067). Each plate included: (1) extracted samples and controls, 6 replicates, 80 μ l well⁻¹; (2) RNA and DNA standards, 2 to 4 replicates, 80 µl well⁻¹. Half of the replicates of each sample/control were designated for RNA determination (hereafter RNA samples/ controls) and half for DNA determination (hereafter DNA samples/controls). DNA samples, DNA controls, and DNA standards received 5 µl well⁻¹ of RNase followed by 25 min incubation at 37°C. At the end of the incubation, 80 µl well⁻¹ of EtBr were added to all wells and the plate was incubated for another 5 min at room temperature in darkness. The plate was then scanned with 0.5 s well-measurement time, 10 measurements per well. Total DNA was calculated based on the DNA-EtBr standard curve. Total RNA was estimated by calculating the difference between RNA and DNA sample fluorescence. From this difference, RNA concentrations in the samples were calculated based on the RNA-EtBr standard curve. Recoveries were determined by spiking 6 samples (3 for RNA and 3 for DNA) of positive controls. RNA and DNA standard stocks were added to this homogenate at concentrations of half the expected values for the homogenate. The final yields of RNA and DNA recovery were 94.1 ± 4.3 and $93.3 \pm 5.8\%$, respectively. Accordingly, the concentrations in the samples were corrected for percent recovery of internal standards.

Statistics. Statistical tests were conducted with GraphPad Prism 3.03 (GraphPad Software). Unless specified otherwise, data are presented as means \pm SD. When comparing 2 groups, an unpaired *t*-test was applied followed by an *F*-test to compare variances. The effects of temperature and food levels were examined by 2-way ANOVA. Data were tested for homogeneity of variances using Bartlett's test. Linear regressions were fitted by the least-squares method; ANCOVA was used to compare slopes and intercepts of regression lines. To determine whether the data differ significantly from a straight line, a *runs* test was applied. In all cases significance was accepted when p < 0.05.

RESULTS

Environmental and feeding conditions

Due to strong winds and low temperatures in the summer of 1998, the mixing layer was very uniform, with temperatures between 14 and 17°C. The thermocline was situated around 15 to 17 m depth. Concentrations of chl a, particulate organic carbon (POC) and particulate organic nitrogen (PON) were fairly

homogenous in the mixed layer. The chl a concentration was on average 2.4 mg m^{-3} , mean standing stocks of POC and PON were $640 \text{ mg C} \text{ m}^{-3}$ and $80 \text{ mg N} \text{ m}^{-3}$. respectively. Particulate phosphorus in the mixed layer ranged from 6 to 11 mg P m⁻³ (Olesen et al. 1999). The phytoplankton community was dominated by cyanobacteria and nanoflagellates <15 µm. Filamentous cyanobacteria (>90 µm; Aphanizomenon sp. and Nodularia spumigena) and picocyanobacteria (3 to 10 µm; mostly compact and loose colonies of Cvanodiction imperfectum, C. planctonicum, and Cyanonephron styloides) exceeded 70% of the phytoplankton biomass in the top 10 m of the water column, often with picocyanobacteria as the single most important group. The total phytoplankton biomass averaged $67.5 \text{ mg C m}^{-3} \text{ or } 7.45 \text{ g C m}^{-2} \text{ over the entire water col-}$ umn (S. Hajdu & H. Höglander, Stockholm University, pers. comm.).

Variability of EPR and SEPR

In the experiments with incubation in ambient water, EPR and SEPR averaged 8.3 and 0.28 eggs female⁻¹ d⁻¹, respectively (Fig. 2A,B). Corresponding values in the experiment with variable temperature and food availability were 5.0 and 0.18 eggs female⁻¹ d⁻¹ (Fig. 2A,B). There were no significant differences in either EPR or SEPR between the incubations in ambient water and the T × F experiment (unpaired *t*-test, p > 0.05 in all cases). In neither experiment were statistically significant correlations detected between EPR or SEPR and the prosome length of the females (p > 0.05 in all cases; Fig. 2A,B; size range 0.65 ± 0.02 mm, n = 33).

Variability of RNA-DNA concentrations and RNA:DNA ratios

In the incubations in ambient water, RNA and DNA concentrations averaged 79.5 and 25.6 μ g mg C⁻¹ (Fig. 2C,D), respectively. In the T × F experiment, RNA and DNA concentrations averaged 73.9 and 24.0 μ g mg C⁻¹, respectively (Fig. 2C,D). Corresponding RNA:DNA ratios varied from from 2.2 to 4.7 in ambient water and from 1.9 to 5.0 in the T × F experiment, with mean values of 3.0 and 3.1, respectively (Fig. 2E). Although in all the experiments variability in DNA concentration was much lower than that of RNA and of RNA:DNA (Fig. 2C-E), the amount of DNA per individual varied to as high as 1.3-fold (0.027 to 0.035 μ g). Neither nucleic acid concentrations nor their ratios differed significantly between the experiments with ambient water and the T × F experiments



Fig. 2. Acartia bifilosa. (A) Egg production rates (EPR, eggs female⁻¹ d⁻¹), (B) weight-specific egg production (SEPR, d⁻¹), (C) RNA and (D) DNA concentrations (μ g mg C⁻¹), and (E) RNA:DNA ratio in relation to female prosome length (PL, mm; mean \pm SD). Open and closed symbols represent data from the T × F experiment and from incubations in ambient water, respectively. For each variable, the average value and coefficient of variation for the pooled data set are shown in the upper left corner of the graph

(unpaired *t*-test, p > 0.05 in all cases). In neither of the experiments were statistically significant correlations detected between RNA-DNA concentrations or RNA:DNA ratios and prosome length (p > 0.05 in all cases; Fig. 2C–E).

Effects of temperature and food availability

Variation in temperature and feeding conditions resulted in substantially different female growth

rates (Table 1, Fig. 3). A 2-way ANOVA, with feeding regime and temperature as factors, and SEPR, RNA and DNA concentrations and RNA:DNA ratios as response variables, revealed highly significant effects of food levels on weight-specific fecundity, RNA concentration and RNA:DNA ratios (Table 1, Fig. 3A,C). At all food levels, both growth rates, RNA concentrations and RNA:DNA ratios appeared to be somewhat higher at 16 than at 9°C (Fig. 3A,C), but the differences were never significant (Table 1). There was a significant temperature effect, but also a significant interaction between temperature and food level in relation to DNA values (Table 1), suggesting that their effects on DNA concentration are complex. Simple-effect ANOVAs were conducted as follow-up tests because of the significant interaction. The DNA levels of copepods at either temperature did not differ significantly between the food levels (1-way ANOVA, p > 0.2 in both cases). However, while at low and medium food concentrations there were no significant differences between the temperatures (unpaired *t*-test, p > 0.6 in both cases), at high food levels, DNA values were significantly higher at 9 than at 16°C (unpaired *t*-test, $r^2 = 0.81$, p < 0.02; Fig. 3B).

Table 1. Acartia bifilosa. 2-way ANOVAs, for weight-specific egg production (SEPR, d⁻¹), RNA and DNA concentrations (µg mg C⁻¹) and the RNA:DNA ratio in females from the T × F experiment. Significant effects are indicated with asterisks (*p < 0.05, **p < 0.01, ***p < 0.001)

Variable,	df	SS	MS	F					
sources of variation									
SEPR									
Interaction	2	0.00019	0.00010	0.05357					
Food	2	0.1093	0.05488	30.25***					
Temperature	1	0.00211	0.00211	1.170					
Error	12	0.02169	0.00181						
DNIA									
Interaction	2	104.2	07 16	2 005					
Theraction	2	194.5	97.10	2.903					
Food	2	5464	2732	81.68					
Temperature	1	1.531	1.531	0.04578					
Error	12	401.4	33.45						
DNA									
Interaction	2	21.28	10.64	3.994*					
Food	2	1.440	0.7200	0.2703					
Temperature	1	20.48	20.48	7.688*					
Error	12	31.97	2.664						
ρνιλισινίλ									
Intoraction	2	0.4416	0 2208	1 227					
Food	2	0.4410	4 522	1.337					
Toolu	4	9.044	4.522	27.37					
Temperature	1	0.09480	0.09480	0.5739					
Error	12	1.982	0.1652						



Fig. 3. Acartia bifilosa. $T \times F$ experiment: relationship between weight-specific egg production (SEPR, d⁻¹) and (A) RNA and (B) DNA concentration (µg mg C⁻¹), and (C) RNA:DNA ratios in females incubated at 2 different temperatures (9°C: closed symbols; 16°C: open symbols) and 3 food levels (low, medium and high)

RNA-DNA concentrations, RNA:DNA ratios and egg production rates

There were significant positive relationships between female egg production rates and nucleic acid levels, as well as between egg production and RNA: DNA ratios for individuals incubated at both ambient conditions and in the $T \times F$ experiment (Table 2, Fig. 4). Because neither the slopes nor intercepts differed significantly between the regression lines for any of the conditions tested, the data were pooled for fitting common regression lines for RNA, DNA and the RNA:DNA ratio. As a result, not only RNA and the RNA:DNA ratio, but also DNA concentrations became significantly correlated with weight-specific egg production (Table 2, Fig. 4). The regressions between SEPR and RNA and between SEPR and the RNA:DNA ratio explained 92 and 75%, respectively, while regression between SEPR and DNA explained only 42% of the total variance. Since DNA concentration was found to be affected by temperature at the high food levels (Table 1), data obtained at 16° C in the T \times F experiment were combined with field measurements conducted at similar ambient temperatures, and corresponding regressions were calculated for SEPR-DNA and the SEPR-RNA:DNA ratio relationships (Table 2). By doing so, these relationships were strengthened: 80 and 77% of the total variance in SEPR was now explained by variations in DNA concentration and the RNA:DNA ratio, respectively. Still, the RNA concentration, which explained 88 to 95% of the total variance in SEPR (Table 2), and had narrower confidence limits of the regression line (Fig. 4A), was the best predictor of female production rates.

DISCUSSION

Relationships between the RNA content and/or the RNA:DNA ratio and growth rate have been demonstrated for a variety of aquatic metazoans (see Elser et al. 2000 for a summary), including copepods at different ontogenetic stages, i.e. females of Acartia grani (Saiz et al. 1998) and, more recently, juvenile stages of Calanus finmarchicus (Wagner et al. 2001). In this study, I demonstrate a similar relationship in females of A. bifilosa under summer temperatures and feeding conditions. This allows RNA-based growth indices to be used in the prediction of egg production rates in copepods. In particular, the regressions derived here can be used to assess the in situ reproductive growth of A. bifilosa under environmental conditions comparable to those observed during this study. Furthermore, since A. bifilosa is a common species for temperate waters and estuaries, this study provides a data set illustrating trends that might apply to other copepods with similar ecological preferences.

Because of high wind-induced mixing and thermocline erosion, environmental conditions during the study period were rather unusual: the maximum surface temperature approached the long-term mean and was the lowest observed in 6 previous years in the eastern Gotland basin (HELCOM 2002). Nevertheless, the ranges in egg production and body size were similar to those found for this species in other studies conducted during summer seasons in the Baltic (Schmidt Table 2. Acartia bifilosa. Statistics and parameter estimates of weight-specific egg production rate (SEPR, d⁻¹) regressions on RNA-DNA concentrations (µg mg C⁻¹) and RNA:DNA ratios for field-collected females (qq, field), for females used in T × F experiment (qq, T × F), for all the experiments (qq, all data), and for data obtained at 16°C in T × F experiment combined with field measurements conducted at similar ambient temperatures (qq, ambient T°C). In no case was significant deviation from linearity in the slope found. ns: p > 0.05, *p < 0.01, **p < 0.001

Independent variables and experimental groups included in regression	Slope	Intercept	r ²	Significance $F_{\rm df}$	of slope p
RNA					
٥٥, field	0.0040	-0.082	0.95	$99.62_{1.2}$	**
$\phi \phi$, T × F	0.0042	-0.134	0.88	31.591,4	**
çç, all data	0.0043	-0.125	0.92	94.141,8	***
DNA					
qq, field	0.034	-0.645	0.75	$6.007_{1.2}$	ns
$QQ, T \times F$	0.003	-0.110	0.01	$0.0116_{1.4}$	ns
çç, all data	0.027	-0.471	0.42	5.8321,8	*
çç, ambient T°C	0.038	-0.739	0.80	20.611,5	**
RNA:DNA					
oo, field	0.154	-0.217	0.93	$93.06_{1.2}$	*
$\phi \phi$, T × F	0.090	-0.103	0.82	$18.74_{1.4}$	*
çç, all data	0.116	-0.152	0.75	$24.16_{1,8}$	**
çǫ, ambient T°C	0.112	-0.131	0.77	15.991,5	*

et al. 1998, Koski & Kuosa 1999), as well as in other temperate estuaries (Uriarte et al. 1998, Burdloff et al. 2002). However, significant seasonal and geographical variations in all of these variables have been observed for Acartia bifilosa in the Baltic Sea (Viitasalo et al. 1995). Therefore, there remains a need to confirm that growth-RNA relationships exist in individuals with different life histories under various seasonal and trophic conditions, and a need to describe these relationships. In particular, contradictory results have been presented on the importance of female size for egg production in copepods (Peterson et al. 1991), and virtually nothing is known about variations in weight-specific guantities of nucleic acids and their ratios as a function of within-stage body size. Although in my study none of the variables were found to correlate significantly with body length (Fig. 2), this may not be the case over a wider size range. It would be desirable to carry out a study similar to the one presented here during a spring season, when the A. bifilosa population consists mostly of large, overwintered females (Viitasalo et al. 1995) adapted to low temperatures and low food concentrations from the winter period. Such individuals might have different physiological and metabolic rates than the summer generation, which consists of small-sized individuals (Viitasalo et al. 1995; this study) adapted to higher temperatures and moderate-to-high feeding conditions. The differences in morphology and life history may result in different growth patterns, metabolic

rates, cell biochemistry, and altered relationships between growth and nucleic acid levels, as found for *Calanus* (Wagner et al. 2001, Hansen et al. 2003).

If considered separately, both temperature and food availability can affect growth and egg production of Acartia bifilosa (Uye 1981, Saiz et al. 1997, Koski & Kuosa 1999). Several studies, however, indicate that temperature is a more important factor than food on a seasonal scale, while food conditions modulate egg-production dynamics mainly at shorter time scales (Uye 1981, White & Roman 1992). Moreover, as indicated previously, a significant interactive effect of temperature on the RNA-EPR relationship has been found in A. grani (Saiz et al. 1998). In my study, the response to increased food levels was clearly pronounced, while the response to temperature was not (Table 1, Fig. 3). In the $T \times F$ experiment, egg production responding to increased food availability was similar to the generally

observed functional response of A. bifilosa egg production, with the average egg production increasing up to chl a levels of ~12 mg m⁻³ (Koski & Kuosa 1999). Unexpectedly, SEPR estimates for individuals incubated in the ambient water shortly after collection covered a wider range than those in the $T \times F$ experiment (Figs. 2 & 4). Moreover, maximal SEPR values of females incubated in media containing a 5-fold concentration of ambient seston assemblage were ca. 2× lower than those in the females collected from the mixing layer (Figs. 2 & 4), where chl a values were nearly invariant. Several studies have shown that Acartia species prefer ciliates to phytoplankton, and their egg production is enhanced by ciliate feeding (Stoecker & Sanders 1985, White & Roman 1992). However, while preparing the food media for the $T \times F$ experiment, the ambient water was filtered to remove large colonies of phytoplankton, microzooplankton, and copepod eggs. As a result, ciliates were likely to become significantly reduced in the filtrate: due to their fragile nature, they easily burst when passing the sieve. Thus, a likely explanation to the larger variation and higher egg production rate in ambient water is that in the T × F experiment, the media was severely depleted in ciliates and had a lower food quality than the ambient plankton assemblage. This could potentially hamper egg production in the $T \times F$ experiment; the effect, however, was consistent among treatments and therefore did not affect the main result. Changes in both RNA and the



Fig. 4. Acartia bifilosa. Regressions between weight-specific egg production (SEPR, d⁻¹) and (A) RNA and (B) DNA concentration (μ g mg C⁻¹), and (C) RNA:DNA ratios from field-collected females (closed symbols) and those from the T × F experiment (open symbols). Each data point is a mean ± SD of 3 to 5 replicates (see 'Materials and methods'). Solid lines are least-squares regressions; dotted lines show respective confidence intervals

RNA:DNA ratio were significantly correlated with changes in EPR and SEPR (Fig. 2, Table 2), and followed the same pattern. However, while both egg production rates and RNA-based indices in *A. bifilosa* strongly responded to variations in food abundance, they were virtually temperature-independent (Table 1) — at least during this time and in this area. This is in agreement with the results of Koski & Kuosa (1999), who found no significant differences in egg production rate in the 7 to 18°C range in *A. bifilosa* from the summer populations in the Gulf of Finland. It has also been suggested that the temperature optimum for this species in the Baltic spans the 6 to 16°C interval (Sidrevics 1980), indicating a high physiological plasticity. Furthermore, the lack of temperature-dependence is not unexpected, considering vertical distribution of the A. bifilosa population and the temperature conditions during the study period. According to our survey of vertical distribution of plankton during the cruise, up to 65% of older copepodites and adults stayed near the thermocline, where temperatures were around 10°C, while the remaining part of the population occurred mostly in the upper waters with temperatures between 13 and 16°C (author's pers. obs.). Nevertheless, while the use of RNA-growth regression without a temperature variable is an empirical convenience, it may not hold over a wider temperature range; precise temperature functions would require more extensive experimental data.

Despite egg production not being directly related to temperature, there appears to be a change in growth response with temperature on a cellular level, as indicated by the DNA response. Copepods are known to exhibit determinate cell numbers (McLaren & Marcogliese 1983); therefore, all adult females of the same species have a similar number of somatic cells after the last moult, regardless of their body size. Hence, given the facts that: (1) individual DNA content varied 1.3-fold between the analyzed females, (2) DNA concentrations correlated with growth rates (Fig. 4B), and (3) a combination of 16°C and the high food level in the T×F experiment resulted in a significantly higher DNA concentration in female bodies (Fig. 3B), the assumption of the invariant cellular DNA content in Acartia bifilosa, which is an important part of the rationale for using RNA:DNA ratio for growth assessment, has failed.

The intra-population and intra-individual variations in nuclear DNA content have been described for many plant and animal taxa (see review by Gregory & Hebert 1999), including several calanoid species (McLaren et al. 1989, Escribano 1992). The DNA content of cells within an individual organism is believed to be manipulated in 2 ways. The first involves endopolyploidy and expansion of DNA in somatic cells; this process is especially common among arthropods (Lecher et al. 1995). The average proportion of polyploid nuclei in, for example, adult Daphnia pulex, was found to be as high as 27 % (Korpelainen et al. 1997), or even close to 50%, with ploidy levels varying among tissues from 2 to 2048 (Beaton & Herbert 1999). Moreover, ploidy levels were affected by growth rate and age, with adults expressing more extensive polyploidy than juveniles (Beaton & Herbert 1999). The second form of intra-individual DNA content modulation is chromatin diminution, when large amounts of DNA present in the zygote are deleted from early somatic cell lines (but not germline cells), resulting in gametic genomes that are substantially larger than expected from the somatic cell genome size (Gregory & Hebert 1999). The chromatin diminution has been described for several species of copepods, and mature females and males showed many gonadal nuclei with elevated amounts of DNA, suggesting that cycles of DNA endoreduplication may occur in germ cells during gametogenesis (Wyngaard & Rasch 2000). Genome size modulations may also result from the coordinate replication of some repetitive elements in the genome, in particular, those responsible for rRNA transcription and, therefore, potentially be related to protein synthesis and growth rates (White & McLaren 2000, Gorokhova et al. 2002). Standiford (1988) suggested that during oogenesis in the copepod Acanthocyclops, when demand for rRNA is high, oocyte contains a large amount of extra rDNA sequences supplying all the rRNA needed by the embryo. Once oogenesis is complete, the extra rDNA sequences are then diminuted. Therefore, actively reproducing females are likely to have higher DNA levels. Indeed, recent studies have shown that in Calanus, ovarian cells undergoing oogenesis alter RNA:DNA ratios (Biegala et al. 1999). Thus, there are several possible explanations, none of them exclusive of one another, to account for the observed correlation between DNA concentration and growth rate in Acartia bifilosa (Table 2, Fig. 4B). However, regardless of the cause, the variable nuclear DNA content implies that for predicting growth rates, the RNA index is likely to be more reliable when the concentration is expressed per biomass unit rather than as RNA:DNA ratios. I found RNA concentration per carbon weight to have a higher predictive value than the RNA:DNA ratio, even when the regression SEPR-RNA:DNA was restricted to data obtained at similar temperatures (Table 2, Fig. 4). These findings agree with earlier studies that have shown RNA concentration per biomass unit to be a more sensitive indicator of nutritional condition than RNA:DNA ratios in lobsters (Parslow-Williams et al. 2001), shrimps (Moss 1994a,b), and fish (Ferguson & Danzmann 1990).

One of the criticisms of using RNA-based indices as measures of growth rate has been that they may appear to correlate with growth rates purely as a function of ontogenetic change. It was suggested that a true test should include correlation analysis of nucleic acids and growth rates in individuals of the same size and/or developmental stage growing at different rates (Runge & Roff 2000). This type of test was conducted in the present study and the results provide further evidence that RNA content does reflect reproductive growth rates in copepods, and may serve as a basis for *in situ* production assessment. Further experiments are, however, needed (1) to establish similar relationships for juveniles of different developmental stages, as they are likely to be stage-specific (<u>Wagner et al. 2001</u>), and (2) to evaluate variations in growth-related life-history traits and their relationships with nucleic acid concentrations under varying environmental conditions.

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